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EDITORIAL

Dear Readers,

I am delighted to publish the Thirtieth Volume of Nepalese Veterinary Journal (NVJ) with several articles in the new research frontiers. This journal is aimed at disseminating the technologies and information available to the wider audience within and outside the country with the hope that the information and technologies thus disseminated will be helpful not only in improving the production and productivity from healthy animals but also for improving the quality of animal products in food chains and therefore, is aiming for the realization of one world one health concept. National livestock development requires continuous and dedicated efforts of veterinarians involved in this endeavor from diverse perspective and effective execution of programs at all levels. Our efforts so far has produced some tangible outputs which need to be strengthened further to contribute for national development through major improvement in sustainable production of livestock, poultry, fisheries and apiaries with no compromises on animal health, welfare and mother nature, and importantly meeting food and nutrition security besides uplifting the peoples' livelihoods dependent on animals.

Many individuals contributed for production and editing of this journal and the list is extensive to name all of them. I would like to express my sincere appreciation to all individuals who contributed directly or indirectly by sharing the information and knowledge for the publication of this journal.

I am also grateful to the authors for their contributions, which will increase our knowledge and understanding for improving animal, human and environmental health of the country and to the editorial board members for their invaluable time for critically reviewing the papers. Let us continue work together to achieve the common goals of food security for all and towards one health initiatives.

Editor in Chief

Review Article

GLOBAL CLIMATE CHANGE AND ITS IMPACTS ON DAIRY CATTLE

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ABSTRACT

Anthropogenic production of greenhouse gases (CO_2, CH_4, N_2O, etc) and its cumulative accumulation in the earth's atmosphere has caused the temperature of earth's atmosphere to rise continuously causing multifaceted effects to the animals. Atmospheric warming due to global climate change resulting thermal stress is one of the greatest climatic challenges faced by dairy cattle affecting animal health and welfare. This is the major issue of economic importance to dairy farmers due to loss in production, reduced reproductive performance of the animals and increase incidence of disease and mortality in the area of the world where ambient temperature often exceeds upper critical temperature. Impacts of thermal stress range from simple physiological and metabolic disturbances in animals to severe heat stroke and death. This review describes the impacts of global climate change and resulting thermal stress in dairy cattle.

Key words*:* Global climate change, global warming, thermal stress, dairy cattle

INTRODUCTION

Warming of climate system of the earth is a unanimously accepted reality (IPPC, 2007b) and probably one of the most prominent challenges for scientists, development workers, policy makers and other relevant stakeholders regarding development and sustainability in international and national arena during past several years. Intergovernmental panel on climate change (IPPC) has described climate change as any anthropogenic or naturally occurring alteration in the climate over time (IPPC, 2007b). The World Bank has published 'world development report 2010' with the title "development and climate change" as an example to depict the importance of this issue.

Global warming is attributable to increase in atmospheric concentration of green house gases (mainly CO_2, CH_4 and N_2O) as a result of human activities since the industrial revolution (IPPC, 2007b, World Bank, 2010). Concentration of total green house gases in the atmosphere has been increased by more than 75% from 1970 to 2004 (Barker *et al.,* 2007) (Figure 1). These trace gases have significant contribution to increase radiative forcing at the atmosphere (Lashof and Ahuja, 1990) resulting in net positive forcing of +1.6 W m^{-2} since 1750 (Solomon *et al.,* 2007). Emissions of green house gases (GHG) at current rate would result in more warming of global climate in 21st century than during 20th century (IPPC, 2007b). Atmospheric temperature of the earth has been increased by 0.74±0.18°c in 20th century and predicted to be increased by 1.8 to 4°c by the end of 21st century (IPPC, 2007b). Scientists have envisioned that increase in average global temperature above 2°c may be beyond the bearable limit of present-day societies causing extended and widespread societal and environmental disruptions (Richardson

et al., 2009). Warming of global climate has multifaceted effects in many natural, economic and social systems including ecosystem, agriculture, health, soil, water resources etc. across the world (IPPC, 2007a) and these effects will most probably be continued for centuries in future (IPPC, 2007b). Among several global fingerprints of climate change; increase in global mean surface temperature (Figure 1) and global ocean temperature, sea-level rise and arctic sea ice extent, ocean acidification, and more frequent extreme climatic events are some of the key variations in natural environmental system of the earth (Richardson *et al.*, 2009).

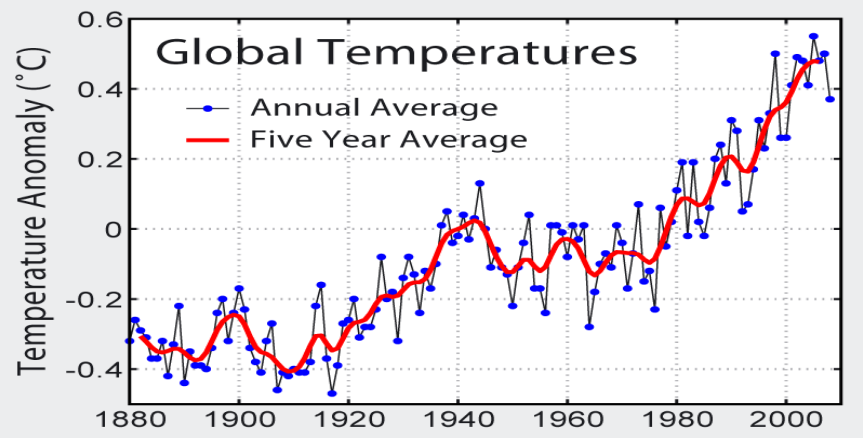

Figure 1**.** Instrumental record of global average temperatures as compiled by National Aeronautics and Space Administration (NASA). Anomalies in temperature are depicted with the zero of the coordinate as the mean temperature from 1961 to 1990. Source: http://www.globalwarmingart.com

Global climate change and animals

Climate change has complicated impacts on animals affecting distribution, growth, incidence of diseases, availability of prey, productivity and even extinction of species in extreme cases due to habitat loss (Gitay *et al.*, 2002, Nardone *et al.*, 2010, Meynecke, 2004, Franco *et al.*, 2006). Although both domestic and wild animals ranging from insects, amphibians, birds to mammals have been reported to be affected by global climate change, information on direct impact of climate change on animals is scarce (Burns *et al.*, 2003, Carey and Alexander, 2003, Chambers *et al.*, 2005, Nardone *et al.*, 2010, Walther *et al.*, 2002). Most of the impacts of climate change are attributable to increased ambient temperature.

Climate change has complex impacts on domestic animal production system affecting feed supply, challenging thermoregulatory mechanism resulting thermal stress, emerging new diseases due to change in epidemiology of diseases and causing many other indirect impacts (Thornton *et al.*, 2009). Thermal stress is one of the greatest climatic challenges faced by the domestic animals (West, 2003) and global climate warming may further aggravate the condition and even provoke new episode of thermal stress condition. Global warming has two way effects on animal production system. In one hand it directly affects the health, reproduction, nutrition etc. of the animals resulting in poor performance, inferior product quality, outbreak of novel diseases, etc. while on the other hand there are indirect effects on animal production due to change in soil fertility, decrease in preferred vegetation, rangeland degradation, desertification and decrease in production of feed stuffs (Nardone *et al.*, 2010).

Impact of global warming on dairy cattle

When ambient temperature increases, animal body attempts to regulate the core body temperature by altering physiological and metabolic function (Bernabucci *et al.,* 2010). Many of the behavioural, health and performance disturbances are attributable to these physiological and metabolic alterations (Bernabucci *et al.,* 2010, Wheelock *et al.,* 2010). Increased ambient temperature affects animal production system by affecting the health, reproduction, nutrition etc. of the animals resulting in poor performance, inferior product quality, outbreak of novel diseases, etc. (Nardone *et al.,* 2010, Thornton *et al.,* 2009).

Dairy cattle are particularly more susceptible to increased ambient temperature than other ruminants because of their high metabolic rate and poor water retention mechanism in kidney and gastrointestinal tract (Bernabucci *et al.,* 2010). Similarly, neonatal, postpubertal and lactating cattle are especially prone to thermal stress (Collier *et al.,* 1982a). Effects of thermal stress vary among individuals according to breeds, production level, prior experience etc. (Kadzere *et al.,* 2002). *Bos indicus* (Zebu) cattle are more thermotolerant than *Bos taurus* cattle due to possession of thermotolerant gene by zebu cattle (Hansen, 2004).

Intensification of thermal stress and more frequent occurrence of this problem is probably the most obvious consequence of global climate change in dairy cattle which is attributable to increased atmospheric temperature. The possibility that the problem of thermal stress to become more prominent with increased atmospheric temperature in future cannot be overlooked. Thermal stress is not only the concern of only tropical region but also a matter of major apprehension in sub-tropical and temperate regions too as frequent spells of high ambient temperature and gradual rise in global atmospheric temperature is being experienced in many of the temperate regions of the world due to global climate change (Nardone *et al.,* 2010).

Impacts of increased ambient temperature on dry matter intake (DMI)

Decrease in feed intake is one of the thermoregulatory physiological attempts of animals which decrease the metabolic rate, hence reducing metabolic heat production (Blackshaw and Blackshaw, 1994). Reduction in dry matter intake (DMI) indirectly helps to maintain core body temperature by reducing generation of heat during ruminal fermentation and nutrient metabolism.

Feed consumption by dairy cattle starts to decline when average daily temperature reaches 25 to 27°C (Beede and Collier, 1986) and voluntary feed intake can be decreased by 10-35% when ambient temperature reaches 35°c and above (Conrad, 1985). Mallonee *et al* (1985) reported that during hot weather feed consumption in cows is reduced by 56% during the day time in no-shed condition as compared to cows kept in shed. In the same study, feed consumption was increased by 19% during the night time and overall feed consumption is 13% less in cattle kept in no-shed condition as compared to cattle kept in shed.

Decrease in dry matter intake is more prominent in animals fed with roughage based diet than in animals fed with concentrate based diet (Beede and Collier, 1986). Similarly, reduction in DMI is more severe and rapid when food is poorly digestible (Beede and Collier, 1986). Decreased rumen motility due to thermal stress together with increased water intake results in

gut-fill which in turn reduce feed intake (Attebery and Johnson, 1969, Beede and Collier, 1986). Thermal stress may have direct effect in appetite centre in hypothalamus to inhibit feed intake (Baile and Forbes, 1974). Also, decrease in feed intake is more prominent in *Bos taurus* cattle than in *Bos indicus* cattle (Blackshaw and Blackshaw, 1994).

Impacts of increased ambient temperature on physiological parameter

Different physiological and metabolic alteration can be seen in animals under thermal stress many of which are body response to maintain the core body temperature constant. Rectal temperature and respiration rate of the animals are often higher in thermally stressed animals than those in the animals in thermoneutral zone (Mallonee *et al.,* 1985, Roman-Ponce *et al.,* 1977, Collier *et al.,* 1982b, McGuire *et al.,* 1991, Wheelock *et al.,* 2010). Increase in respiration rate and rectal temperature are more severe in *Bos taurus* cattle than those in *Bos indicus* cattle when exposed to similar thermal stress indicating more thermotolerant nature of *Bos indicus* (Gaughan *et al.,* 1999). Similarly heart rate of the animal under thermal stress is higher to ensure more blood flow towards peripheral tissue to dissipate heat from body core to the skin and then to the ambient.

Respiration rate of the animal can be used as an indicator of severity of thermal load but several other factors like animal condition, prior exposures to high temperature etc. should be considered to interpret the respiration rate (Gaughan *et al.,* 2000).

Effects of thermal stress on endocrine system

Process of adaptation and acclimation to thermal stress in animal is generally mediated by alteration in hormonal profile in the body (Bernabucci *et al.,* 2010). Levels of secretion of different endocrine glands and activity of hormones have been found to be altered during both active and chronic thermal stress.

Thermal stress in animal has found to alter the activity of thyroid gland resulting reduced concentration of thyroxine (T_4) and increased concentration of triiodothyronine (T_3) in plasma (Collier *et al.,* 1982b, Nardone *et al.,* 1997, McGuire *et al.,* 1991). It has been speculated that reduced thyroid activity reduces GI tract motility and rate of ingesta passage (Beede and Collier, 1986). Similarly, secretion of adrenal hormone aldosterone is decreased due to thermal stress which causes reduced sodium reabsorption in kidney tubules resulting electrolytes imbalance (Collier *et al.,* 1982a). Level of catecholamines (adrenaline and noradrenaline) and glucocorticoides (hydrocortisone) were found to be sharply increased when Holstein cattle were exposed to high ambient temperature (40–43°c) with glucocorticoides level returning to normal after long heat exposure but level of catecholamines remained persistent (Alvarez and Johnson, 1973). Likewise, level of prolactin hormone was found to be increased during thermal stress (Ronchi *et al.,* 2001). In contrast, secretion of plasma somatotropin was marginally reduced during thermal stress which is independent of reduced dry matter intake (McGuire *et al.,* 1991)

Impact of increased ambient temperature on energy balance and metabolism

Thermal stress condition results in 20-30% more maintenance energy requirement ensuing reduced amount of net energy for growth and production (Collier *et al.,* 2005, Little and Campbell, 2008). Increased expenditure of energy for maintenance together with reduced

intake of energy results in negative energy balance which is responsible for many of the consequences of thermal stress (Baumgard *et al.,* 2007, Rhoads *et al.,* 2009, Bernabucci *et al.,* 2010). Thermal stress independent negative energy balance condition causes lower blood insulin and decreased tissue sensitivity to insulin (Bernabucci *et al.,* 2010) but thermal stress causes increased level of circulating insulin and increased insulin response (Wheelock *et al.,* 2010, Itoh *et al.,* 1998)

Thermal stress causes reduction in blood glucose and non esterified fatty acid (NEFA) level due to reduction in hepatic glucose synthesis (Baumgard *et al.,* 2007, Rhoads *et al.,* 2009, Wheelock *et al.,* 2010). Reduction of non esterified fatty acid (NEFA) level during thermal stress is peculiar and contrasting than expected because increased level of catecholamines and glucocorticoides were supposed to cause lipolysis and mobilize adipose tissue (Bernabucci *et al.,* 2010). This phenomenon proved direct effect of thermal stress which is independent of reduced dry matter intake (Bernabucci *et al.,* 2010, Wheelock *et al.,* 2010). Reports about level of growth hormone (GH) during thermal stress are inconsistent as both increase and decrease secretion of this hormone in response to thermal stress has been reported (McGuire *et al.,* 1991, Rhoads *et al.,* 2009).

Impact of thermal stress on electrolyte and acid base balance

Increased potassium loss through skin due to increased sweating (Jenkinson and Mabon, 1973, Mallonee *et al.,* 1985) together with increased urinary sodium excretion due to lower aldosterone during thermal stress results in electrolyte imbalance in rumen fluid and plasma (Collier *et al.,* 1982a). Decreased net mineral intake due to reduced appetite (Blackshaw and Blackshaw, 1994) and reduced absorption of minerals during hot ambient temperature (Kume *et al.,* 1987) results further imbalance in electrolytes and chemical reaction in blood and rumen. Similarly, hyperventilation due to increased respiratory rate reduces the level of bicarbonate (HCO_3^-) in blood resulting respiratory alkalosis (Roman-Ponce *et al.,* 1977).

Impacts of thermal stress on animal health

Thermal stress may have both direct and indirect effects on animal health. Direct effects of thermal stress range from simple physiological disturbances to organ dysfunction and death (Nardone *et al.,* 2010, Bernabucci *et al.,* 2010). Reduction in feed intake together with diversion of more energy to maintain normal body function creates negative energy balance which compromise health and deteriorate animal body condition score (Brosh *et al.,* 1998, Avendano-Reyes *et al.,* 2010, Baumgard *et al.,* 2007, Bernabucci *et al.,* 2010). Immunity of nutritionally challenged animals with poor body condition is compromised making animals prone to infectious diseases (Markusfeld *et al.,* 1997). Reduced disease resistance of the animals, enhanced multiplication of microorganisms and altered vector population cause increased incidence of certain diseases like mastitis during summer when ambient temperature is high (Hogan *et al.,* 1989, Waage *et al.,* 1998, Chirico *et al.,* 1997). Furthermore, high ambient temperature and moisture level creates the environment suitable for fungus growth in feed and feedstuffs which may lead to mycotoxicosis in animals due to mycotoxin produced by fungi (Cotty and Jaime-Garcia, 2007, Hussein and Brasel, 2001).

Mortality of the animal is reported to be directly related with temperature humidity index (THI) above certain break point (Vitali *et al.,* 2009). In a 6-year extensive study in Italy, it was found

that mortality rate in dairy cows is the highest in summer and the lowest in spring (Vitali *et al.*, 2009). From this study, Vitali and his colleagues (2009) reported that mortality in dairy cattle increases sharply when maximum and minimum temperature humidity index (THI) increases from 80 and 70 respectively. The same study specified 87 and 77 as upper maximum and minimum critical THI above which the mortality reached maximum. Similarly, calves born in summer and winter have higher mortality rate (Martin *et al.*, 1975).

Excessive water loss through sweating and panting during thermal stress may cause cardiovascular disturbances (Silanikove, 1994). Similarly, modification in glucose and fatty acid metabolism together with reduced liver function and oxidative stress during thermal stress causes more incidences of metabolic disorders resulting reduced productivity and reproductive efficiencies (Nardone *et al.*, 2010).

Impacts of thermal stress on rumen health and pH

Increase in ambient temperature causes panting and increased respiration rate as an attempt to maintain body temperature through evaporative cooling (Schneider *et al.*, 1988, Wheelock *et al.*, 2010). Increased respiration rate leads to hyperventilation and increased exhalation of CO_2 resulting low level of bicarbonate (HCO_3^-) in blood (Schneider *et al.*, 1988). Increased secretion of bicarbonate (HCO_3^-) from kidney and decreased secretion of HCO_3^- in saliva are some of the consequences of hyperventilation (Nardone *et al.*, 2010, Schneider *et al.*, 1988). Buffering action of saliva is impaired due to this which results in disturbances in rumen pH - (Nardone *et al.*, 2010). Lower volume of saliva due to less feed intake as an attempt to reduce metabolic heat production further intensify the instability in rumen acid base balance (Nardone *et al.*, 2010). The imbalance in rumen PH may leads to rumen acidosis (Kadzere *et al.*, 2002), laminitis and reduction in milk fat production (James, 1997). Attebery and Johnson (1969) reported decreases in amplitude and frequency of rumen contractions when Holstein cattle were exposed to 38°C ambient temperature for 5 days. Similarly, rumination also decreases during thermal stress (Little and Campbell, 2008).

Impacts of thermal stress on proportion of VFAs produced in the rumen

Total amount of volatile fatty acids (VFAs) and proportion of different VFAs is altered when ambient temperature is above the thermoneutral zone for cattle (Kelley *et al.*, 1967). Kelley *et al.*, (1967) reported that molar proportion of acetate, propionate and total VFAs altered from 94.7, 33.3 and 147.9 to 47.2, 10.6 and 66.3 mEq/L respectively when ambient temperature was raised from 18.2 to 37.7°c and feed intake was controlled at constant level by force feeding through rumen cannula (Figure 2). From this experiment, it is evident that molar percentage of acetate is increased and that of propionate is decreased when cattle undergoes thermal stress condition.

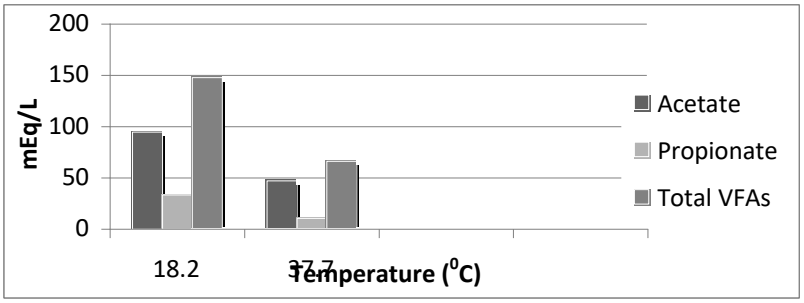

Figure 2: Proportion of VFAs produced in the rumen at different ambient temperature, Data from Kelly *et al* (1986)

Impact of thermal stress on nutrient absorption from GI tract

Although digestibility of feed was reported to be increased at higher ambient temperature (Warren *et al.,* 1974), absorption of nutrient from gastrointestinal tract is impaired during thermal stress. When ambient temperature is more than the normal body temperature, the blood circulation to the skin and peripheral tissue increases with vasodilation of peripheral blood vessels to transfer more heat from core to the skin surface and to hasten evaporative and convective heat loss from skin thereby reducing blood supply to visceral organs including GI tract (Oakes *et al.,* 1976, Roman-Ponce *et al.,* 1978). Reduction in intestinal blood flow may reduce the absorption of nutrients from the intestine.

Impact of thermal stress on immunity

Development of resistance against disease in calves is largely influenced by amount of immunoglobulin present in colostrum. Passive transfer of immunity from dam to neonatal calves through colostrum is found to be decreased with increased ambient temperature (Donovan *et al.,* 1986) and concentration of immunoglobulins (IgG and IgA) in colostrum is lower when cow is exposed to high ambient temperature during late pregnancy and early postpartum period (Nardone *et al.,* 1997). In contrast to above result, Lacetera *et al.,* (2005) have found that level of IgM secretion in periparturient cows calved in summer is higher than the cows calved in spring. Altered metabolic status of the animals may also cause reduction in immunity making animals more susceptible to diseases (Nardone *et al.,* 2010). Decline in immune function is breed dependent characteristics therefore different breeds may have different immunological response to high ambient temperature (Lacetera *et al.,* 2006). Increased incidence of mycotoxicosis during hot ambient temperature also compromise immunity in animals (Oswald *et al.,* 2005).

Impacts of thermal stress on reproduction

Thermal stress causes imbalance in secretion of reproductive hormones (Ronchi *et al.,* 2001). High ambient temperature has been reported to increase incidence of ovarian cysts (Ronchi *et al.,* 2001). Plasma progesterone level in animals under high ambient temperature is low as compared to animals under thermal comfort (Ronchi *et al.,* 2001). Badinga *et al* (1993) have reported that high ambient temperature causes poor quality of ovarian follicles resulting poor reproductive performance in cattle. Fertility of cattle is also reduced due to low intensity and duration of estrus caused by reduced luteinizing hormone (LH) and estradiol secretion during thermal stress (Rensis and Scaramuzzi, 2003). Reduced libido, decreased length and intensity

of heat and increased embryonic mortality in cattle suffered with thermal stress reduce reproductive efficiency (Collier *et al.,* 1982a, Ryan *et al.,* 1993, Little and Campbell, 2008).

Conception rates were reported to be less in cattle under thermal stress as compared to those in cattle in thermoneutral zone (Dunlap and Vincent, 1971, Roman-Ponce *et al.,* 1977). Thermal stress prior to and immediately after artificial insemination (AI) causes reduction in conception rate in high producing lactating cows (Ricardo *et al.,* 2004). In addition, thermal stress also causes decrease reproductive efficiency by increasing calving interval due to reduction in 90-day non-return rate after calving (Yaser *et al.,* 1999). Similarly, calves borne from dams under thermal stress were found to be of lower body weight than those from normal cows followed by reduced lactational performance of the dams due to carryover effects of thermal stress during prepartum period (Collier *et al*., 1982b, Collier *et al.,* 1982a). Similarly, climatic warming also affects reproductive performance in bulls. Concentration of semen, motility and spermatozoa per ejaculation is lower in summer than in winter (Mathevon *et al.,* 1998). Impaired spermatogenesis during thermal stress results in poor quality semen (de Alba and Riera, 1966). In addition, defects in spermatozoa are higher during summer than during winter (Nichi *et al.,* 2006).

Impacts of thermal stress on milk production

Reduction in milk production is one of the major economic impacts of climatic stress in dairy cattle. Decrease in milk yield due to thermal stress is more prominent in Holstein than in Jersey cattle (Sharma *et al.,* 1983). Decreased synthesis of hepatic glucose and lower non esterified fatty acid (NEFA) level in blood during thermal stress (Baumgard *et al.,* 2007, Rhoads *et al.,* 2009, Wheelock *et al.,* 2010) causes reduced glucose supply to the mammary glands resulting low lactose synthesis which in turn ensues low milk yield (Nardone *et al.,* 2010). Reduction in milk yield is further intensified by decrease in feed consumption by the animals to compensate high environmental temperature (Nardone *et al.,* 2010, Blackshaw and Blackshaw, 1994).

Reduced milk production due to thermal stress is attributable only partly to decrease in feed intake (Rhoads *et al.,* 2009). Actually 35% of reduced milk production is due to decreased feed intake while remaining 65% is attributable to direct effect of thermal stress (Rhoads *et al.,* 2009). Other factors resulting reduced milk production during thermal stress are decreased nutrient absorption, effect in rumen function and hormonal status and increased maintenance requirement resulting reduced net energy supply for production (Wheelock *et al.,* 2010, Bernabucci *et al.,* 2010).

Milk production in cow has been found to be reduced when ambient temperature and temperature humidity index increases above critical threshold (West, 2003, Bohmanova *et al.,* 2007). Thermal stress during 60 days prepartum period negatively affects postpartum milk production (Moore *et al.,* 1992) and cows parturated during summer produce less milk as compared to other season (Ray *et al.,* 1992). Similarly, quantity of milk protein and solid not fat (SNF) have been found to be reduced during thermal stress in dairy cattle (Bernabucci *et al.,* 2002, Rhoads *et al.,* 2009, Ominski *et al.,* 2002). Mallonee (1985) reported 20% less milk yield in cattle kept in sun than milk yield in cattle kept in shed. Similarly, Roman-Ponce (1977) found 10.7% higher milk production in cows kept in shed than that in cows kept in sun during hot weather.

CONCLUSION

Atmospheric temperature of the earth has been increased due to cumulative effects of greenhouse gases in the atmosphere emitted from different industrial and agricultural activities of human. Warming of the climate system of the earth has multifaceted affects on animals. Although information about exact impacts of the global climate change on animal production system and the animals are scarce, intensification and increase frequency of thermal stress is the most prominent impact of global warming in dairy cattle resulting in different physiological, metabolic and production disturbances. Importance of thermal stress has been increased to the dairy farmers in tropical, subtropical and even in temperate region of the world due to atmospheric warming. Although effects of thermal stress at farm level can be ameliorated by altering microclimate in the farm and applying nutritional management strategies long term sustainability of the system requires reduction in greenhouse gas emission.

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GROSS AND HISTOPATHOLIGAL CHANGES IN EXPERIMENTAL AFLATOXICOSIS IN RABBITS

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ABSTRACT

A total of eighteen healthy rabbits of 3-6 months of age with body weight of 1-1.5 kgs were divided into 3 groups of (G1, G2 and G3) 6 rabbits each fed with diets containing 0.1, 0.2 and 0.3 ppm of aflatoxin respectively for a period of 30 days. Gross and histopathological changes were recorded after feeding aflatoxin at different levels. In 0.1 ppm group the livers were mottled with distended gall bladder and in 0.2 ppm besides the above changes numerous pale areas and hardening of the consistency of the liver were observed, while in 0.3 ppm , the liver was atrophied with the wrinkling of the glisson's capsule and a scattered pale foci on the surface. Microscopically, hyperaemia, cell swelling and cytoplasmic granularity were consistently present in all the groups, while the hydropic change, focal necrosis, Kupffer cell hyperplasia with portal infiltration of round cells were observed in low dosed group. The changes were superimposed with bile duct hyperplasia, portal fibrosis, cholangitis and cholangiofibrosis.in high dosed group. Lungs in all dose levels showed sub pleural haemorrhages and emphysema. Microscopically, oedema, focal haemorrhages and emphysema were mild in 0.1 ppm group, where as these changes were pronounced in the 0.3 ppm group. Kidneys in 0.1 ppm group showed enlargement with hyperaemia while in higher dosed groups a few pale spots and granularity of the cortex were observed. Microscopically, glomerular hyperaemia, swelling of the tuft and filling up of the Bowman's capsule besides tubular degeneration were observed in 0.1 and 0.2 ppm groups. Tubular degeneration, necrosis and interstitial infiltration were prominent in 0.2 ppm group and these changes were pronounced along with interstitial fibrosis and medulllary haemorrhage among rabbits fed with 0.3 ppm level. The intestine in all the groups showed congestion and diffuse catarrhal changes. Meninges in all the treated animals showed varying degrees of congestion, while splenomegaly was occasionally observed in 0.3 ppm group. Microscopically catarrhal changes mild epithelial necrosis and desquamation in the intestine were observed in all the groups. Hyperaemia alone was observed in the brain with 0.1 ppm treatment, however mild neuronal degeneration and necrosis observed in 0.2 ppm were superimposed with gliosis, vacuolation astrocyte proliferation and perivascular cuffing in 0.3 ppm group. In the spleen hyperaemia and lymphoid depletion were observed in all the groups, while reticular hyperplasia developed as a sequalae of changes in the 0.3 ppm group. The activity of GGT was moderate in the livers of 0.3 ppm group animals which also histologically revealed cholangitis, cholestasis and cholangiofibrosis apart from hepatitis

INTRODUCTION

Aflatoxicosis as a separate disease entity had its first report by Blount in 1960 in turkeys from England (turkey "X" disease), when the exact etiology was not known. Subsequently many others reported on aflatoxicosis from different parts of the world affecting several species of animals. The disease is due to the mycotoxin commonly called "Aflatoxins" which are the toxic metabolites of certain strains of the moulds *Aspergillus flavus* and *Aspergillus parasiticus*. This condition was found to be one of the major causes of economic losses to the poultry and livestock industry. The fungi producing aflatoxins are known to be the usual contaminants of feeds and fodder such as ground nut, cotton seed meal, maize, sorghum, etc. due to their ubiquitous presence in nature. These fungi can affect growing crops secondary to primary infections with bacteria or viruses during growth, harvest or storage and produce toxins. The presence of mycotoxins in grains, pulses and feed poses serious problems in quality control usually encountered by producers, manufacturers and handlers.

The hepatotoxic, teratogenic, mutagenic, immunosuppressive and carcinogenic characteristics of the aflatoxin drew the attention of scientific world. However, acute or chronic aflatoxicosis may occur depending on the level of toxins in the feed and species of the animals affected more often resulting in moderate to severe hepatic damage. Ducklings and rabbits are reported to be more sensitive to these toxins. Poor growth rates and moderate to high death rates have been encountered depending on the species susceptibility and the concentration of toxin uptake.

The rabbits as a species once grouped under experimental animals has now assumed the status of food source and listed in commercial livestock industry. They are bred for their valuable fur and meat owing to its rapid and high fecundity potentials and rabbitary on commercial lines is expected to ease the demand pressure on chicken and mutton. In recent years, rabbit farming is now gaining recognition as an economic livestock industry.

ICAR, India has succeeded in selecting a broiler type of rabbit that achieves two kilos of body weight in just twelve weeks. Consequently the large scale rabbit farming has been intensified in India particularly in north eastern states, Jammu and Kashmir, Kerala and Tamil Nadu. Aflatoxicosis in rabbits can result in a threat for rabbit farming since commercial feeds used for rabbit may contain varying amounts of toxins.

This study was undertaken by feeding mash feed containing various levels of aflatoxins to experimentally produce sub acute and chronic aflatoxicosis in rabbits and study the pathology. Detailed documentation on the pathogenesis of aflatoxicosis in rabbits is scanty in literature. This study was designed to experimentally induce aflatoxicosis in rabbits and record the pathogenesis with suitable parameters at varying levels of the toxin incorporated in the feed. The study considered to assess the gross and histopathological changes in rabbits after experimental feeding in rabbits.

MATERIALS AND METHODS

Culture of *Aspergillus parasiticus* NRRL2999 was received from Food and Drug Toxicology Research Centre, National Institute of Nutrition, Hyderabad, India. *Aspergillus parasiticus* (NRRL 2999) culture was maintained and sub cultured once in 15 days in potato dextrose agar (Shotwell *et al.,* 1966). Aflatoxin was produced on rice (Shotwell *et al.,* 1966). One hundred gram (100 gm) of rice was taken in 500 ml of Erlenmeyer flasks, and soaked in 40 ml of distilled water for two hours with frequent shaking. The flasks were autoclaved, cooled and inoculated with *Aspergillus parasiticus* (NRRL2999). They were kept at room temperature and hand shaken vigorously six to ten times a day. After 48 hrs of inoculation mould growth was seen as white spots on the surface of the rice, later turning to bright yellow in colour. Subsequently in about a week time the colour became brown. On the tenth day, mouldy rice was dried in hot air oven overnight at 60°c after which it was ground to a fine powder. The powdered mouldy rice was analysed for the aflatoxin content by the following method:

Extraction of aflatoxin

Slurry was prepared by blending 25g of mouldy rice powder with 100 ml of distilled water for three minutes. The extraction was done by blending with 150 ml of acetone for two minutes and filtering through Whatman No.1 filter paper. The extract was purified by taking 75 ml of filtrate in 250 ml conical flask and swirling it after adding 1.5g of Cupric carbonate. In another 250 ml conical flask, ferric gel was prepared after adding 15 ml of Ferric chloride solution (0.41M) to 85 ml of Sodium hydroxide (0.2M) and swirled. The ferric gel was immediately transferred to the flask containing the extract and the contents were mixed thoroughly and allowed to stand for two minutes with occasional swirling. It was filtered through Whatman No.1 filter paper and 100 ml of the filtrate was mixed with 100 ml of Sulphuric acid(0.03%) in a 500 ml separating funnel and the extraction was done thrice using 20ml, 20 ml and 10 ml of chloroform each time, collecting the lower chloroform layer. The combined extract was transferred to a 250 ml separating funnel containing 100 ml of Potassium hydroxide (0.02M) and Potassium chloride (1%) mixture and gently swirled for 10 seconds. The lower chloroform layer was collected in a 100 ml beaker through a funnel containing a bed of anhydrous sodium sulphate. The final extract was evaporated to near dryness in a water bath at 40°C and transferred to a vial and dried completely. Known quantity of chloroform was added to dilute the extract and used for quantitation in thin layer chromatography (TLC).

Estimation of aflatoxin by Thin Layer Chromatography (TLC)

TLC plates were prepared (0.25mm thickness) by using Silica gel G: distilled water (2:1) slurry. Applicator was used for spreading the gel on the plate. They were activated in a hot air oven at 110 degree C for an hour. The samples and standards were spotted on the plates and developed in Chloroform and Acetone (90:10) mixture for 45 minutes. The plates were then removed dried and examined under long wave (365 nm) ultraviolet light in a chromato-view cabinet. The aflatoxin content (B1) was calculated according to Association of Official Analytical Chemists (AOAC) (1980) specifications.

Formula for calculation:
Aflatoxin content in ppm = S×Y×V⁄Z×W
Where, S= Comparable volume of standard in μ ml
Y= Concentration of standard in ng/ml
V= Volume in ml of the solvent required
Z= Comparable volume of sample in μ ml
W= Effective weight (gm) of the sample

Preparation of the diet
Special rabbit feed was obtained from the poultry Research Station, Nandanam, Madras. The feeds were analysed to eliminate the presence of aflatoxin. Weighed amounts of powdered rice containing known amount of aflatoxin were incorporated into the aflatoxin free diet so that the dietary aflatoxin level was at 0.025, 0.1, 0.2 and 0.3 ppm by mixing thoroughly.

Experimental animals
A total of eighteen rabbits of 3-6 months of age with body weight of 1-1.5 kg were received from Livestock Research Station, Kattupakkam, Chennai and from the Department of Laboratory Animal Science, Madras Veterinary College. The rabbits were housed in individual cages with *ad libitum* supply of water. Animals were kept on a control uncontaminated diet for a 10 days period and base values of haematological and biochemical parameters were taken before they were subjected to different treatments.

Experimental design
The trial consisted of 3 groups of (G1, G2 and G3) 6 rabbits each fed with diets containing 0.1, 0.2 and 0.3 ppm of aflatoxin respectively for a period of 30 days.

Pathology
At the end of each experimental period the rabbits were sacrificed. A detailed post mortem examination was conducted and the gross changes were recorded. The animals which died during the trial were also subjected for post mortem examination. Representative pieces of livr, lungs, heart, kidney spleen, duodenum and pancreas were collected in ten percent buffered formalin and embedded in paraffin for histopathological studies. Sections were cut and stained by Haematoxylin and Eosin.

Histochemical localization of Gamma-glutamyl transpeptidase (GGT)
Histochemical demonstration of GGT was carried out in both cryostat sections and paraffin embedded sections as per the methods of Ruttenbery *et al* (1969) and Albert *et al* (1961). Tissues pieces were taken from all the lobes of liver. Cryostat sections were prepared from fresh tissue stored in -20°c mounted on slides, fixed with chilled acetone and stained.

For paraffin embedded sections tissues were fixed in 80% alcohol for 24-48 hours at 4°c, dehydrated in two changes of absolute alcohol, cleared in benzene and impregnated with low melting paraffin at a temperature below 55°C. They were embedded in hard paraffin.
Sections of 5μm thickness were cut, mounted and deparaffinised with benzene before staining. Sections were incubated at room temperature in a freshly prepared solution containing:

- γ- glutamyl-4-methoxy-B napthalamide 20 mg
- Glycyl glycine 10 mg
- Fast blue BBN 10 mg
- Tris HCl Buffer (pH7.4) 5 ml
- Saline(0.9%) 14 ml

After incubating for 15-30 minutes, the slides were rinsed in saline for 2 minutes and then transferred to 0.1M copper sulphate solution for 2 minutes. After another saline rinse, the sections were rinsed in distilled water, counter stained with Haematoxylin, washed, dried and mounted in glycerine jelly. The sites of enzyme were stained yellowish red.

RESULTS AND DISCUSSION

In 0.1 ppm aflatoxin fed group, the livers were mottled with distended gall bladder while in 0.2 ppm group, numerous pale areas and hardening of the consistency of the liver were observed besides the above changes. In 0.3 ppm fed group, the liver was atrophied with the wrinkling of the glissons capsule and a scattered pale foci on the surface.

Microscopically, hyperaemic, cell swelling and cytoplasmic granularity were consistently present in all groups, while the hydropic change, focal necrosis, Kupffer cell hyperplasia with portal infiltration of round cells were observed in low dosed group. The changes were superimposed with bile duct hyperplasia, portal fibrosis, cholangitis and cholangio-fibrosis in high dosed group. Occasional microgranuloma formation was also observed. With AFB1 treatment, icterus (Clark *et al*., 1980) and liver atrophy with sclerosis (Parenti *et al*., 1975; Stracciari, 1988) were observed. Hyperaemia of central veins, granular degeneration of hepatic cells (Antyukov, 1964), hepatic cell necrosis and bile duct hyperplasia (Newberne and Butler 1969), were observed as in present study. In addition to the above changes, hepatocytomegaly (Edds, 1973) increase in proportion of binucleate hepatocytes and hyperplasia of biliary ductular epithelium (Clark *et al*., 1980) we also observed in the present study. The above structural changes correlated well with the functional derangement of liver *viz.*, hypoproteinemia, hypoalbuminemia, hypercholesterolemia, increased serum activity of AST and ALT. Coles (1986) observed that these biochemical alterations reflected hepatic damage. Further the cholangitis fibrosis with stasis of bile observed in this study could be the probable elevation of GGT levels in the treated groups. Bauer *et al* (1974) also reported that GGT level was elevated markedly in intrahepatic cholestasis.

Necrosis of the hepatocytes, hepatomegaly, proliferation of the bile ducts and lobular atrophy were related to the dose of aflatoxin in the feed and their cytotoxic effect (Newberne and Butler, 1969). Similarly in the higher levels severe changes in hepatocytes including a large increase in binucleate hepatocytes, cholangitis and ductular epithelial hyperplasia were directly related to the increased doses of aflatoxin in feed (Clark *et al*., 1980).

Lungs in all dose levels showed sub-pleural haemorrhages and emphysema. Microscopically, oedema, focal haemorrhages and emphysema were mild in 0.1 ppm group, where as these changes were pronounced in the 0.3 ppm group. The oedema and haemorrhages observed in this study might stimulate the changes as to the effect of aflatoxin that induced phlebitis and veno-occlusions in buffalo calves (Moorthy *et al*., 1984). The pulmonary haemorrhage noted in

this study appeared to be aggravated by the decrease in coagulation time and reduction in platelet count induced by aflatoxin.

Kidneys in 0.1 ppm group showed enlargement with hyperaemia while in higher dosed groups, a few pale spots and granularity of the cortex were observed. Microscopically, glomerular hyperaemia, swelling of the tuft and filling up of the Bowman's capsule besides tubular degeneration were observed in 0.1 and 0.2 ppm groups. Tubular degeneration, necrosis and interstitial infiltration were prominent in 0.2 ppm group and these changes were pronounced along with interstitial fibrosis and medulllary haemorrhage among rabbits treated with 0.3 ppm level. Clark *et al* (1982) also reported similar haemorrhages, while Gill *et al* (1985) observed degenerative changes leading to necrosis in the kidney of rabbits. Cysweski (1982) reported a moderate toxic tubular nephrosis in horses. Benjamin and George (1967) also reported fat droplets in tubular epithelial cells and moderate congestion.

The intestine in all the groups showed congestion and diffuse catarrhal changes. Meninges in all the treated animals showed varying degrees of congestion, while splenomegaly was occasionally observed in 0.3 ppm group. Microscopically, catarrhal changes, mild epithelial necrosis and desquamation in the intestines were observed in all groups.

Hyperaemia alone was observed in the brain with 0.1 ppm treatment, however mild neuronal degeneration and necrosis observed in 0.2 ppm were super imposed with gliosis, vacuolation astrocyte proliferation and perivascular cuffing in 0.3 ppm group. Spleen hyperaemia and lymphoid depletion were observed in all groups, while reticular hyperplasia developed as a sequel of changes in the 0.3 ppm group. Antyukov, (1964) reported fibrinous inflammation in stomach and catarrhal inflammation in intestine. Morisse *et al* (1981) observed splenomegaly besides gastritis, typhlitis and colitis.

The activity of GGT was moderate in the livers of 0.3 ppm group animals which also histologically revealed cholangitis, cholestasis and cholangio-fibrosis apart from hepatitis. This enzyme was reported to be elevated in the serum following obstruction of bile flow.

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BIOLOGICAL CHARACTERIZATION OF AVIAN INFLUENZA VIRUSES SUBTYPE H9N2

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ABSTRACT

Biological diversities and genetic mutation of avian influenza viruses (AIV) have posed a continuous threat to human and animal health worldwide. Comparison of 3 isolates of AIV subtypes H9N2, KBNP-0028, SNU 8011 and an inactivated vaccine strains A/Ck/Kor/01310/01 were performed. Antigenic relationship, viral shedding, and tissue tropism were examined. In cross haemagglutination inhibition (HI) test isolate, SNU 8011 showed higher HI titers with all isolates. Comparison of virus shedding from cloaca and oropharynx revealed that both isolates could be more frequently isolated from upper respiratory tract (90-100 %) constantly from 1 day post inoculation (DPI) to 5 DPI than from gastrointestinal tracts (10-60 %). Moreover, isolate KBNP-0028 was recovered from all organs including bone marrow, heart, brain and kidneys, indicating higher ability for broad tissue dissemination than that of a recent isolate SNU 8011. Recovery rate was also higher in respiratory tract but viral titers were higher in caecal tonsil in both isolates. In the comparison of growth kinetics and replication among two isolates and vaccine strain, isolate KBNP-0028 replicated earlier than others and with higher titer than recent isolate SNU 8011.

INTRODUCTION

Avian influenza (AI) is an acute contagious respiratory viral disease caused by influenza virus. Among respiratory viruses, influenza viruses have unique features with the segmented RNA genome and highly antigenic diversity having enveloped, single stranded, negative sense RNA genome with eight segmented gene belonging to Orthomyxoviridae family (Swayne & Halvorson 2008). Influenza viruses have been classified in three serotypes A, B and C. Type A is responsible for highly pathogenic avian influenza (HPAI) and human pandemics worldwide associated with severe morbidity and mortality. Influenza viruses infecting poultry can be divided in two groups. One is very virulent viruses, anciently called fowl plague and now termed as "Highly pathogenic avian influenza" (HPAI) in which mortality may reach as high as 100 percent. The other is "Low pathogenic avian influenza" (LPAI) which causes disease in milder form, however some times may cause high mortality if concurrent infection occurs with other secondary pathogens. HPAI viruses are H5 and H7 subtypes to date. However, there are LPAI viruses within the H5 and H7 subtypes as well. After continuous circulation of LPAI in chickens or other domestic birds a strain can mutate in a highly pathogenic influenza A strain (Alexander, 2000). Influenza A viruses have been isolated from many species including humans, pigs, horses, mink, marine mammals and wide range of domestic and wild birds. The first isolation of highly pathogenic influenza virus was from Scotland in 1959 from chicken (Swayne & Halvorson 2008). Waterfowls, shorebirds and gulls have been known as the main

natural reservoirs of the influenza viruses. All influenza viruses infecting mammalian species originate from wild birds (Webster *et al.,* 1992).

Currently, AI subtype H5N1 has been causing as the most significant problem worldwide in poultry industries. Meanwhile, another challenge is the extensive spreads of AIV subtype H9N2 in the Asian and Middle East countries since their first detection from turkeys in Wisconsin, USA in 1966 (Homme *et al*.,1970). In Asia, H9N2 subtypes of viruses have been already reported from Korea, China, Hong Kong, Saudi Arabia, India, Pakistan, Bangladesh, Nepal, Vietnam, Iran, UAE, Israel and Jordon (Mo *et al.,* 1997, Naeem *et al.,* 1999, Alexander, 2000, Nili and Asasi, 2002, Al-Natur and AO-Shehada, 2005, Pant and Selleck, 2005, Aamir *et al.,* 2007, and Nagarajan *et al.,* 2009).

MATERIALS AND METHODS

1. Experimental design and challenge of chicken

Two groups of 3 weeks-old specific pathogen free (SPF) chicken (Hy-vac., Iowa, US), 10 chickens in each group were housed in P3Lab type negative pressure maintained chicken isolator. Both group of chicken was challenged with each isolate KBNP-0028 and SNU 8011 at a viral dose of 106.5 EID50/0.1ml (Egg infectious dose 50 percent) per bird by intra nasal/ocular route.

2. Viruses

The low pathogenic avian influenza viruses (H9N2) A/Ck/Korea/KBNP-0028/00 (denoted KBNP-0028) and A/Ck/Korea/SNU/8011/08 (denoted SNU 8011) were isolated in routine diagnostic works and maintained at Avian Disease Laboratory, College of Veterinary Medicine, Seoul National University, Korea. EID50 was calculated according to formula previously described (Reed and Muench, 1938).A/Ck/Kor/01310/01 (vaccine strain) was used for comparison.

3. Serology

Haemagglutination (HA), haemagglutination inhibition (HI) and agar gel precipitation tests (AGP) were carried out as described in the protocol (OIE, 2008).

4. Re-isolation and titration of virus

Cloacal swabs and oro-pharyngeal swabs were collected on 1 day post inoculation (DPI), 3rd DPI and 5th DPI from each bird. All chicken were numbered individually for sampling purpose. On 5th DPI, all the chicken were sacrificed and various tissues were collected aseptically to prevent cross contamination. All the swab samples were suspended in PBS (pH 7.2) and treated with 1/10th volume of 10x-X concentrated antibiotics and antimycotics (WHO, 2002). Similarly, tissues were homogenized using PBS as 10 percent W/V and same methods applied as for swab samples. All the samples were inoculated in 10 day-old specific pathogen free embryonated chicken eggs (SPF ECEs, Hy vac., Iowa, US). Viral titrations were performed from all pooled tissues samples in SPF ECEs.

5. Comparison of antigenicity

Cross haemagglutination inhibition assay (HI) was conducted as described in standard protocol (OIE, 2008) using 4 HA unit of respective antigens. Antigens of isolates KBNP-0028, 01310

and SNU 8011 were prepared by inactivating the allantoic fluid with 0.1 percent formalin at 200 C for 10 hrs. The positive sera were prepared by injecting the inactivated virus emulsified with oil adjuvant in 6 week-old SPF chicken. Three weeks after injecting respective antigens, the chicken were bled and sera were harvested.

RESULTS

Virus replication

Three isolates were inoculated in 10 day-old SPF ECEs and HA titer was determined in every 4 hours, starting from 8 hours to 32 hours. Isolate KBNP-0028 showed earlier replication in embryos with higher titer. Recent isolate SNU 8011 appeared weaker in replication time and HA titer as well than others. Although the vaccine strain showed late replication in embryos, HA titer was equal to that of KBNP-0028 (Figure 1). Further more, the viral growth reached

Antigenic relationship among the isolates

Cross HI test showed similar pattern of inhibitory reactions indicating those isolates were antigenically similar (Table 1). Although, recent isolate SNU 8011 showed marked HI inhibition reaction with homologous and heterologous virus isolates equally to log2 values of 2^9 whereas the other two isolates showed limited reaction with heterologous isolates. Particularly, vaccine strain 01310 showed very weak HI reaction with log2 values of 2^4 with KBNP-0028.

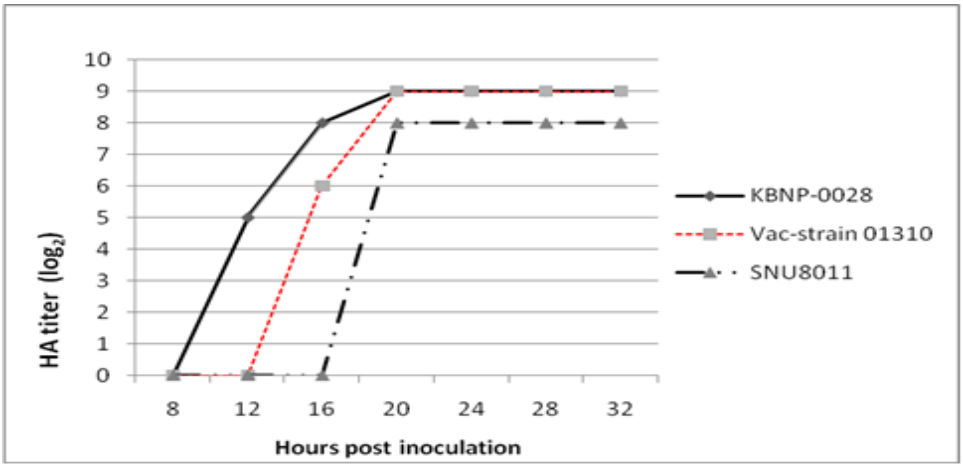

Figure 1: Growth kinetics of three strains of low pathogenic avian influenza virus subtype H9N2 Each virus was inoculated in SPF eggs and HA titers were determined in every 4 hours starting from initial 8 hours. Inoculum of each virus was 106.5 EID50/0.1ml/egg.

Table 1. Antigenic relationship among avian influenza virus H9N2 by cross haemagglutination inhibition test

	Avian influenza viruses		
Anti-sera	**KBNP-0028**	**01310**	**SNU 8011**
KBNP-0028	256*	32	32
01310	16	64	32
SNU 8011	512	512	512

*HI antibody titers. Sera were obtained at two weeks after injecting formalin inactivated AIVs as antigen.

Comparison of virus shedding and tissue tropism

Both strains of viruses were recovered from the oropharyngeal swabs commencing within 24 hours of challenge to 5th DPI consistently at 90 % to 100 % level with higher HA titer in contrast to cloacal swabs at 10 to 60 % level (Table 2). In addition on 5th DPI, recovery rate was higher for both isolates along with higher HA titer.

Isolate KBNP- 0028 was recovered from all the tissues (Table 3), bone marrow (2/10, 0), brain (2/10, 0), caecal tonsil (6/10, 104.5 EID50/0.1 ml), heart (3/10, 0), kidney (2/10, 103.9), lungs (9/10, 101.9), spleen (5/10, 100.7), and trachea (10/10,102.3). In contrast, isolate SNU 8011 was recovered from caecal tonsil (5/10,103.9 EID50/0.1 ml), lungs (5/10,101.1) spleen (3/10,100.7) and trachea (9/10, 101.1). Viral titer was found higher in caecal tonsil in both isolates. Viral titer from kidney was higher in KBNP-0028 in comparison to recovery rate which was 2/10; titer was 103.9 EID50/0.1ml.

Moreover, isolate SNU 8011 was not recovered from bone marrow, brain kidney and heart tissues. Though, isolate KBNP-0028 was recovered from all theses tissue but viral titer was not in detectable level in bone marrow, brain and heart (Values within the parenthesis are number of the virus isolated/ number tested and viral titer in each pooled tissues sample expressed as log 10 EID50/ 0.1 ml).

Table 2. Comparison of virus shedding after experimental infection with two avian influenza subtype H9N2 virus isolates in 4-wk-old SPF chicken

AIV	**OP swabs**			**Cl swabs**		
Isolates	**1 DPI**	**3 DPI**	**5 DPI**	**1 DPI**	**3 DPI**	**5 DPI**
KBNP-0028	9/100	10/10	10/10	1/10	4/10	6/10
SNU 8011	10/10	10/10	9/10	1/10	3/10	5/10

DPI=Days post inoculation, OP swabs=Oropharyngeal swabs, Cl swabs=Cloacal swabs.
0Number of shedding/Number tested.
Chicken were challenged with each AIV isolate. Inoculum of each virus was 106.5 EID50/0.1ml/bird by intra nasal/ocular route.

Table 3. Recovery of avian influenza virus subtype H9N2 from different internal organs after experimental infection with each virus in 4-wk-old SPF chicken

Isolates	Internal organs							
	BM	**Br**	**CT**	**Hrt**	**Kid**	**Lu**	**Sp**	**Tr**
KBNP-0028	2/10*(0)	2/10(0)	6/10(4.5)	3/10(0)	2/10(3.9)	9/10(1.9)	5/10(0.7)	10/10(2.3)
SNU 8011	0/10(0)	0/10(0)	5/10(3.9)	0/10(0)	0/10(0)	5/10(1.1)	3/10(0.7)	9/10(1.1)

Tissues-BM=Bone Marrow, Br= Brain, CT=Caecal Tonsil, Hrt=Heart, Kid=Kidney, Lu=Lungs, Sp=Spleen, Tr=trachea.

*Number of the virus isolated/number tested. Values in parenthesis are viral titer from each pooled tissues expressed as log 10 EID50/0.1 ml.

DISCUSSION

LPAI, H9N2 is an economically important disease and remains a continuous threat to commercial poultry production worldwide and since last decade it has shown more devastating effects in Asia causing high mortality (Naeem *et al.,* 1999) and continuous evolution in domestic poultry (Li *et al.,* 2005, Kim *et al.,* 2006, Lee *et al*., 2007, Tosh *et al.,* 2008, Kim *et al.,* 2009).

However, in an experimental infection SPF chicken, neither mortality nor clinical illness was found. It has been frequently reported that secondary pathogens such as E coli, Staphylococcus aureus, Mycoplasma gallisepticum, Ornithobacterium rhinotracheale and infectious bronchitis virus played a significant role in aggravating the clinical condition of the birds earlier infected with AIVs, H9N2 (Matrosovich *et al.,* 2001, Nilli & Asasi 2002, Bano *et al*., 2003, Kishida *et al*., 2004, Nishida *et al.,* 2004, Haghighat-Jahromi *et al.,* 2008, Saad 2008).

In growth kinetics of three isolates, they differed in replication time though peak HA titer was reached 20 hours post infection. In antigenic analysis, vaccine strain 01310 showed partial cross reactivity to heterologous isolates. In general higher HI titer indicates higher protection efficacy. AIVs circulating in their natural host have shown static evolution that has a higher rate of genetic conservation. However, interspecies transmission to pass the species barrier is likely to lead the viruses to significant changes in their genetic and antigenic properties (Liu *et al.,* 2004, Lee *et al.,* 2007). Virus recovery from oropharyngeal and cloacal swabs were 90-100 % and 10-60 %, respectively, indicating that the viruses could efficiently replicate in respiratory tract in compared to the digestive tract. Similar results have been reported from previous studies (Tumpey *et al.,* 2004, Choi *et al.,* 2008).

KBNP- 0028 showed wider range of tissue tropism and replicability. Although low recovery rate (2/10) and higher viral titer (103.9 EID50/0.1 ml) was shown in kidney. However, recent isolate SNU 8011 could not be recovered from bone marrow, kidney, heart and brain. In a study reported from Pakistan, H9N2 AIV was re-isolated persistently from bone marrow following challenge infection (Ejaz *et al.*, 2007) indicating marked biological variation among the same subtypes.

Nepal has already faced the devastating effects of H5N1 and H9N2 virus has already been reported serologically (Pant *et al.*, 2007, Shrestha *et al.*, 2010).

In conclusion, antigenic and biological diversities exist among the AI subtype H9N2 viruses. There might have been significant changes in biological and genetic characteristics of AIVs H9N2 in the field's necessity, intensive active surveillance among poultry farms, live bird market and wild birds population in routine basis.

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EFFECT OF SEASONS ON FEED INTAKE AND FCR INHUBBARD FLEX BROILER IN CHITWAN

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ABSTRACT

This study was conducted at a commercial broiler farm, Mangalpur, Chitwan from August 8, 2008 to May 16, 2009 in order to compare the effect of seasons Rainy (August), Winter (December) and Summer(April) on feed intake and FCR in Hubbard Flex Broiler. Eight thousand, straight run Hubbard broiler chicks were reared in each season i.e. winter (December to January), summer (April to May) and rainy (August to September). Birds were fed starter ration (2900 Kcal/Kg energy and CP 22%) up to 21 days and finisher ration (3000 Kcal/Kg energy and 20% CP) from 21 to 42 days in all the three seasons in similar managemental conditions. They were fed ad libitum feed and water throughout the experimental period. The experiment was carried out in a completely randomized design (CRD) having three treatments and eight replications comprising 1000 chicks in each replication. The result showed that the average feed consumption was significantly higher in birds reared in dump winter season in entire six weeks, whereas, summer season showed lowest feed consumption at 5th and 6th week. In 6th week of the trial period during winter (T1) had lowest FCR 2.03, summer (T2) had highest FCR 2.65 whereas rainy (T3) had 2.60.

INTRODUCTION

Livestock contributes approximately 31 percent to Agricultural gross domestic product (GDP); out of which poultry sector has a share of approx 8% (Dhakal *et al*., 1999). Poultry industry has great role in national income of the country contributing 3.5-4% in GDP. In addition to ration and management, climate is another factor influencing production potential of poultry species. Season has significant role in production performance of commercial broilers. Weather severities during season leads to production risk in most livestock activities (Bassam and Al Aharafat, 2009).For the developing countries like ours where there is very less controlled environmental condition even at the commercial production facilities, the birds reared are directly in contact with the external environment. Therefore, the great share of poultry farmers is getting the business away from profitable margin**.** There is lacking of preplanning among farmers for mitigating stress due to climatic severities. As a result there could be loss of performance due to great variation of temperature and humidity during different seasons of a year. Temperature is an important factor in the growth, feed intake and body composition of broilers (Howlider and Rose, 1987). In context of Chitwan, the temperature ranges from 7°c to 42°C from cool season to hot season of the year. Hence, this study aims to analyze the effect of different seasons such as winter (December to January), summer (April to May) and rainy (August to September) in feed intake and FCR in Hubbard Flex broilers.

MATERIALS AND METHODS

The experiment was carried out in a completely randomized design (CRD) having three treatments and eight replications comprising 1000 chicks in each replication at commercial broiler farm Mangalpur, from August 2008 to May 2009. The location of the site was 27^0 40' N latitude and 84^0 19 E longitude with an elevation of 228 meter above sea level. This farm was consrructed for broiler birds rearing in deep litter system. Eight thousand (N=800) day old Hubbard broiler chicks were purchased from Avinash Hatchery Pvt. Ltd., Narayangadh, Chitwan. The chicks were fed starter ration containing 22% CP and 2900 Kcal/Kg energy and finisher ration containing 20% CP and 3000 Kcal/Kg of energy. Starter ration was fed for 21 days and finisher ration from 21 to 42 days. Following ration with nutrient composition was fed to the birds in each season. (Table 1 and 2)

Table 1. Percent ingredient composition of broiler starter and finisher rations.

Ingredients	Starter ration (Upto 21 days)	Finisher ration (21 to 42 days)
Maize	46.5	51.5
Rice polish	5	5
DOC	8	7
Soyabean meal	35.1	29.5
Soya-oil	0.9	2.0
Rapeseed meal	1	1
L/st	1.4	1.4
DCP	0.8	0.5
Lysine	0.5	0.8
Methionine	0.5	1
Salt	0.3	0.3
Enzyme with phytase	200g/ton	200g/ton
Total	**100.00**	**100.00**

Table 2. Calculated nutrients composition of broiler starter and finisher rations

	Starter Ration		Finisher Ration	
Nutrient required	Required	Available	Required	Available
ME, Kcal/Kg	2900	2903	3000	2993
CP	22	21.9	20	19.89
CF	3	4.9	3	4.7
Ca	1.00	0.95	1.00	0.87
P	0.75	0.87	0.75	0.76
Lysine	1.00	1.5	1.00	1.69
Methionine	0.50	0.86	0.50	1.32
Meth+Cys%	0.75	0.75	0.75	0.68
Arginine	1.11	1.14	1.11	1.02
Tryptophan	0.20	0.25	0.20	0.22
Threonine	0.70	0.76	0.70	0.69
Isoleucine	0.66	1.1	0.66	1.00
Valine	0.68	0.96	0.68	0.86
Linoleic acid	1.40	1.2	1.4	1.27

Body weight gain

The initial and cumulative weekly body weights of birds were taken using digital balance. Body weight gain was obtained by subtracting previous weight of the birds from their corresponding body weight for each week to find out daily gain or weekly gain.

Weekly weight gain = Final weight of birds – Initial weight of birds

Feed and water consumption

The feed consumption by birds in different treatments was calculated on weekly basis. Feeding was done every day between 6 to 7 A.M. and 6 to 7 P.M. Drinkers were thoroughly cleaned before watering each time. Fresh water was made available *ad libitum* during the experimental periods. Daily feed consumption was recorded by subtracting feed offered and feed left over

Weekly feed intake = Weekly feed offered- Weekly feed left over

Feed conversion ratio (FCR)

The weekly feed conversion ratio in different treatments was determined by dividing the weekly feed intake by their respective weight gain.

$$\text{Feed conversion efficiency (FCR} = \frac{\text{Average daily feed intake (g)}}{\text{Average daily gain (g)}}$$

Temperature

The average maximum and minimum temperature were recorded on weekly basis. Similarly RH of outside environment during the experimental period was collected from nearest meteorological station National Maize Research Programme (NMRP), Rampur. The average maximum temperature, the average minimum temperature and relative humidity during winter were recorded to be 25°c, 12°c and 98% whereas during summer season 35°c, 18°c and 68% were the maximum temperature, minimum temperature and relative humidity. Similarly, during rainy season 34°c, 25°c and 87% were maximum temperature, minimum temperature and relative humidity during the experimental period.

RESULT AND DISCUSSION:

Feed consumption:

Table 3. Average daily feed consumption (g) of Hubbard broilers at Mangalpur (g)

	Periods in week					
	1st	2nd	3rd	4th	5th	6th
Treatments						
Winter (T1)	24.63a	49.68a	80.22a	106.87a	142.75a	153.34a
Summer (T2)	19.68c	39.13b	65.77b	83.91b	107.62c	122.16c
Rainy (T3)	20.86b	35.14c	60.57c	72.67c	117.16b	125.63b
Probability	0.00	0.00	0.00	0.00	0.00	0.00
F-value	372**	6792**	4961**	7525**	13699**	1035**
CV %	1.74	0.62	0.59	0.65	0.36	1.12
Lsd(p<0.05)	0.39	0.27	0.42	0.59	0.46	1.56

Means in column with different super script differ significantly (p<0.05), *significant at 5% (p<0.05), **significant at 1% (p<0.01), nsnot significantly different (p>0.05)

Average daily feed consumption (g) of Hubbard Flex broiler during different seasons of the year was recorded and calculated.Feed intake was significantly (p<0.01) highest in the entire 6 weeks of winter (T1) season from 26.63 gm in first week to 153.34 gm in 6th week whereas in summer (T2) 2nd, 3rd and 4th week had higher feed consumption as compared to rainy (T3) birds. All experimental groups recorded significant (p<0.01) difference in feed consumption pattern season-wise. Rahman *et al* (2007) in one of the experiments in Bangladesh found that feed intake per bird was significantly (p<0.05) different in Hubbard broiler in three different seasons of the year on birds rearing from 0 to 31 day. Average feed consumption per day was 88±3.32, 75.35±2.03 and 81.17±6.51g in winter, summer and rainy seasons, respectively. Variation in the data is due to rearing period of birds and temperature difference during the rearing period of broiler at rural environment of Bangladesh and Chitwan. The mean weekly feed consumption of Hubbard broiler at different seasons are presented in fig. 1.

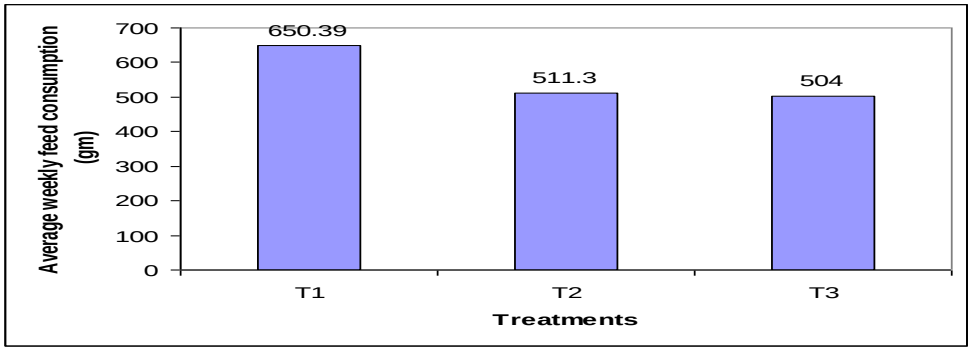

Figure 1: Overall mean of the weekly feed consumption of the Hubbard broiler (g) reared in different season.

FCR:

Average weekly feed conversion ratio (FCR) of Hubbard broiler (g)

	Periods in week					
	1st	2nd	3rd	4th	5th	6th
Treatments						
Winter (T1)	1.58a	1.82a	2.09b	2.60a	2.03b	2.03c
Summer (T2)	1.52a	1.48b	1.74c	1.99b	2.56a	2.65a
Rainy (T3)	1.27b	1.41	2.20a	1.70c	2.54a	2.60b
Probability	0.00	0.00	0.00	0.00	0.00	0.00
F-value	57.08**	321**	61.86**	380**	507**	319**
CV%	4.21	3.3	4.26	3.17	1.58	2.26
LSD($p<0.05$)	0.07	0.07	0.08	0.07	0.03	0.06

Means in column with different super script differ significantly ($p<0.05$), *significant at 5% ($p<0.05$), **significant at 1% ($p<0.01$), ^{ns}not significantly different ($p>0.05$).

The result of the present study revealed that there was gradual increase in FCR from week 1st to week 4th and decline 5th and 6th week in winter season whereas in summer season second week has lower FCR as compared to 1st week. From the second week onwards, there was increasing trend up to 6th week. In case of rainy season there was drop in FCR value only in 4th week otherwise there was increment from 1st week to 6th week. Raju *et al* (1996) conducted trial on two different seasons' summer and winter, and found that there was significant improvement in winter as compared to summer season. Similarly finding obtained by Cooper *et al* (1998) is in alignment with the result of above experiment wherein the 28- to 49-d experimental period, the birds in the 21°c environment required 2.05 g feed to obtain a 1-g increase in body weight (BW) as compared to 2.37 g of feed needed by the birds in the 32 ^{2070} environment. Similarly, Donokh (1989) found depression in growth rate, feed intake and efficiency in feed utilization for 30°c and 35°c temperature treatment. He obtained this result when birds were reared at 20°c, 25°c, 30°c and 35°c temperature treatment.

CONCLUSION

The climate of winter season was suitable for commercial broilers in Chitwan condition. Best production performance was achieved during this season as compared to summer and rainy. Though the feed consumption was highest during this season, the consumed feed was best utilized by the birds thereby reducing FCR.

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ANTIMICROBIAL RESISTANCE PATTERN OF ESCHERICHIA COLI ISOLATES FROM CHICKEN AND HUMAN SAMPLES IN CHITWAN

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ABSTRACT

A study was conducted at National Avian Laboratory (NAL), Bharatpur, Chitwan from October 2007 to May 2008 with an objective to find out the antimicrobial resistance pattern of E. coli isolates from chicken and human in Chitwan District, Nepal. One hundred fifty samples of dead-in-shell embryos (DSE) from chicken hatcheries, 50 sample from clinically affected chicken and 35 samples of human hospital isolates suspected to E. coli in primary culture of urine or stool were collected for this study. Isolation and identification of organisms were made by examination for cultural characteristics in MacConky agar, Gram's stain and biochemical tests. A total of 84 isolates (23 from layer DSE, 19 from broiler DSE, 22 from clinically affected chicken and 20 from human) were kept for sensitivity test against nine antimicrobials: Ampicillin (A), Ceohalexin (Cp), Ciprofloxacin (Cf), Cotrimoxazole (Co), Enrofloxacin (Ex), Gentamycin (G), Doxycycline (Do), Nitrofurantoin (Nf) and Tetracycline (T). The sensitivity tests were done by standard disk diffusion method. During isolation of organisms, it was found that prevalence rates of E. coli in broiler and layer DSE were 31.6 and 25.5%, respectively with overall prevalence rate of 28%. All isolated of E.coli showed resistance to 2 or more antimicrobials and a pattern of multiple drug resistance were observed. In-vitro pathogenicity tests were done by taking 10 samples from each of layer dead - in- shell embryos (LDSE), Broiler dead - in- shell embryos (BDSE) and clinically affected chicken. Among chicken isolates, the highest rate of resistance were against A (100%), followed by Cp (95.31%), T (78.12 %), Do (64.06%) and Co (51.66%). However, chicken isolates were highly sensitive to Cf and Ex, (0% resistance each), G (4.68%) and Nf (6.25%) antibacterials. In human isolates the highest rates were against A, Cp, Do, and T (100% resistance each) followed by Cf, Ex and G (80% each) and Nf (60%). In this study A, Cp, Do and T have similar pattern of resistance ($p>0.05$) in both human and chicken isolates. Antimicrobial resistance pattern among all chicken isolates were similar against all antibiotics with no significant differences ($P>0.05$). Similar pattern of resistance of E. coli among chicken isolates suggest that there were common sources of resistant bacteria from environmental contamination and use of similar antibiotics. E. coli isolates from human showed significantly higher ($P<0.05$) antimicrobial resistance profiles against fluroquinolones antibiotics and Gentamycin than those from chicken in Chitwan and the pattern reflects consequences of human drug use habit in the study areas. Higher percentage resistance against A, Cp, T, and Do in chicken correlate with their high level of use and lengthy practice. However, similar pattern in human isolates against these antibiotics were possibly due to transmission of resistance organisms through chicken via food chain (meat) and/or environmental contamination. Therefore, a detail investigation is needed to assess the public health aspect of the use of the antimicrobials in

chicken and other food animals. Such a high percentage of antimicrobial resistance in E. coli organism indicates an alarming situation for both chicken and human health.

1 INTRODUCTION

In Nepal, poultry farming comprises of the rearing of parent stock for day old chick production, commercial layers for hybrid table egg and broiler chickens for meat within an intensive farming in cage or in deep litter system. Such poultry intensively kept are more susceptible and are likely to face outbreak of many diseases and parasites in their life.

Among various diseases, colibacillosis is one of the important diseases of poultry which is caused by various strains of *E. coli* bacteria. *E. coli* has been associated with a variety of diseases in chicken, including enteritis, arthritis, omphalitis, coligranuloma, septicemia, salpingitis and complicated air sacculitis.

Colibacillosis can be controlled with antibiotic therapy but a significant increase in drug-resistant strains of *E. coli* has complicated the problem in the poultry industry (Roy *et al.,* 2004). Antimicrobial resistance has been recognized as an emerging problem worldwide in both human and veterinary medicine, and misuse of antimicrobial agent considered the most important factor for the emergence, selection, and dissemination of antimicrobial resistant bacteria (Witte, 1998).

It has been proved that the use of anti-microbial drugs in food animals and chicken have been shown to lead to resistant strains of pathogens which may be transmitted to humans through food chain but the contribution from the use of anti-microbial agents in food animals leading to resistance in bacteria infecting humans or vice versa in developing countries like Nepal still remain unclear due to the lack of research works.

Therefore, there is need to continue investigating the resistance profiles of *E coli* and other enteric pathogens from extensively reared animals and birds that receive antibiotics for growth promotion and disease treatment as a means of understanding the human/animal drug resistance interaction. Considering the above facts, the present study has compared anti-microbial resistance profiles of *E. coli* isolates from chicken and human in Chitwan District, Nepal.

2. MATERIALS AND METHODS

2.1. Sample collection

One hundred fifty unhatched eggs or dead in shell embryos (DSE) were randomly collected from chicken hatcheries located at Chitwan. The eggs with DSE were swabbed with alcohol and aseptically opened to collect samples using sterile swabs. Likewise, fifty samples were obtained from dead birds (DB) brought for necropsies collected from different commercial chicken farms of Chitwan and National Avian Laboratory(NAL), Bharatpur, Chitwan. The samples were obtained from heart blood or liver by using sterile swabs.

Similarly, thirty five human isolates were obtained from the diagnostic laboratories of Narayani Samudaik Hospital and Bharatpur Hospital, Chitwan. All the samples were processed as per the

standard microbiological procedures for isolation and identification of *E coli* organisms. The numbers of *E. coli* isolates obtained were 23, 19, 22 and 20 from layer DSE, broiler DSE, diseased chicken and human respectively.

2.2 Antimicrobial drug sensitivity test

Once *E. coli* isolates were isolated and identified from each sample collected, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial agent sensitivity profiles of the *E. coli* isolates for 9 antimicrobial agents: Gentamicin (G), Ciprofloxacin (Cf), Enrofloxacin (Ex), Cotrimoxazole (Co), Cephalexin (Cp), Doxycycline (Do), Tetracycline (T), Ampicillin (A) and Nitrofurantoin (Nf).

The *E. coli* isolates were inoculated in tryptic soy broth and incubated at 37°C for 5 hrs. Cotton swabs were used for streaking the broth on to Mueller-Hinton agar plates. After air drying, antibiotic discs were placed 30 mm apart and 10 mm away from the edge of the plate. Plates were incubated at 37°C for 18 to 24 hrs. The zone of inhibition and resistance was measured and interpreted according to the recommendation of the disc manufacturer (Hi media Laboratories, Mumbai, India).

2.3 In *vitro* pathogenicity test

For i*n vitro* pathogenicity test, a total of 30 *E. coli* isolates i.e. 10 isolates from each of clinical cases of colibacillosis, layer DSE and from broiler DSE were subjected to Congo red, hemolysis and motility tests.

2.4 Data analysis

Prevalence rates of *E. coli* bacteria in layer and broiler DSE were calculated and the differences were determined by using Chi- square test. Similarly, susceptibility data were recorded quantitatively by measuring the diameters in millimeter using a vernier caliper. Significant differences in mean percentage resistances were determined across the samples categories using Tukey-Kramer procedure (PH Stat-2, Version 2.5, Prentice Hall Inc., 2003).

3 RESULTS AND DISCUSSION

3.1 Prevalence of *E. coli* in DSE

In this study, a total of 28% of the DSE were affected with colibacillosis. The prevalence rate was higher in the broiler DSE (31.6%) than the layer DSE (25.56%). According to the annual technical report of NAL (2006), out of 525 samples obtained from post mortem cases, 55.81% were *E. coli* positive. Lower prevalence rate (7.61%) was obtained in a study conducted in different sources of water in Chitwan (Ghimire et al., 2006). Similarly, in a study at Veterinary Teaching Hospital, IAAS, Rampur, about 12% (11/90) of the samples taken from postmortem cases were found positive for *E. coli* (Sapkota *et al.*, 2006) which is comparatively lower than this study. A higher prevalence rate in this study indicates poor hygienic condition of chick production in the hatcheries and post death contamination to some extent.

3.2 In vitro pathogenicity test

Present study of *in vitro* pathogenicity test showed that two isolates from layer DSE 20% (2/10), one from broiler DSE 10% (1/10) and two from clinical cases 20% (2/10) were haemolytic *E.*

coli (Table 1). Similar results were obtained by Ragi *et al* (2003) in clinical cases of collibacillosis (10%, 2/20), DSE from Simtu and NAPRI farm (0%, 0/28) in Zaria-Nigeria.

Table 1. Results of *Invitro* pathogenicity test on *E. coli* isolates from DSE and clinical cases of colibacillosis in chicken.

Pathogenicity test	Layer DSE (%)	Broiler DSE (%)	Clinical Cases (%)
Haemolysis	2/10(20)	1/10(10)	2/10(20)
Congo red dye	7/10(70)	8/10(80)	10/10(100)
Motility	10/10(100)	10/10(100)	10/10(100)

The result of the Congo red (CR) binding assay indicates that 100% of the clinical cases produced CR positive, while 70% and 80% from layer DSE and broiler DSE were CR positive, respectively (Table 1). The result is in contrary with Ragi *et al* (2003) who found only 55% (11/20), 50% (14/28) and 42.2% (19/45) CR positive in the *E. coli* isolates obtained from clinical colibacillosis, DSE of Simtu Farm and NAPRI Farm, respectively. A number of workers have reported that CR binding did not correlate with pathogenicity. But the loss of CR binding parallel to the loss of virulence for chickens and mice (Yoder, 1989). Berkhof and Vinal (1986) evaluated CR dye as a marker for pathogenicity of avian *E. coli* strains. They found that CR binding *E. coli* caused septicaemia, whereas non CR binding *E. coli* were non pathogenic.

All the isolates in this study showed 100% motility which is in agreement with Ragi *et al.* (2003) who obtained 100% motility in the isolates from both DSE of NAPRI Farm and clinical colibacillosis and 99.8% motility in DSE of Simtu Farm.

3.3 Antimicrobial resistance pattern

In chicken isolates the highest rate of resistance were against A (100%), followed by Cp (95.31%), T (78.12%), Do (64.06%), Co (51.6%), Nf (6.25%) and G (4.68%). In human isolates, the highest rates were against A, Cp, Do, and T (100% resistance each) followed by Cf, Ex and G (80% each), Nf (60%) and Co (55%) (Table 2).

Table 2. Antimicrobial resistance in *E. coli* isolates from DSE, DB and human.

Antimicrobial	Layer DSE	Broiler DSE	Diseased Birds	Overall (Chicken)	Human
Ampicillin (A)	23(100)	19(100)	22(100)	64(100)[a]	20 (100)[a]
Cephalexin (Cp)	23(100)	17(89.47)	21(95.45)	61(95.31)[a]	20 (100)[b]
Ciprofloxacin (Cf)	0(0)	0(0)	0(0)	0(0)[a]	16(80)[b]
Cotrimoxazole(Co)	10(43.4)	11(57.89)	12(54.54)	33(51.56)[a]	11(55)[a]
Doxycycline (Do)	15(65.21)	12(63.15)	14(63.63)	41(64.06)[a]	20(100)[a]
Enrofloxacin (Ex)	0(0)	0(0)	0(0)	0(0)[a]	16(80)[b]
Gentamycin (G)	2(8.69)	0(0)	1(4.54)	3(4.68)[a]	16(80)[b]
Nitrofurantoin (Nf)	0(0)	2(10.52)	2(10.52)	4(6.25)[a]	12(60)[b]
Tetracycline (T)	18(78.26)	14(73.68)	18(81.81)	50(78.12)[a]	20(100)[a]
Total samples	23	19	22	64	20

Figure in the parenthesis indicate percentage.
Figures with different superscripts in the same row are highly significant ($p<0.01$).

Similar pattern of resistance was found (Roy *et al.,* 2006) with highest rate against A and T (100% each) in Japanese quail. Antimicrobial resistances were detected in *E. coli* isolates obtained from domestic and wild animal faecal sample and human septage in Michigan, USA with highest percentage of resistance to T (27.3%) followed by Cp (22.7%) and Co (13.3%) (Sayah *et al.,* 2004) and the results agreed with findings of other studies on antimicrobial resistance of *E. coli* from a variety of sources throughout the world (Letholainen *et al.,* 2003). The result of high level of resistance against these antibiotics would be due to long term use of inexpensive first readily available line broad spectrum antibiotics (Meng *et al.,* 1998). The higher resistance against A, Cp, T and Do in chicken correlates with their high level of use for long duration. However, similar pattern in human isolates against these antibiotics was possibly due to transmission through food chain (meat) and environmental contamination.

In all chicken isolates, of *E. Coli* antibiotics namely Cf and Ex (both 0% resistance), G (4.68% resistance) and Nf (6.26%) were highly sensitive. Similar results were obtained in Nigeria with very low resistance against Cf (0%), G (3.2%) and Nf (9.7%) to *E. coli* isolated from DSE from chicken hatcheries (Okoli, 2006). But this result is in contrary to the result of Roy *et al.* (2006) who found higher resistance percentage against Cf (67.7%), G (61.3%) and Nf (51.3%) in *E. coli* isolated from Japanese quail and their environments.

Mean disk diffusion zone sizes were also examined for differences between types of samples (Table 3). No significant differences ($P> 0.05$) were observed between chicken and human isolates in the sizes of diffusion zone for three agents namely A, Co and Do. The diffusion zone were significantly highest ($P<0.01$) in chicken isolates than in isolates of human against Cp, Cf, Ex, G and Nf.

Table 3. Mean disk diffusion diameter and resistance break points for *E. coli* isolates

Antimicrobials	Mean disk diffusion zone diameter (mm)						P value
	Resistance break points	LDSE	BDSE	Diseased birds	Overall	Human	
Ampicillin	13	6.04a	6.1a	6.18a	6.1a	6a	0.2726
Cephalexin	14	10.74a	11.74a	11.59a	11.35a	7.55b	<0.01
Ciprofloxacin	15	23.32a	22.79a	21.84a	22.65a	11.89b	<0.01
Cotrimoxazole	10	14.19a	11.5a	12.88a	12.85a	13.02a	0.9115
Doxycycline	12	12.43a	12.68a	13.04a	12.71a	10.7a	0.3255
Enrofloxacin	15	21.45a	21.95a	22.06a	21.82a	11.6b	<0.01
Gentamycin	12	15.39a	16.05a	15.77a	15.73a	10.6b	<0.01
Nirofurantoin	14	16.42a	16.04a	15.86a	16.1a	13.75b	<0.01
Tetracycline	14	12.73a	12.76a	12.29a	12.59a	8.1b	0.0040
Total sample		23	19	22	64	20	

Legends: LDSE: layer, BDSE: broiler dead-in-shell embryos.
(Figures with different superscripts in the same row are highly significant ($p<0.01$).)

In this study, all isolates of *E.coli* showed resistance to two or more antimicrobials and a pattern of multiple drug resistance were observed. In all isolates from chicken, highest percentage of organisms showed resistance to four antimicrobial agents combinations (ampicillin, cephalexin, tetracycline and doxycycline) with percent of resistance 34.78, 36.84 and 59.09 for LDSE, BDSE and DB isolates respectively (Table 4).

Table 4. . Percentage of multi-drug resistance to *E. coli* isolates

Source	n	Percentage of isolate resistant to agent/s					
		One	Two	Three	Four	Five	> Five agents
LDSE	23	0	8.62(2)	26.08(6)	34.78(8)	30.43(7)	0
BDSE	19	0	5.26(1)	36.84(7)	36.84(7)	21.05(4)	0
DB	22	0	9.09(5)	13.63(3)	59.09(10)	18.18(4)	0
Human	20	0	0	0	0	5(1)	95(19)
All	84	0	5.95(5)	19.04(16)	33.33(28)	19.04(16)	22.62(19)

The percent ages of organisms resistant to three agents in chicken were 26.08, 36.82 and 13.63 for LDSE, BDSE and DB respectively. Whereas in human all isolates showed resistance to 5 or more than 5 antimicrobials i.e. 95% showed resistance to more than 5 antimicrobials and rest were resistant to 5 agents. The result is in contrary to the study at Michigan where a quite low percentage of multi-drug resistant *E. coli* were isolated (Sayah *et al.,* 2004) in which the

percentage of *E. coli* isolates resistant to non of the agent were 44.44%, followed by one agent (27.78), two agents (11.1%), and three agents (4.44%), respectively. Therefore, the present study suggested critical situation of the presence of multi-drug resistant *E. coli* in Chitwan.

CONCLUSION AND RECOMMENDATION

Prevalence of *E. coli* in DSE in both broiler and layer hatcheries were high in Chitwan which indicate poor hygienic conditions in the chick production. The *in vitro* pathogenicity tests showed high CR uptake and motility reflecting pathogenic nature of the isolates. Higher resistance against A, Cp, T and Do were found in both chicken and human isolates. The results correlate with their high level of use in prevention and treatment of diseases in chicken. However, similar pattern of resistance in human isolates against these antibiotics would be due to transmission through food chain (meat and environmental contamination).

The regulatory authority should take appropriate action for the prudent use of antimicrobials. People awareness programs regarding consequences of antimicrobial misuse should be conducted. Use of antibiotic growth promoter in animal feeds should be minimized and the emphasis should be given to non- antibiotic growth promoters. Further studies are recommended in order to correlate antimicrobial use in poultry and development of resistance strains of *E. coli* in human in Chitwan as well as other parts of the country. So, the regulatory authority should take appropriate action in time for the prudent use of antimicrobials. Otherwise, a crisis of antimicrobial chemotherapy may exist in upcoming days.

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STUDY OF TICK FAUNA IN BOVINE, CAPRINE AND CANINE OF KATHMANDU VALLEY

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ABSTRACT

A research was conducted during February to July of 2010 to find the prevalence of tick parasites in livestock of Kathmandu valley. A total number of 100 cattle, 50 goats and 30 dogs were selected from the five different region representing four different direction and Khumaltar as center, from the study area 900 ticks were collected as five ticks per each animal with purposively random method, after collection they were preserved in 70% alcohol containing 5% glycerin until further laboratory analysis. The preserved ticks were examined by morphological characteristics using stereomicroscope device and identified according to Acarology Division, IMR (1995), Kaiser and Hoogstraal (1964), Morel (1989) and MAFF (1986). Available data indicated the prevalence of Boophilus microplus in bovine, caprine and canine were 90%, 70.8% and 32.67%, respectively while that for Rhipicephalus haemaphysalides were 6%, 11% and 37.33%, respectively. The prevalence of Haemaphysalis bispinosa was found to be 3% and 18% in bovine and caprine, respectively whereas it was not identified in canine species. In bovine, the prevalence of Dermacentor albipictus was 1% and in canine 30% were Haemaphysalis leachi.

Key words: prevalence, ticks, bovine, caprine, canine

INTRODUCTION

Ticks are obligatory blood-sucking arachnid arthropods infecting mammals, birds, reptiles and amphibians. They are vectors of diseases, causing anaemia, dermatitis, paralysis, otocariasis as well as loss of production (Schmidt and Roberts 1989). Three families of ticks are established, but two of them are well known and of veterinary importance, Ixodidae (hard ticks) and Argasidae (soft ticks) (Sonenshine, 1991).Ticks are getting attraction of many researchers, as they play a major role of vector in spreading different diseases in the livestock and human beings throughout the world. A research was conducted to determine the prevalence of tick species in Kathmandu valley and vicinity with objectives of to determine ticks species distribution level and sex wise distribution of identified tick fauna in bovine, caprine and canine of Kathmandu valley, which is located in the mid-hill region of the country at an altitude of 1,337 meter above sea level (masl) with an average minimum temperature 9.7°c during winters and average maximum temperature 20.3°c during summer. The average annual rainfall is recorded to be 1200 mm.

METHODOLOGY

A total number of 100 cattle, 50 goats and 30 dogs were selected from the five different region representing four different direction and Khumaltar as center. From the study area 900 ticks were collected as five ticks per each animal with purposively random method. After collection they were preserved in 70% alcohol containing 5% glycerin until further laboratory analysis. The preserved ticks were examined by morphological characteristics using stereomicroscope device and identified according to the figures and key described by Acarology Division, IMR (1995), Kaiser and Hoogstraal (1964), Morel (1989) and MAFF (1986).

RESULTS AND DISCUSSION

Overall prevalence of tick species

The species wise distribution of the tick fauna in Kathmandu Valley

Tick species		Bovine	Caprine	Canine	Total
B. microplus	Male	50	25	7	82
	Female	400	152	42	592
R. haemaphysalides	Male	6	4	0	10
	Female	24	24	0	48
R. sanguineus	Male	0	0	16	16
	Female	0	0	40	40
H. bispinosa	Male	5	5	5	15
	Female	10	40	40	90
D. albipictus	Male	0	0	0	0
	Female	5	0	0	5

Considering the occurrence of the 5 species of the hard ticks *Boophilus microplus* had the higher population frequency than others. The hard tick species found in the Bovine, Caprine and Canine of the Kathmandu valley were *Boophilus microplus, Rhipicephalus haemaphysalides, Dermacentor albipictus, Haemaphysalis bispinosa* and *Rhipicephalus sanguineus*.

The ticks species identified in Bovine of Kathmandu valley were *Boophilus microplus, Rhipicephalus haemaphysalides, Dermacentor albipictus* and *Haemaphysalis bispinosa.* In Bovine among 450 ticks of *Boophilus microplus*, 11.11% (50) of male and 88.89 % (400) were female. Among the 30 ticks identified as the *Rhipicephalus haemaphysalides*, 20% (6) were male and 80% (24) were female among the 15 ticks identified as the *Haemophysalis bispinosa*, 33.33% (5) were male and 66.67% (10) were female. Among the 5 ticks identified as *Dermacentor albipictus* all the ticks were female. It is observed that 90% (450) of the total ticks in Bovine was *Boophilus microplus*, 6% (30) was of *Rhipicephalus haemaphysalides*, 1% (5) was of the *Dermacentor albipictus* and 3% (15) of *Haemaphysalis bispinosa.*

Boophilus microplus was found abundant in all animals. In 89 Bovine out of 100 used for sampling it was infested with *Boophilus microplus* only where as in 11 Bovine it was mixed infestation.

Boophilus microplus, Rhipicephalus sanguineus and *Haemaphysalis bispinosa* were found in Caprine. Among the 177 cases of the *Boophilus microplus* 14.12 % (25) male and 85.88% (152) cases of female was reported. Among the 28 ticks identified as the *Rhipicephalus haemaphysalides*, 14.28% (4) were male and 85.72% (24) were female. Among the 45 ticks identified as the *Haemophysalis bispinosa*, 11.11% (5) were male and 88.89% (40) were female. It was observed that 70.8% (177) of the total ticks in Caprine was *Boophilus microplus*, 11.2% (28) was of *Rhipicephalus haemaphysalides*, and 18% (45) was of the Haemaphysalis bispinosa. In total 50 Caprine only *Boophilus microplus* was found in 40 where as in 10, it was mixed infestation *with Boophilus* as one of them.

In Canine of Kathmandu valley were *Boophilus microplus* and *Rhipicephalus sanguineus.* Among the 49 cases of the *Boophilus microplus* 14.28% (7) male and 85.68% (42) cases of female was reported. In total of the 58 *Rhipicephalus haemaphysalides* identified 17.24% (10) were male and 82.76% (48) were female. Among the 56 ticks identified as the *Rhipicephalus sanguineus*, 28.57% (16) were male and 71.43% (40) were female. Among the 45 ticks identified as the *Haemaphysalis leachi (?)*, 11.11% (5) were male and 88.89% (40) were female. It was observed that 32.67% (49) of the total ticks in Canine was Boophilus microplus, 37.33% (56) was of *Rhipicephalus haemaphysalides*. In Canine, *Boophilus microplus* is found. In 10 Canine out of 30 Canine used for sampling has infested with the *Boophilus microplus* only. And in 20 Canine out of 30 the Boophilus and other species (mixed infestation) were found.

In Nepal, five genera were identified viz.*Boophilus, Hyalomma, Rhipicephalus, Haemaphysalis* and Ixodes. Among them *Boophilus microplus* was the most abundant in all agro climatic zones and in most of the farm animals namely bovine, buffalo, goat, pig and rabbit (Shrestha *et al.*, 2003). During this study only four genera of the hard ticks were found in tick fauna in bovine, caprine and canine of Kathmandu valley.

In Kathmandu, in Bovine and goat, *Boophilus microplus, Boophilus annulatus, Ixodes ovatus* and *Rhipicephalus sanguineus* were identified, while in Lalitpur *Boophilus microplus* and *Rhipicephalus sanguineus* and in Arghkhachi *Boophilus microplus* and *Haemaphysalis bispinosa* were identified (Shrestha *et al.*, 2005).

A number of studies have been conducted in different geographical areas of Pakistan to investigate the prevalence of ticks on livestock. Although infestation with a single species of tick was recorded, most of the animals examined during the study had mixed infestation with different tick species. In this study it was found that the mixed infestation with *Boophilus microplus* was noticed in all study animals (Kaiser and Hoogstraal, 1964).

The cattle tick, *B. microplus* is predominant, which is similar to this result, followed by *R. haemaphysalides, H. bispinosa, D. albipictus in* case of Bovine, in the case of caprine *B. microplus* is predominant followed by *R. haemaphysalides, and H. bispinosa*. While in case of Canine *B. microplus was* predominant followed by *R. sanguineus.* (Ghosh and Bansal, 2007).
It had been reported that there were 349 cases of theileriosis, 34 cases of anaplasmosis and 3963 cases of babesiosis in Bovine from the Kathmandu valley in 10 year period from 2000 to 2009 (VEC, 2008/09). The large value of the *Boophilus microplus* in the kathmandu valley can

be used to define the large cases of babesiosis as it is the vector. The vector *Hyalomma, for Theileriasis* in cattle was not identified in this study. The case of *Theileriasis* recorded in Kathmandu valley may be due to the animal brought from endemic areas of the disease.

The data of June 1988 the mean maximum temperature 28.4˚c and that of same month of 2008 is 29.3˚c. This gives the increase of the Kathmandu Valley with 0.9 in 10 year period. This increased value can be defined for the pre dominance of the single host tick i.e. *Boophilus microplus* which found in all most all samples of the bovine, most of samples of caprine and canine comparing with other multi host ticks.

The similar report is also found giving the progressive displacement of multi host ticks by a single host tick population at Lele, Lalitpur in dogs and goats over three years period (Shrestha, 2010). This supports the climate change pattern in country; climate change is altering the distribution, incidence and intensity of animal and plant pests and diseases such as bluetongue, a sheep disease that is moving north into more temperate zones. Temperate countries and regions will be more vulnerable to invasions by exotic arthropod-borne virus diseases and parasites. Change in climate resulting in changes in species composition will augment the emergence of unexpected events, including the emergence of new diseases and pests (Khanal *et al.,* 2010).

CONCLUSIONS

This study is an attempt to identify and map the distribution of tick species in the livestocks of Kathmandu valley. The tick species identified in Kathmandu valley were *Boophilus microplus, Rhipicephalus haemaphysalides, Dermacentor albipictus, Haemaphysalis bispinosa* and, *Rhipicephalus sanguineus.*

Based on these data, we can recommend that livestocks in Kathmandu valley are highly parasitized with ticks. The infestation and other climatic conditions of Kathmandu valley are major predisposing factors for endemic outbreak of Anapalasma, Babesia and others protozoal diseases. This not only threats livestock health, rather it can also result in outbreaks of protozoal and other zoonotic diseases. So there is need of an immediate action towards controlling and reducing tick infestation in livestocks. Due to limited time of research, the research may not fully denote the overall status of tick infestation in livestocks of Kathmandu valley, so an intensive research should be carried out.

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GENETIC DIVERSITY OF FIVE INDIGENOUS GOAT POPULATION OF NEPAL

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ABSTRACT

The project allowed an in-depth study of the genetic diversity of indigenous goat breeds of Nepal in relation to different lineages derived earlier. The main goal of the present study is to characterize the domestic goat at Mitochondrial DNA (mtDNA) diversity. The mitochondrial hypervariable region I (HVR 1) with 625bp in 75 individuals belonging to five Nepalese goat populations from different geographic regions were sequenced and analyzed. Seventy haplotypes were identified in the studied samples. The haplotype diversity and nucleotide diversity are 0.984±0.005 and 0.02625±0.036, respectively. Phylogenetic analyses revealed that there were three mtDNA lineages (A, B and D) identified in Nepalese goat, in which lineage A is predominant, followed by lineage B and lineage D is at lower frequency. These results showed the multiple maternal origins of domestic goats. There was no significant geographical structuring in Nepalese goat populations as the result of the extensive transportation of goats from one place to another. Moreover, it was presumed that Nepalese goat breeds are more influenced by Indian goat but out of 76 sequenced samples, only 8 shared the same haplotype. This shows that Nepalese goats are having own uniqueness.

Keywords: *Capra hircus*, mt DNA, lineages

1. INTRODUCTION

The goat *Capra hircus* is an important livestock species in Nepal. Since it can be sold in any time, it is popularly known as the living bank; moreover as Live ATM it is very easy to sell goat due to its acceptance by all communities. Archaeological evidence indicates that the goat was one of the first animals to be domesticated in the Fertile Crescent Region around 10,000 years ago (Porter, 1996; Pringle, 1998; Zeder and Hesse, 2000). Some studies have suggested that second domestication was in Pakistan for cashmere breeds (Meadow, 1996; Porter, 1996). It has been suggested that at least two wild species of the genus *Capra* have contributed to the gene pool of domestic goats (Clutton-Brock, 1981), supported by molecular data (Mannen *et al*., 2001).

Mitochondrial DNA (mtDNA) contains highly informative polymorphic sites and its simple maternal inheritance without recombination makes it useful for the population studies in many organisms (Luikart *et al.,* 2001). Luikart *et al* (2001) carried out a worldwide survey of domestic goat mtDNA diversity and identified seven different lineages (haplogroups). Lineage A was the most diverse and widely distributed across all continents. Lineage B was predominantly found in eastern and southern Asia, including China, Mongolia, Laos, Malaysia, Pakistan, and India; also in Spain. Lineage C was found in low frequencies in China, Mongolia, Switzerland, Spain, Slovenia, Pakistan, and India. Divergence times for these three lineages

were estimated as more than 200,000 years ago among the five lineages, suggesting multiple maternal origins. Lineage D was rare found only in Pakistani, Indian and Chinese local goats. Lineage E was very rare, only found in India. Naderi *et al* (2007) also reported Lineage F and Lineage G, each having high haplotype diversity. Only 8-10% of the mtDNA variation is seen among continents or geographical regions due to extensive intercontinental transportation and movement within the regions and countries of goats (Luikart *et al*., 2001; Sultana *et al*., 2003; Joshi *et al*., 2004.

Nepal has good population of indigenous goat. Four known indigenous goat breeds were identified and characterized in phenotypic and chromosomal level (Neopane *et al.,* 2005). Terai goat is well adapted to topography and climate of Terai region. Similarly, Khari goat is well adapted to hilly region throughout the country. This breed is mainly producing meat and well known for its prolificacy. Chyangra and Sinhal are inhabited in high hills. Although neighbouring countries, mainly in China, Pakistan and India are way ahead on phylogenetic study Sultana *et al*., 2003; Joshi *et al*., 2004. A little attention has been paid to study to the genetic diversity of Nepalese goat. The main objective of this study was to examine genetic diversity and phylogeography of five different Nepalese goats populations based on the analysis of mtDNA D-loop hypervariable region.

2. MATERIAL AND METHODS

2.1. Population sampling

Seventy five blood samples of five representative goat populations were collected. Bloos samples were collected from three distinct groups of Khari goats from East, West and Central hilly region. Sinhal and Terai goats that seemed true to the type with the phenotypic characteristics. The individuals were unrelated genetically based on the information from the owners and also were cross-checked with neighbors. The details of breeds, geographic regions and sample sizes are listed in Table 1.

Total genomic DNA was extracted from blood following the instructions of the manufacturer (TIANamp Blood DNA kit from Tiangen Biotech (Beijing) Co., Ltd.).

2.2. DNA amplification and sequencing

Mitochondrial HVR1 of goats was amplified using the primers: forward 5' CATTACACCGCTCGCCTAC 3' and reverse 5' GGGCTGATTAGTCATTAGT 3' (Wu, Y.P. *et al*., 2009). PCR amplification was carried out in 50 µl reaction mixtures including each primer (1µl of a 10 µmol/L solution), dNTPs (4 µl of a 2.5 mmol/L solution), 5 µl of 10X buffer and 1.5 µl of 5U/l µTaq DNA polymerase (Tiangen Biotech, Beijing, China). The PCR conditions were an initial denaturing step at 95°C for 5 minutes followed by 35 amplification cycles (94°C for 30s, 56.2°C for 30s and 72°C for 30s) and a final extension at 72°C for 10 min in a Programmable Thermal Controller. All the PCR products were sequenced as instructed in Genomics.com.cn.

2.3. Analysis of sequence data

Six-hundred and twenty-five base pairs from HVR1 region of five different populations of Nepalese goats were edited using Chromas version 2.23. These sequences were aligned along

with 22 reference sequences recommended by Naderi *et al* (2007) using ClustalX program. The NJ tree was constructed using the program Mega 3.0 (Kumara *et al.*, 2004). The Median joining (MJ) network was drawn using the program Network 4.2 (Bandelt *et al.*, 1999).

3. RESULTS

3.1. Sequence variation

Seventy five mtDNA HVR1 sequences belonging to five different Nepalese goat populations were examined. Comparison of these sequences revealed 45 different haplotypes. Number of haplotypes found in each group ranged from 6 to 15 depending on the difference in number of samples and the diversity ranging from 0.89 to 0.971. Nucleotide diversity values ranged from 0.0153 to 0.03195 (Table 1). The analysis of Haplotype diversity and Nucleotide diversity showed that Nepalese Goat Khari Bandipur (NGKB) was least variable group and NGKI was high diverse group.

Table 1: Breed names, geographic regions and sample sizes and Haplotype and Nucleotide diversities

Breed	**Abbr.**	**Geographic distribution**	**No. of samples (n)**	**No. of Haplotypes**	**Haplotype diversity (±S.E.)**	**Nucleotide diversity (±S.E.)**
Khari Bandipur	NGKB	Central Development Hill Region	20	14	0.947±0.034	0.03195±0.027
Khari Salyan	NGKS	Far West Dev. Hill Region	14	10	0.945±0.045	0.01769±0.022
Khari Ilam	NGKI	Eastern Dev. Hill Region	14	8	0.890±0.06	0.0153±0.021
Sinhal	NGS	Central Development Alpine Region	8	6	0.893±0.111	0.02926±0.022
Terai	NGT	Western Terai Region	19	15	0.971±0.027	0.02543±0.024
Total			75	47	0.984±0.005	0.02625±0.036

3.2 Phylogenetic analysis

A neighbour-joining phylogeny was constructed for the Chinese goats and 22 reference sequences. In the tree, three different clusters (A, B and D) were present. Lineage A was predominant and followed by Lineage B. Lineage D represents only one sample. Lineages A and B was found in all breeds. Only one sample of Khari belonged to Lineage D.

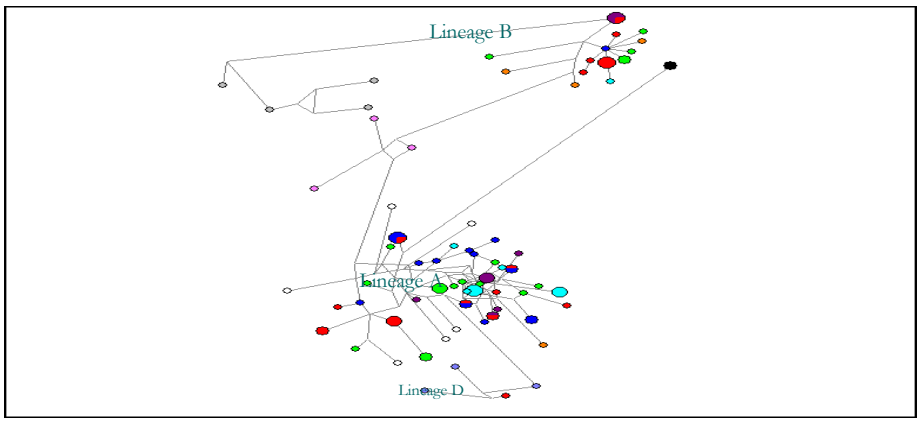

Figure1: Median joining (MJ) network showing genetic relationships among Nepalese goat haplotypes for mtDNA HVR1.

3.4 Comparison with goat sequences from neighboring countries

Presuming the possibility of the interference in the indigenous goat from the goats of neighboring countries, Nepalese goat sequences were compared with Indian, Pakistani and Chinese (Tibet) goats. Only a few Nepalese goat sequences shared with others. These showed the probable uniqueness in the Nepalese goat (Table 2).

Table 2 Comparison of Nepalese goat with goat from neighbor countries

Country	Shared sequences	Samples shared
India	8 out of 75	6 Terai (12, 4, 26, 18, 19, 21) and 2 Khari Salyan (48, 37)
Pakistan	1 out of 75	Khari Salyan (37)
China (Tibet)	2 out of 75	Terai (4) and Khari Salyan (37)

DISCUSSION

The mitochondrial DNA D-loop region is very polymorphic as reported by many authors (Chen *et al.*, 2005; Keyser-Tracqui *et al.*, 2005). Nepalese goat mtDNA sequences showed notable diversity. Forty-five haptotypes were identified in 75 samples. The wide variablity Nepalese goat mtDNA sequences maybe caused by the multiple origin of the population, in accordance with the results of other authors (Liu *et al.*, 2006; Chen *et al.*, 2005) studying different goat populations.

Some haplotypes were shared by individuals of different breeds from different geographical regions. These results suggested that there was no correspondence between the geographical regions of origin and among the breeds. The weak phylogeographic structure could be explained by the frequent human movement from one place to other. The goat is known to be the most adaptive species in the new environment may be the reason for multiple origin of the breed. Thus, gene flow or gene exchange occurred among different populations (Wen *et al.*, 2004). However, since there were few samples of Nepalese goat sequences shared with foreign goat sequence, it seems that there is uniqueness in the Nepalese population which needs to be studied in detail.

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EFFECT OF STINGING NETTLE FEEDING ON PRODUCTIVITY AND IMMUNE STATUS IN LAYING HENS

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ABSTRACT

The effect of feeding stinging nettle on productivity and immune status in laying hens of 52 weeks of age was investigated in a 10 weeks long trial at Animal Health Research Division, Khumaltar, Nepal. A total of 80 laying hens and eight cocks of Black Australorp breed were divided into four groups: T1 (Induced molting by feed restriction), T2 (6% nettle supplementation twice weekly), T3 (12% nettle supplementation once weekly) and C (Control);each group consisting of 20 laying hens and two cocks. Parameters like egg productivity, egg quality and immune status were recorded. Egg quality in terms of eggshell thickness and egg albumin height of seven eggs from each group were measured by using Spherometer. On the 4th week of the trial, ten eggs from each group were collected and stored at room temperature to simulate rural areas without cold storage for 5 weeks to observe the internal quality of eggs. The birds were re-vaccinated on 7th week of the trial with live B1 strain of New Castle Disease (ND) vaccine. Blood samples were collected on the day of ND vaccination and on day 28 post-vaccination. Differential leukocyte count (DLC) and antibody level against ND were determined for assessing the immune status of birds. Available data revealed that egg shell thickness and calcium content were higher in T2 (0.34 ± 0.04 mm and 1.21%) followed by T3 (0.3 ± 0.05 mm and 1.13 %), T1 (0.28 ± 0.03 mm and 1.01%) and C (0.27± 0.023 mm and 1.02%) while albumin height was highest for T3 (7.09 ± 1.05 mm) followed by T2 (6.59 ± 1.08 mm), C (5.72 ± 1.03 mm) and T1 (5.02 ± 0.68 mm). Data on antibody titer and DLC were also slightly higher for T3 and T2 than T1 and C. These findings indicated that nettle supplementation increases the egg quality besides improving the immune status of laying hens.

INTRODUCTION

Increasing demand of poultry products has led to unnecessary use of hormones and other chemicals to boost the productivity and production capability of birds. The indiscriminate use of such production boosters will result in serious effects on birds' health; decrease production period and alter physiological state leading to a compromised immune system. Moreover, rise in preference of consumers towards organic products has prompted to seek an alternative for such production boosters. Many herbal and other plant extracts are presumed to have positive influence in productivity.

There is dearth of information on effect of herbal supplementation on production performance of poultry. The first evidence of use of nettle in birds is by Juliette (1984) whereby nettle was used to treat worms in birds. In recent days, some researchers have documented the

immunomodulating property and improvement of blood lipid profile (Daher *et al*., 2006) of aqueous and ethanol extract of nettle and many other beneficial effects on experimental animals. However, no such evidences are found in relation to productivity of birds.

Grela *et al* (2006) have documented that a supplement of herbs or herbal extracts (nettle, oregano, Echinacea, garlic and lemon balm) can exert a positive effect on the health and reproductive traits of sows. Pilot research done at Agricultural Research Station, Pakhribas, Nepal had shown that once weekly feeding of 12% nettle powder to layers improved the egg laying capacity by 35% besides keeping the bird healthy (Khanal, 2005a). Furthermore, pigs fed with cooked nettle at 30% level had shown a significant weight gain with a good body musculature. Nettle fed pork was found tastier in sensory evaluation (Khanal, 2005b).

The usual practice of induced moulting for enhancing laying performance in commercial layers has been termed inhumane and criticized by the animal welfare groups. The present exploratory research was aimed at evaluating the effect of abundantly available stinging nettle (Urtica dioica) feeding as an alternative to induced molting for enhancing the productivity and the immune status of laying hens.

MATERIALS AND METHODS

Experimental Design

A total of 80 Australorp laying hens and 8 cocks at 52 weeks age were procured from the Swine and Avian Research Programme (SARP), Khumaltar and randomly allocated into four groups comprising of 20 hens and 2 cocks in each group. The first group consisted of induced moulting (T1) while the second group (T2) received 6% nettle powder twice weekly, the third group (T3) received 12% nettle once weekly and the last group served as the control group (C). Nettle feeding was switched between control and 12% fed group after one month but no change was done in T1 and T2 groups.

Feeding Schedule

At the time of purchase, the birds were fed commercial pellet layers ration at the rate of 150 gm per bird per day, but it was decreased to 120 grams per bird after a month. Clean drinking water was provided *ad libitum* after disinfection with Chlorine (Water guard). In the induced molting group (T1), water was withdrawn for 3 days followed by feed for 10 days. However, *ad libitum* drinking water was provided during feed withdrawal period. Feeding was resumed in T1 group with 50 gram feed per bird per day with gradual increment up to normal level as in other groups.

Assessment of egg production and quality

Eggs were collected twice a day; in the morning and in the evening. Weekly production performance was recorded. The egg albumin height was measured on 7 eggs from each group. Four eggs from each group were subjected to proximate analysis to determine egg protein, fat and calcium levels. The egg shell thickness was measured using gauge spherometer. For assessing egg quality, ten eggs from each group were collected and stored at ambient temperature for five weeks. After five weeks of storage, the eggs were broken and interior was observed.

Assessment of Immune Status

Blood samples of five birds from each group were collected randomly on the day of vaccination against ND. Using 23 gauge needles, 2 ml of blood was drawn from the jugular vein. The blood samples were again collected on 28 days post-vaccination with ND oral vaccine (La Sota Strain). Thin smears of blood were also prepared for differential leucocytes count (DLC) while serum was used to determine the hemagglutination (HA) titre of antibodies against ND vaccine using standard procedure as described by OIE Terrestrial Manual (OIE, 2004).

RESULTS AND DISCUSSIONS

Egg Production

Table 1: Trend of weekly egg production in control and treatment groups

Week	T1 (Induced molted)	T2 (6% nettle twice weekly)	T3 (12% nettle once weekly)	C (Control)
Pre trial	**134**	**95**	**121**	**102**
1	29	87	91	99
2	10	82	100	97
3	16	71	108	98
4	68	70	106	99
Sub total	**123**	**310**	**405**	**393**
After 4 weeks, 12% nettle fed group (T3) was switched to control (C) group)				
5	66	61	78	61
6	87	55	100	80
7	79	49	91	78
8	75	26	77	79
9	96	39	71	80
10	85	40	58	72
Sub total	**488**	**270**	**475**	**450**
Grand total	**611**	**580**	**880**	**843**

Molted birds (T1) showed a rapid decline in egg production during molting period and reached its peak production at 9th week after resumption of normal level of feeding. Oguike *et al* (2005) had also found similar trend of post molting production and attained a peak egg production by the second month.

Bird of T2 group developed the habit of eating their own eggs and due to which the data on overall production was not comparable with other treatment groups.

In T3 group, the average weekly egg production was increased while supplementing nettle at 12% rate. Improvement on egg laying performance may be attributed to presence of high amount of Ca, P, vitamins and non-specific immunomodulators in the nettle that activates the gene responsible for egg laying (Khanal, 2008). He further indicated that a positive response of nettle on the performance of ready to cull hens that had a significant increase in production

after nettle supplementation. In control group, the numbers of egg laid was lesser than that of T3 group.

On the 5th week of trial, the birds showed symptoms of salmonellosis and treatment was done accordingly to all groups by providing Neodox forte (Vetcare, India Ltd.) in drinking water for 5 days. Due to salmonellosis the egg production was declined on the fifth week, which could not achieve its peak production even after treatment in all groups. Despite of manifestation of salmonellosis in all groups, T3 (12% supplemented group) still maintained higher egg production. Holt *et al* (2006) reported that the long-term feed withdrawal has been shown to increase ileocecal intestinal colonization and fecal shedding of *Salmonella enterica* serovar *Enteritidis* in challenged hens and the outbreak of Salmonellosis in the present study might be attributed to this fact.

Assessment of Egg Quality

Table 2: Measurements of egg quality parameters

	T1 (Induced Molting)	T2 (6% UD)	T3 (12% UD)	C (Control)
Albumen Height (mm)	5.02 ± 0.68	6.59 ± 1.08	7.09 ± 1.05*	5.72 ± 1.03
Shell Thickness (mm)	0.28 ± 0.03	0.34 ± 0.04*	0.30 ± 0.05	0.27 ± 0.023
Calcium Content (%)	1.01±0.17	1.21±0.09	1.13±0.21	1.02±0.18

Albumen height

Internal egg quality is frequently assessed by measurements of inner thick albumen often measured as a function of the height of the inner thick albumen (Silversides and Villeneuve, 1994) which decreases with decreasing egg freshness (Pappas *et al.,* 2005). The average albumen heights in nettle supplemented birds were 7.09 ± 1.05 mm in T3 (12 % supplemented group) which was significantly higher ($p<0.05$) than C (control: 5.72 ± 1.03 mm) group (Table 2) whereas the molted group had the lowest (5.02 ± 0.68 mm). These findings on albumin thickness indicate that nettle supplementation in diet of layers can be beneficial in improving the egg quality.

Shell thickness

The average shell thicknesses in both nettle supplemented groups (T2 and T3) are 0.34 ± 0.04 mm ($p<0.05$) and 0.3 ± 0.05 mm, respectively which are higher than 0.28 ± 0.03 mm in T1 (induced molted) and 0.27 ± 0.023 mm in C (control) groups as depicted in Table 2. These differences are attributable to higher calcium content in stinging nettle that provided a better dietary source of calcium for eggshell formation. Ronald *et al* (1994) have also documented that adequate calcium in poultry diet enhances shell quality.

Calcium content of egg

Egg calcium contents in nettle supplemented groups are 1.21 ± 0.09 % in T2 and 1.13 ± 0.21 % in T3) that are higher than in non supplemented groups 1.01 ± 0.17 % in T1 and 1.02 ± 0.18 % in C) as shown in Table 2. The proximate analysis of nettle powder fed to birds has shown very high amount of calcium 1.11% on dry basis. This abundantly higher amount of calcium in egg may be attributable to abundant calcium available in nettle.

Effect of storage on egg quality

Fig 1: Showing egg contents of T1 group after 5 week of storage

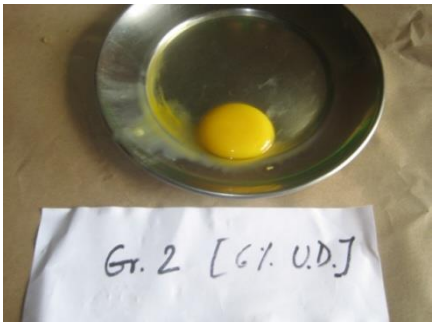

Fig 2: Showing egg contents of T2 group (biweekly 6% nettle supplemented) after 5 of week storage

Fig. 3: Showing egg contents of C (Control) group after 5 week storage

Fig. 4: Showing egg contents of T3 group (once weekly 12% nettle supplemented) after 5 week storage

The visible changes on egg quality after 5 week storage at room temperature are shown in Figures 1-4. These changes include decrease in albumin content and rupture of vitelline membrane in nettle non supplemented groups (T1 and C), while albumin content was not decreased in nettle supplemented group and with a more intact egg yolk depicting well maintained vitelline membrane integrity. Decrease in vitelline membrane integrity of eggs was also recorded by Kirunda and McKee (2000) upon one week storage of hen eggs at 25°c. The decrease in vitelline layer strength observed during storage is associated with the dissolution of the chalaziferous layer of the albumen, which occurs during long-term storage (Fromm, 1967; Heath, 1976). In the process of aging, water is also displaced from the egg albumen to the yolk (Trziszka and Smolinska, 1980). The excess water in the egg yolk causes the vitelline membrane to stretch and lose elasticity. Moran (1936) observed a similar decrease in elasticity of the vitelline membrane with age. As nettle supplementation increased the thickness of eggshell, thicker shell preserved the water loss and maintained egg quality. This would be a boon to Nepal and other third world countries where many rural areas are without electricity and refrigerator to preserve table eggs for longer duration.

Antibody titre against New Castle Disease

Table 3: Antibody titre against ND in different groups

	T1	**T2**	**C**	**T3**
0^{th} day	2^7	2^7	2^7	$\mathbf{2^3}$
10^{th} week	2^7	2^8	2^8	2^8

The result of hemagglutination inhibition (HI) test showed that birds fed with nettle powder had higher antibody titre than induced molted layers as shown in Table 3 at the end of 10^{th} week. Similar titres were obtained in broilers supplemented with nettle powder by Piya (2006) and Maharjan (2008). Rathore *et al* (1987) considered New Castle disease HI titer above 2^4 as protective level. In both T2 and T3 groups, the antibody titer was found higher than in T1 (molted group). It was not known why the final titre in control group also remained elevated as that of T2 and T3. The direct challenge experiment would help to assess the protective effect of stringing nettle. On the event of possible ND outbreak, chicken supplemented with nettle would be prepared to defend from the disease due to maintenance of protective level of antibody.

Differential Leukocyte Count

Table 4. Patterns of lymphocytes and Heterophils in different groups

Groups	**Lymphocytes (%)**	**Heterophils (%)**
T1 (IM	68±1	22.67±0.58
T2 (6% UD)	70±2.73	20.33±1.53
T3 (12% UD)	71.33±51	20.33±1.53
C (Control)	69.33±1.53	23.33±2.3

Nettle Supplemented groups had comparatively higher proportion of lymphocytes than non-supplemented groups. A decrease in peripheral lymphocytes was observed by Safamer (2008) in broilers challenged with aflatoxin B_1. It was observed that immune stimulation causes peripheral lymphocytosis with more number of reactive lymphocytes (Kahn, 2005). Wagner *et al* (1989) has also demonstrated an increased lymphocyte proliferation by nettle extract on experimental animals. These facts indicated that nettle feeding has an immunomodulating action on immune system.

CONCLUSIONS

The present findings indicated that nettle supplementation can improve egg quality and the vitelline membrane integrity besides enhancing antibody titre against New Castle disease. Maintenance of vitelline membrane integrity is very important in case of breeder hens. Nettle can also increase the calcium content of egg and has positive influence on egg albumen thus favouring for more nutritious table egg. Also nettle increases the eggshell thickness, which can subsequently reduce the cases of broken eggs and subsequent breakage losses. The effect of nettle on total egg productivity could not be documented due to outbreak of salmonellosis, development of egg eating habit in T2 group and occasional encroachment by rodents in poultry shed besides shorter duration of research (10 weeks).

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EFFICACY OF DIFFERENT ANTICOCCIDIAL DRUGS ON EIMERIOSIS OF RAINBOW TROUT *(Oncorhynchus mykiss)*

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ABSTRACT

Eimeriosis is one of the most economically devastating diseases of fresh water fish. Since last decade its outbreak causes epidemic threat in rainbow trout (Oncorhynchus mykiss) production in Nepal. Emergence of resistance to anticoccidial drugs currently used by trout entrepreneurs had stimulated the search for safe and effective drugs to control the disease. Studies have been carried out to isolate and identify the organism responsible for coccidiosis in trout, the degree of infestation and to recognize the most suitable drugs as control measure.

Eimeria positive rainbow trout showed yellow diarrheal syndrome with gross mortality reached up to 37.6% (average 27.4). Intestinal examination of infected trout revealed that average oocyst shedding per gram fecal (OPG/g) was 5504 ± 2669 as pathogenic sign of eimeriosis. Eimeria aurati (average 20.2 µm x16.23 µm) parasite was isolated from infected trout for the first time in Nepal. Three synthetic anticoccidial drugs commonly used in poultry were tested for their efficacy to prevent and control coccidian parasite. Drug bioassay trial was carried out with 11150 different sized trout (278 gm ± 270) in commercial private farms at Nuwakot. Anticoccidial drugs namely Supercox, Coctreat-EP and Duaprim was mixed in the trout feed at three different doses viz. 60 mg/kg, 80 mg/kg and 100 mg/kg body weight. Fish were fed with treated feed for 7 days, breaking 2 days after 3 days of feeding.

Insignificant numbers of oocyst (40±34.6 and 100±34.6 OPG/g) were detected at 9th day post treatment with Supercox and Coctreat-EP, respectively. The shedding of oocyst (4840 ± 541.11 OPG/gm) was higher in fish treated with Duaprim and similar to that of untreated group of fish. Coccidian prevalence rate (7.5 and 10%) and cumulative mortality rate (1.9±0.3 and 4.2 ± 0.5) was significantly reduced ($p<0.05$) in trout population treated with Supercox at dose 60 mg/kg body weight per day and Coctreat-EP at dose 100 mg/kg body weight per day respectively. Based on the efficacy trial, the application of Supercox at 60 mg/kg body weight per day to treat the fish against eimeriosis would be effective and economical.

Key words: Eimeriosis, *Eimeria aurati*, Rainbow trout, Anticoccidial drugs, Efficacy, Treatment

INTRODUCTION

Among coccidia (Sub-Class), several parasites of the Genus *Eimeria* have been described from the fish of different geographical regions of the world (Lom & Dykova, 1981; Lukes, 1993;

Sitja-Bobadilla *et al* (1996), Molnar and Pellerdy (1970), Molnar and Fernando, (1974), Molnar and Hanek (1974). As regards to the biodiversity of *Eimeria*, Davies and Ball (1993) were notified more than 130 *Eimeria* species of Phylum Apicomplexa were described from a wide range of fish hosts and geographic locations. Species-rich *Eimeria* is a protozoan parasite that may prove to be one of the limiting pathogens of both marine and cultured freshwater fish that are subjected to crowding and intensive management (modern fish farming) and other factors contributing to diseases in fish like those noted in mammals and birds, i.e. age, immune status, nutrition, and stress (Pellerdy, 1974; Awadallah *et al.,* 1998; Gregory, 1990; Bauer *et al.*, 1981). *Eimeria* spp. is quite commonly reported in the intestine of freshwater fish (Marwan and Khidr 2001). Among fresh water fish, eimeriosis caused by *Eimeria* is a long known and rather common disease of rainbow trout (*Oncorhynchus mykiss*) and transmission process of this coccidial infection to cultured fish may be direct by oral ingestion of oocyst that are shed with contaminated faeces in environment (Tyzzer *et al.*, 1932, Brown *et al.*, 1996).

In Nepal, pathogenecity of eimeriosis has been considered as one of the economically important problems (Rayamajhi and Dhital, 2008) and most of the farmers' complaints that the growth and yield performance of rainbow trout in semi intensive system had been significantly affected. So far, determined attempt has been made to treat this disease (Rayamajhi *et al.*, 2010). Recently, farmers paid much attention to used synthetic compounds (amprolium) as anticoccidial drugs that is commonly used in poultry. Unfortunately, possibility of increased resistance of trout coccidian to anticoccidial drugs may be a major drawback in coccidiosis control. Therefore, the purpose of this study was to isolate and identify the organism responsible for coccidiosis in trout, the degree of infestation and to recognize the efficient commercial drugs for controlling eimeriosis in naturally infected Rainbow trout fish.

MATERIALS AND METHODS

From December 17 to 23, 2010, a total of 25 intestines of yellow diarrheal syndrome affected rainbow trout were sampled from 5 commercial private trout farm at Nuwakot. Collected trout intestine samples were stored in vials of Normal saline solution and 10% formaldehyde for oocyst observation and species identification. Paraffin sections 3-5 µm thick were stained with hematoxylin-eosin. The sampled intestine was opened in longitudinal direction and the mucus and fecal material drawn off. Quantitative fecal examination was performed by McMaster technique to determine the number of oocyst per gram of feces (OPG) as per standard procedure (MAFF, 1986) with some modification. According to McMaster technique, 1 gram of feces was homogenized into 14 ml of distilled water. The solutions placed into a 15 ml centrifuge tube and centrifuged at 2000 rpm for 2 minutes. The supernatant was decanted without disturbing the pellet. Oocyst is counted from each fecal pellet sample in Mac-Master chamber and rest of the pellet samples were kept in 2.5% Potassium Dichromate ($K_2Cr_2O_7$) solution for oocysts sporulation at 26^0C in an incubator. Species identification based on morphological characteristics of oocyst was followed from the key of Steve *et al* (1984).

For coccidia control measures a total of 11,150 grown to brood trout (278 gm ± 270), were used in a drugs bioassay trial. About 54.71 % trout were treated with Supercox, 26.01 % with Coctreat-EP and 19.28 % trout with Duaprim as feed additive (Table 1). Therapeutic doses of the anticoccidial drugs, mixed in the trout feed at three levels viz. 60 mg/kg, 80 mg/kg and 100

mg/kg BW, were fed for 7 days, breaking 2 days after 3 days of feeding. Each drug was tested twice at a time (2 replicates of each drugs/site). During drugs trial fish mortality was recorded daily. Three to four fishes from each treated group were sacrificed at the 9^{th} days of treatment for the confirmation of oocyst (OPG/g). Efficacy of tested drugs against emeriosis was analyzed by one-way ANOVA with SPSS software. Active ingredients, trade name, manufacturers of anticoccidial agents and their use levels are given in Table 2.

RESULTS

Eimeria positive rainbow trout showed sluggish behavior, blackish in color, anorexia and yellow diarrheal syndrome with gross mortality reaching up to 37.6% (average 27.4 ± 28.7) (Table 1). Fecal examination of infected trout revealed that average oocyst shedding per gram of fecal (OPG/g) was 5504 ± 2669 which was pathogenic. *Eimeria aurati* (average 20.2 µm by 16.23 µm) parasite was isolated from the intestine of infected trout for the first time in Nepal. Dimensions (µm) and shape index of *Eimeria* species oocyst shown in Table 3. Oocyst sporulation of *E. aurati* took place in 5 to 7 days in 26°C. Four sporocysts developed in each oocyst (Figure 1). Oocyst shape-index (ratio length/width) was 1.24 µm, n=18. There was no sporocyst residum or stieda body seen in the 18 oocyst studied. Infected intestinal villi showing oocyst in various stages of sporulation (some are indicated by arrows) as listed in histopathological study (Figure 2).

Results of drugs bioassay trial revealed that trout group treated with Supercox at dose 60 mg/kg body weight per day and Coctreat-EP at dose 100 mg/kg body weight per day showed significant reduction ($p<0.05$) of trout cumulative mortality rate (1.9 ± 0.3 and 4.2 ± 0.5), coccidian prevalence rate (7.5% and 10%) as well as high reduction in the number of oocyst (40±34.6 and 100±34.6 OPG/gm), respectively. The shedding of oocyst (4840 ± 541.11 OPG/gm) was higher in fish treated with Duaprim and similar to that of untreated group of fish (Figure 3). Results of the coccidian prevalence rate and mortality rate of untreated and treated population of rainbow trout are shown in Table 1.

Based on market price and efficacy, the application of Supercox at 60 mg/kg body weight/day to treat the fish against eimeriosis was analyzed effective and cheaper (NRs 140.70/100 kg fish) than Coctreat-EP (174.75/100kg fish). Drugs prices and expenditure /100 kg fish treatment is given in Table 4.

Table 1. Coccidian prevalence rate and mortality rate of control and treated population of rainbow trout in private trout farm in Nuwakot

Observation period	**Drugs applied (mg.kg-1 body weight)**	**Doses (mg. kg-1 body weight)**	**Stocked trout (No)**	**Average weight (g)**	**Mortality % before beginning of medication**	**Cumulative mortality (%) in medication period**	**Prevalence rate (%)**	**Treated population %**
29 Nov-14 Dec.	Coctreat-EP	0.0	2900	120 ± 40	6.7%		50.0 ± 14.1	
Dec. 17-23 2010	"	60.0	1100			12.32 ± 0.5	50	
Dec. 17-23 2010	"	80.0	1300			9.52 ± 3.1	40	26.01
Dec. 17-23 2010	"	100.0	500			4.16 ± 0.5	10	
Nov. 1-Dec. 14	Superco x	0.0	6100	124.17 ± 72.5	60.2%		23±7.8	
Dec. 17-23 2010	"	60.0	1200			1.87 ±0.3	7.5	
Dec. 17-23 2010	"	80.0	2200			1.43 ± 0.1	8.82	54.71
Dec. 17-23 2010	"	100.0	2700			1.01 ± 0.1	7.5	
Nov. 17-Dec. 14	Duaprim	0.0	2150	590 ± 430.3	15.3%		35 ± 7.1	
Dec. 17-23 2010	"	60.0	46			-	40	
Dec. 17-23 2010	"	80.0	1025			25 ± 1.3	40	19.28
Dec. 17-23 2010	"	100.0	1079			18.94 ± 0.4	30	
Total treated trout			11150					
Gross mortality					37.6%			
Average				278.06 ±270.2	27.4±28.7			

Table 2. Active ingredients, trade name and manufacturers of anticoccidial agents tested for efficacy for the control of eimeriosis caused by *Eimeria* species

Active ingredient, Generic or Chemical name (Manufacturer)	Trade name	Composition	Route of application	Use level (mg)
Diaveridine & Sulphaquinoxaline premix (Vet.)	Supercox	Each 100 g contains: Diaveridine hydrochloride equivalent to Diaveridine 3.3 g Sulphaquinoxaline Sodium equivalent to Sulphaquinoxaline 18.7 g	Feed	60,80,100
Amprolium, Ethopabate & Sulphaquinoxaline	Coctreat-EP	Each gram contains: Amprolium HCL 100 mg Ethopabate 5 mg Sulphaquinoxaline 60 mg Vitamin K_3 2 mg Vitamin C 20 mg	Feed	60,80,100
Trimethoprim l.P & Sulphamethoxazole l.P. premix	Duaprim	Trimethoprim l.P: 400mg, Sulphamethoxazole l.P.: 2000mg	Feed	60,80,100

Table 3. Dimensions (µm) and shape index of *Eimeria* species oocyst isolates

Isolates	Dimensions		Shape index (ratio length/width) (µm)
	Length of Oocyst (µm)	Width of Oocyst (µm)	
1	17.71	15.18	1.17
2	18.98	16.45	1.15
3	23.23	17.17	1.35
4	23.23	17.17	1.35
5	21.21	17.17	1.24
6	22.77	17.20	1.32
7	22.77	17.20	1.32
8	20.24	17.20	1.18
9	16.45	15.18	1.08
10	20.24	14.42	1.40
11	23.78	17.71	1.34
12	16.45	13.92	1.18
13	25.30	13.92	1.82
14	20.24	16.45	1.23
15	17.71	16.45	1.08
16	17.71	16.45	1.08
17	17.71	16.45	1.08
18	17.71	16.45	1.08
Mean ± SD	20.19±2.79	16.23±1.19	1.24±0.018

Table 4. Economic analysis of anticoccidial drugs (total cost (Rs) of drugs/100 kg fish) for the treatment of coccidian positive fish

Drugs	**Drug quantity (g)**	**NRs/100g drugs**	**Applied does (mg)**	**Drug effect**	**Required drug (mg)/100 kg fish**	**Required quantity (mg)/7 days**	**Total expenditure Nrs/100 kg fish**
Supercox	100	335	60	√	6000	42,000	140.70
"			80	√	8000	56,000	187.60
"			100	√	10000	70,000	234.50
Coctreat-EP	100	249.65	60	NE	6000	42,000	104.85
"			80	NE	8000	56,000	139.8
"			100	√	10,000	70,000	174.75
Duaprim	100	177	60	NE	6000	42,000	74.34
"			80	NE	8000	56,000	99.12
"			100	NE	10000	70,000	123.9

NE: Not effective, √: Effective

DISCUSSION

Based on morphometric characteristics, identified parasite is a member of the genus *Eimeria*, with four sporocysts and lacking of residium or stieda body in the sporocyst (Hoffman, 1965). The species-rich *Eimeria* are considered as significant pathogens of cultured freshwater fish. In the current study gross mortality reached up to 37.6% due to pathological condition caused by *Eimeria aurati*. Among eimerian species, *Eimeria truttae* was also found pathogenic to rainbow trout which causes 27.2% mortality (Saglam and Pala, 2008). Recently much attention has been paid to use synthetic compounds as anticoccidial drugs to minimize mortality.

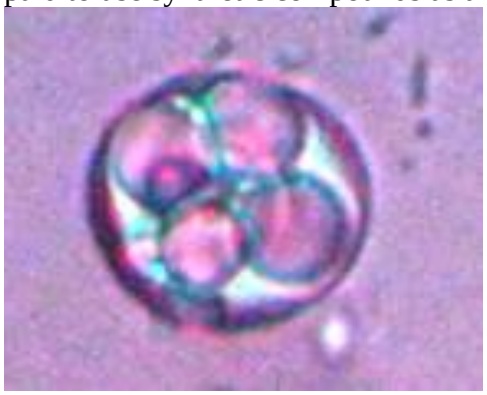

Figure 1. *Eimeria aurati* isolated from intestine of rainbow trout viewed under a microscope.

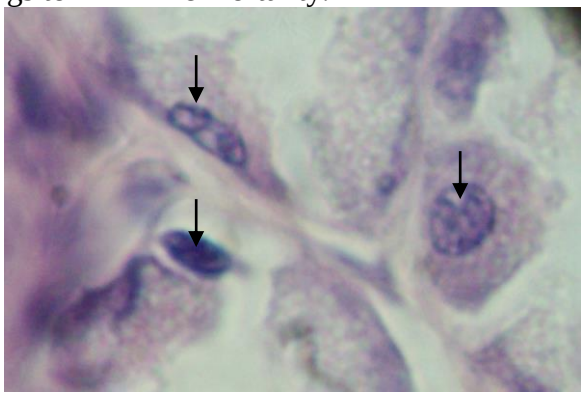

Figure 2. Infected intestinal villi showing oocysts in various stages of sporulation (some are indicated by arrows) in histopathological study.

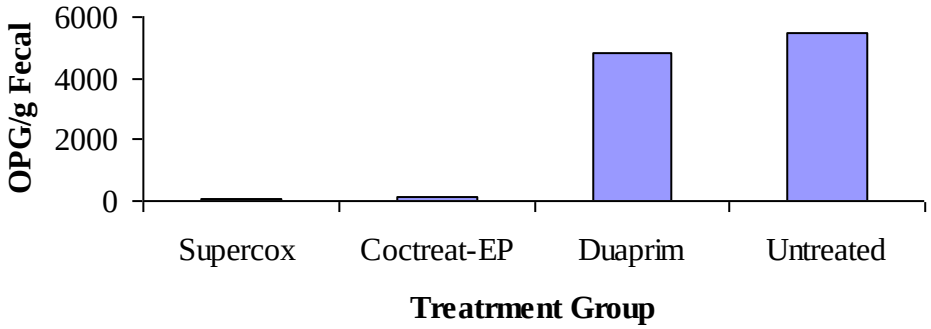

Figure 3. Shedding of oocyst per gram of feces (OPG) in some sampled intestine of untreated and 9th day post treated trout

As drawback, emergence of drug resistance in coccidiosis is a great problem (Bafundo and Jeffers, 1990; Mathis and McDougal, 1982; Chapman and Shirley, 1989). Unfortunately, the emergence of drug-resistant strains of coccidian has made the anticoccidials less effective and this has threatened the economic stability of the industry, especially in developing countries where the problem has become a major concern to resource-poor farmers (Hanan *et al.*, 2009). Therefore, some of other anticoccidial sensitivity testing (AST) is needs to be study to minimize risk of resistance build-up that has been seen in recent past. Among anticoccidials, coccidiostat or coccidiocidal drugs can be utilized. Coccidiostat serves to retard the life cycle or reduce the population of pathogenic coccidian to the point that disease is minimized and host develops immunity. Drugs with a coccidiostatic mode of action arrest the development of certain parasite stages in a reversible way and their withdrawal will still lead to the completion of the coccidiosis life cycle. In contrast, coccidiocidal drugs kill or irreversibly damage most parasite stages with no signs of disease relapse after drug withdrawal. Some products can have coccidiostatic and coccidiocidal properties at the same time (Peek, 2010; Blood and Henderson, 1999). The mode of action of Supercox and Coctreat are to stop the life cycle of oocyst or to kill the parasite. While Duaprim a bactericidal do not stop the population of oocyst as observed in natural condition in fish host. Though it is drug of choice to kill the broad spectrum bacteria flora existed in the gastrointestinal tract of host.

Results revealed the therapy intervention with Supercox and Coctreat-EP significantly reduced ($p<0.05$) the oocyst (OPG/g) discharge through feces and mortality trend (%) at 9th day post treatment compared with untreated group (Figures 3 and 4). These two drugs effectively controlled coccidian in the natural infected trout with *Eimeria* spp. and OPGs remained almost negligible. OPGs in Duaprim treated groups remained almost constant as before the commencement of the treatment.

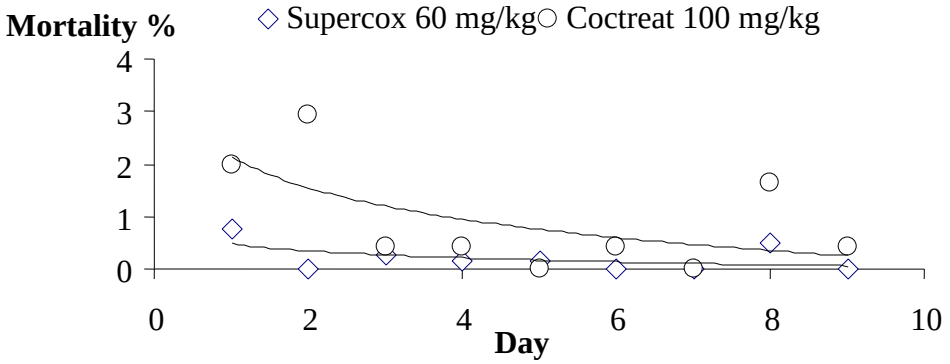

Figure 4. Mortality trend of Rainbow trout treated with Supercox and Coctreat-EP two different doses in private trout farm, Nepal

CONCLUSION

The present findings suggest a high incidence of eimeriosis in rainbow trout, reared in semi intensive raceway farming system of Nepal. Trout of all ages can come down with coccidians, but fingerlings to table size trout are most commonly affected and die of the disease. Results presented in this paper suggest that some of the coccidiostat/coccidiocidal drugs widely used for the therapy and prevention of coccidiosis in poultry can be used successfully for the control and prevention of coccidial infections of fish as well. From the results of the present study, it can be concluded that Supercox and Coctreat-EP commercially economical and gave promising results for controlling *Eimeria* infections. However Duaprim was not effective in controlling and prevention of the eimeriosis.

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EFFECT OF CALCIUM AND PHOSPHORUS SUPPLEMENTED RATION ON MILCH BUFFALOES UNDER FARMERS' MANAGEMENT CONDITION IN KAILALI DISTRICT OF NEPAL

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ABSTRACT

An experiment was conducted in 25 milch buffaloes at Taranagar, Dhangadhi Municipality of Kailali district, Nepal from July 15 to November 16, 2008 in order to assess the effect of calcium and phosphorus supplementation on the milk production, milk fat and solid non fat (SNF) content, serum calcium and phosphorus level, and cost benefit of the Ca and P supplementation under farmer managed conditions. The experiment was conducted using Randomized Complete Block Design with 5 treatments and 5 replications. The treatment groups allotted were: diet without calcium and phosphorus (T_1), diet with 7.5 gm calcium and phosphorus (T_2), diet with 10 gm calcium and phosphorus (T_3), diet with 15 gm calcium and phosphorus (T_4) and diet with 20 gm calcium and phosphorus (T_5). The results of the study revealed that the daily milk yield was significantly ($p<0.01$) higher in T_4 and T_5 (4.45 liter). Significantly higher ($p<0.01$) percent milk fat (5.50) was observed in T_3. The serum calcium (8.76mg/dl) and phosphorus level (4.50 mg/dl) showed significant increase ($p<0.01$) in T_5. The net profit from increment in milk production was (NRs 3420) in T_4 with higher cost benefit ratio (11.4:1). The result showed that supplementation of calcium and phosphorus in the range of 15-20 gm daily was found to be beneficial.

Key words: Calcium, Phosphorus, Buffalo

INTRODUCTION

Kailali is one of the far western Terai districts of Nepal where, as in other parts of the country, buffaloes are kept for milk, meat, draught power, manure, cash income and for traditional values. Low milk production and infertility in buffaloes account for major economic losses in smallholder dairy farms of Kailali.The major constraints to the buffalo milk production and fertility in Kailali district is feed, deficit in both the quantity and the nutritional quality. Feeding practices of buffaloes includes paddy straw, wheat bhoosa and limited green fodder along with no or limited concentrates. Paddy straw and *wheat bhoosa* which contain very little digestible crude protein are also poor sources of energy, minerals, and vitamins. Factors like high lignin content, poor palatability, dustiness, and high oxalic acid content limit their extensive use as cattle feed (Ranjhan, 2003).

Calcium and phosphorous are minerals needed in dairy animal ration in relatively large quantities because of their role in enhancing milk production as well as maintaining reproductive cycle (Schmidt and Vanvleck, 1982). Deficiency of these minerals results in decreased milk production and failure of reproduction. For proper absorption, maintenance of

growth rate and feed utilization, calcium and phosphorus contents in the diet should be within certain proportions. The requirement of calcium and phosphorus in growing, reproducing and milk producing animals is fairly high, as lot of calcium and phosphorous is drained out in milk. The District Livestock Service Office, Kailali has reported the deficiency of calcium and phosphorus as one of the major constraints to performance of milch buffaloes. This study was conducted to find out the effect of supplementation of calcium and phosphorus in the diets of buffaloes under farmers' management conditions.

Objective of the study

The objectives were to study and compare the performance of milch buffaloes fed with different level of calcium and phosphorus supplemented ration.

MATERIALS AND METHODS

A field study was conducted using 25 milch buffaloes of 2nd and 3rd parities under farmers managed conditions from July 16 to 15 November 2008. Farmers were provided with calcium and phosphorus powder prepared from Tri-calcium phosphate after and taught to feed experimental animals at a ratio of 2:1. A randomized complete block design with 5 treatments with 5 replications each was employed. The T_1 (control) comprised normal feeding without calcium and phosphorus supplement and 7.5, 10, 15, and 20 gm calcium and phosphorus were given in T_2, T_3, T_4 and T_5 diets, respectively.

The daily milk yield (liter) of buffaloes was recorded directly by the farmers. Milk samples were taken and examined for fat percent by Gerber's method and SNF percent by Richmond's formula (Aggarwal and Sharma, 1961). Blood samples of buffaloes were collected and examined for serum calcium and phosphorus levels using spectrophotometer and commercial kits as standard. Recorded data were tabulated in Ms-Excel. Analysis of Variance (ANOVA) was done using MSTAT-C version 1.3. Least Significance Difference (LSD) was used to compare the means.

RESULTS AND DISCUSSION

Milk production

The mean milk production, percent fat and SNF of experimental buffaloes presented in Table 1. Results showed that feeding calcium and phosphorous supplemented ration to milk buffaloes significantly ($p<0.01$) increased average milk production as compared to control one. This increase in milk production might be due to better utilization of nutrients. The results agree with the findings of Steevens *et al* (2003), Daniel *et al* (1990) and Block (1984) who reported that feeding calcium and phosphorus diets increased milk production. However, Harris (2003) did not found any effect of calcium and phosphorus supplementation in diets on milk production of Holstein cows.

Table1. Mean daily milk yield (liters), percents fat and SNF of buffaloes fed with different levels of calcium and phosphorus under farmers managed condition

Treatments	Milk yield (liters) and composition (%)		
	Yield	Fat	SNF
T_1 Normal feeding without calcium and phosphorus supplementation (control)	3.44^{c}	4.70^{b}	7.60
T_2 Normal feeding with 7.5 gm calcium and phosphorus supplementation	3.56^{bc}	4.94^{a}	7.87
T_3 Normal feeding with 10 gm calcium and phosphorus supplementation	4.01^{b}	5.50^{a}	7.70
T_4 Normal feeding with 15 gm calcium and phosphorus supplementation	4.45^{a}	5.48^{a}	7.40
T_5 Normal feeding with 20 gm calcium and phosphorus supplementation	4.45^{a}	5.40^{a}	7.67
Probability	0.0001**	0.0001**	0.33ns
CV%	7.65	3.44	2.72
LSD	0.41	0.24	-

*****Significant at 5%, **Significant at 1% and means in column with same superscript is not significantly different.

Milk fat content

The average milk fat percent (5.50%) was significantly ($p<0.01$) higher in T3 as compared to 4.70% in T1 (Control) with no calcium and phosphorous supplementation. The calcium and phosphorus supplementation did not have direct effect on percent fat increment on milk. However, the mean percent fat in milk had significantly increased at all levels of calcium and phosphorus supplementation in diets as compared to control but the percent fat did not differ significantly among treatments irrespective of the level of calcium and phosphorus supplementation in diets. This increase in percent fat in milk might be due to the electrolyte balance which maintained the ratio of acetate to propionate in the rumen. Similar findings was reported by Wu *et al* (2000) who found milk fat increment with increment in calcium and phosphorus levels in diets of cows. The present study contradicts to the findings of Harris (2003) who did not find positive effect of calcium and phosphorus supplementation on percent milk fat of Holstein cows.

Milk SNF content

SNF content of milk was not significantly influenced by the level of incorporation of calcium and phosphorus in diets. Similar findings were reported by Harris *et al* (1964), and Prasad (2001) who found no change in SNF content of milk with the feeding of calcium and phosphorus in diets of cows.

Serum calcium level

There were no significant differences in initial serum calcium levels of buffaloes in all groups of buffaloes. Significantly ($p<0.01$) higher serum calcium level (8.76 mg/dl) after 123 days of experiment was obtained from T_5. However, minimum serum calcium level (5.26mg/dl) was recorded from T_1. The serum calcium value (5.26 mg/dl) in T_1 was significantly ($p<0.01$) lower than in all other groups of buffaloes that were fed 7.5, 10, 15, and 20 gm of calcium and

phosphorus in diets. Similar findings have also been reported by Steevens *et al* (2003), Sunder *et al* (2007), and Bansal *et al* (1978). However, the findings of Awasthi and Kharche (1987) are different who found that serum calcium level did not show any variation in milch buffaloes.

Table 2. Mean serum calcium and phosphorus levels (mg/dl) in buffaloes fed with different levels of calcium and phosphorus under farmers' managed condition

Treatments	Calcium (mg/dl)		Phosphorus (mg/dl)	
	Initial	After feeding	Initial	After feeding
T_1 Normal feeding without calcium and phosphorus supplementation (control)	5.53	5.26[d]	3.34	3.34[b]
T_2 Normal feeding with 7.5 gm calcium and phosphorus supplementation	5.56	5.53[c]	3.09	3.56[b]
T_3 Normal feeding with 10 gm calcium and phosphorus supplementation	5.56	6.68[b]	3.00	3.50[b]
T_4 Normal feeding with 15 gm calcium and phosphorus supplementation	5.68	8.58[a]	3.18	4.60[a]
T_5 Normal feeding with 20 gm calcium and phosphorus supplementation	5.74	8.76[a]	3.48	4.50[a]
Probability	0.78 ns	0.0000**	0.01	0.0000**
CV%	5.54	3.86	8.74	4.57
LSD	-	0.37	-	0.24

*Significant at 5%, **Significant at 1% and means in column with same superscript is not significantly different.

Serum phosphorus level

No significant difference was seen among treatments in serum phosphorus levels of buffaloes in the beginning when they were not fed with phosphorus supplementation in diets. The average serum phosphorus level (mg /dl) initially was lower in buffaloes than normal value (4-6mg/dl) but at end of experiment the mean serum phosphorus value of buffaloes showed an increasing trend following the increasing level of calcium and phosphorus in diets. Results are in agreements with the findings of Biswas and Samanta (2001) and Gowda *et al* (2000) who reported increased serum phosphorus levels in cows fed phosphorus supplemented diets.

Benefit cost analysis of milk production

Table 3 showed that the net profit of Rs.3420/ from increased milk production was highest in T_4. Thus the benefit cost analysis of calcium and phosphorus supplementation showed that a dietary supplementation of 15gm /day was beneficial to milch buffaloes. The benefit from added minerals increased as the levels of calcium and phosphorus in diets were increased. This showed that the animals were not getting sufficient conversion of nutrients in to milk when

diets were deficient in calcium and phosphorus. So, buffaloes need calcium and phosphorus supplementation in diets when they are fed with calcium and phosphorus deficient feeds.

Table 3. Benefit cost analysis of milk production (NRs/Litre) of experimental buffaloes fed with different levels of calcium and phosphorus supplemented diets under farmers managed condition, Dhangadhi, Kailali, 2008

Treatments	Total milk production in 123days (Liter)	Increment over milk production (Liter)	Income from increased milk production @Rs30/ liter	Cost of minerals (NRs)	Net profit from increased milk production (NRs)
T_1 Normal feeding without calcium and phosphorus supplementation (control)	423	-	-	-	-
T_2 Normal feeding with 7.5 gm calcium and phosphorus supplementation	437	14	420	150	270
T_3 Normal feeding with 10 gm calcium and phosphorus supplementation	493	70	2100	200	1900
T_4 Normal feeding with 15 124gm calcium and phosphorus supplementation	547	124	3720	300	3420
T_5 Normal feeding with 20 gm calcium and phosphorus supplementation	547	124	3720	400	3320

The present findings are in agreement with Kincaid *et al* (1981), Kopecek (2000), Wu *et al* (2000), and Prabu (2006), who reported the increased milk and profit from cows fed with different levels of calcium and phosphorus supplemented diets.

CONCLUSION

The present study has revealed that the milk productions in buffaloes were affected by calcium and phosphorus levels in the diet. The optimum levels of calcium and phosphorus for increased profit was within the range of 15-20 gm/day. Hence, the buffaloes fed diets deficient in calcium and phosphorus or reared in poor grazing conditions with no supplemental calcium and

phosphorus should be fed with supplemental calcium and phosphorus in order to increase the milk production, and decrease the intervals between two lactations.

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RETROSPECTIVE EPIDEMIOLOGICAL STUDY OF CATTLE DISEASES IN CENTRAL CATTLE BREEDING AND DAIRY FARM, SAVAR, DHAKA

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ABSTRACT

The retrospective epidemiological study of cattle diseases was undertaken at Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka during July 2006 to June 2007 (12 months) to determine the prevalence and distribution of diseases. In the patient register a total of 1903 sick cattle were considered and 74 types of diseases were identified during this period. The overall occurrence of various diseases was enteritis (8.57%), debility (8.41%), Metritis (5.99%), retention of placenta (5.68%), wound (5.36%), mastitis (5.31%), pneumonia (4.10%), indigestion (3.73%), fever (3.73%), and foot rot (3.57%). Rest of the diseases had lower percentage than 3.57%.Out of 1903 sick animals, 83.2% were female and 16.8% were male cattle. Animals aged between 5-10 years had high prevalence (37.4%) while animal less than 2 years of age had low (8.1%) prevalence. Local Friesian crosses had higher prevalence (42.9%, 32.4%) of diseases than Sahiwal Friesian crosses and local (15.7% and 9.0%) respectively. Prevalence of diseases was high (50.2%) in rainy season (June to October), followed by 29.3% in winter (November to February) and low 20.5% in summer season (March to May). Reproductive diseases (29.9%) were seen highly prevalent among all groups of cattle, which were followed by gastrointestinal diseases (17.0%), skin diseases (15.4%) and infectious diseases (9.1%).

INTRODUCTION

The success of commercial dairy farms is largely dependent on improved health and efficient production techniques. The economics of the dairy industry require producers to operate efficiently to remain competitive. Reproductive and udder performances affect the quantity of milk produced by cow per day of herd life, the number of potential replacements needed to maintain a constant herd size and longevity of the cow in the herd (Congleton and King, 1984). All of these factors alter efficiency and profit.

Veterinary services in Bangladesh have traditionally given emphasis on prevention of major infectious diseases on regional basis, although emphasis is now needed to be given to individual herd. This approach of improving health management relies on the farmer recognizing that there is an abnormality in the herd or individual animal. Efforts are being made to develop new forms of veterinary services which would effectively deal with such problems in countries having successful dairy industries (Cannon *et al*., 1978). These approaches are commonly known as planned, herd health and production programs. Herd health programs are aimed at those conditions in which clinical signs are unapparent and/ or related to putative factors; consequently recognition of abnormalities such as poor performance necessitates a monitoring system that quantitatively detects deviation from optimal

performance (Blood *et al*., 1978). Ideally, the system should have the ability to predict herds' future performances from current and retrospective data.

Bangladesh with her infant stage of dairy industry has yet to develop herd health program mainly because of the absence of surveillance or monitoring system. The development of such system is an evolutionary process based on systematic accumulation of data over a long period which will eventually lead to the development of a herd program suitable for Bangladesh.

The Department of Livestock Services (DLS), Government of Bangladesh has established a Central Cattle Breeding and Dairy Farm (CCBDF) at Savar, Dhaka. This farm is located about 30 km northwest of the capital city of Dhaka. The farm was established in 1973 on 1300 acres of land with assistance of German Agency for Technical Cooperation. The average rainfall and humidity in the farm area are 1788.8 mm and 76.6 percent respectively. The average maximum and minimum temperature in the farm is 37.48°c and 21.1°c respectively. This is the largest farm in Bangladesh and the major objectives of the farm are: (1) To produce crossbred heifers and bulls for distribution to farmers, (2) To collect semen from the proven bulls that are produced and reared in order to support national artificial insemination program, and (3) To supply milk to Dhaka city. During the last 25 years this farm has been maintaining a herd of average 2,500 cattle.

The success of CCBDF partly depends on the sound health management of the animals and efficient production techniques. Diseases are one of the major health constraints in Bangladesh context. Understanding on the prevalence, distribution and determinants or risk factors of diseases in a farm is necessary for undertaking efficient control program. Studying diseases of any species of animal retrospectively is a rapid and cheap means. Analyzing past disease records of the farm may help to undertake appropriate strategy of disease control. Therefore, a retrospective epidemiologic study was carried out.

- To determine prevalence of diseases in cattle and distribution of diseases in different age, breeds and seasonal pattern of diseases.

MATERIALS AND METHODS

A retrospective epidemiologic study of diseases in dairy cattle was done during July 2006 to June2007 data in Central Cattle Breeding and Dairy Farm, Savar, Dhaka.

Study population

The CCBDF, Savar, Dhaka was selected for retrospective epidemiologic study of cattle diseases. During the last 25 years this farm has been maintaining a herd of average 2,500 cattle. Breeding was being performed by artificial insemination. A semi-intensive animal management with an intensive health management was followed in this Government establishment. The animals were housed in tie-stall system in stanchion barns. In order to facilitate proper and easily management of cattle, all the sheds are divided into two sections viz. Byre south and Byre north section.

Sources and nature of data

The retrospective data of 12 months from July 2006 to June 2007 were collected from Patient register of Veterinary hospital, CCBDF, Savar, Dhaka. These data were maintained by farm veterinarians. The data were analyzed and interpreted to determine the prevalence of diseases; seasonal pattern and distribution of diseases. The age and breed of cattle were collected from records available in the Byre section of farm.

Data processing

The data were checked manually for obvious inconsistencies, recording errors or missing data. The potential errors were evaluated and corrected, if possible, following discussion with the relevant veterinarians. Data with suspicious values were excluded. Data were entered in Microsoft Excel 2003 for descriptive study. The breeds were adjusted to 4 for statistical convenience. The diseased cattle were divided into following categories:

(a) Age group: (1) A1: Day old to 2 year (2) A2: 2-5 years (3) A3: 5-10 years (4) A4: >10 years

(b) Genotype group: (1) Local/ Indigenous - L (2) Local x Friesian - LF (3) Sahiwal x Friesian - SLF (4) Miscellaneous - Misc.

(c) Season: (1) Summer (March to May) – Sm (2) Rainy (June to October) – Rn (3) Winter (November to February) – Wi

RESULTS

In total 74 diseases or disorders in cattle were identified from CCBDF disease register. The overall incidence of diseased cattle have been summarized and presented in table-1. It is evident from table-2 that among 74 types of diseases, 10 diseases were most prevalent viz. enteritis (8.57%), debility (8.41%), metritis (5.99%), retention of placenta (5.68%), wound (5.36%), mastitis (5.31%), pneumonia (4.10%), indigestion (3.73%), fever (3.73%), and foot rot (3.57%).

Table 1: Overall Statistics of Diseased Cattle

Categories	**No. of case attended**	**Percentage (%)**
Male	319	16.8
Female	1584	**83.2**
Local (L)	171	9.0
Local x Friesian (LF)	617	32.4
Sahiwal x Friesian (SLF)	298	15.7
Miscellaneous/ Mixed	817	**42.9**
2 Year (A_1)	524	27.5
2 - 5 Years (A_2)	514	27.0
5 – 10 Years (A_3)	711	**37.4**
>10 Years (A_4)	154	8.1
Summer (March to May)	390	20.5
Rainy (June to October)	956	**50.2**
Winter (November to February)	557	29.3
Diseases of Gastrointestinal system	324	**17.0**
Diseases of Musculoskeletal system	133	7.0
Skin diseases	293	15.4
Diseases of Respiratory system	94	4.9
Diseases of Reproductive system	569	**29.9**
Deficiency and Metabolic diseases	173	9.1
Diseases of Eye and Ear	31	1.6
Infectious diseases	207	10.9
Miscellaneous diseases	79	4.2
Total Number of cases	**1903**	

Table 2: Occurrence of diseases and disorders in cattle affecting different systems/ organs

System/ organ	Name of diseases	Case attended	%	Age				Breed				Season				
				A1	A2	A3	A4	L	LF	SLF	Misc	Sm	Rn	Wi	Total	Percentage
Diseases of Gastrointestinal system	Stomatitis	8	0.42	1	2	5	0	0	2	2	4	0	1	7	324	17.03
	Indigestion	**71**	**3.73**	11	**37**	20	3	8	16	21	**26**	14	**28**	**29**		
	Tympany	47	2.47	5	24	10	8	6	13	8	20	5	**33**	9		
	Enteritis	**163**	**8.57**	**143**	6	14	0	0	**82**	31	**50**	18	**115**	30		
	Dysentery	11	0.58	9	2	0	0	0	5	2	4	3	6	2		
	Worm infestation	24	1.26	7	8	9	0	0	12	3	9	9	11	4		
Diseases of Musculo-Skeletal System	Arthritis	43	2.26	8	5	27	3	1	16	8	18	10	19	14	133	6.99
	Lameness	57	3.00	**23**	3	**29**	2	**2**	**25**	14	16	6	**38**	13		
	Hoof infection	1	0.05	0	0	1	0	0	0	0	1	1	0	0		
	Broken horn	24	1.26	2	5	15	2	2	11	1	10	9	11	4		
	Horn lengthening	1	0.05	0	0	1	0	0	0	0	1	0	1	0		
	Infection of Horn	1	0.05	0	0	1	0	0	0	0	1	0	0	1		
	Tail gangrene	5	0.26	0	2	3	0	1	1	0	3	3	2	0		
	Yolk gall	1	0.05	0	0	0	1	1	0	0	0	0	1	0		
Skin diseases	Abscess	35	1.84	**19**	5	9	2	0	10	11	14	8	15	12	293	15.40
	Carcinoma at gluteal region	1	0.05	0	1	0	0	0	0	0	1	0	1	0		
	Allergic dermatitis	32	1.68	6	3	**20**	3	1	17	0	14	13	11	8		
	Hump sore	32	1.68	0	**13**	14	5	**12**	**11**	2	7	**16**	**16**	0		
	Subcutaneous edema	2	0.11	0	1	1	0	0	1	0	1	1	1	0		
	Edema	6	0.32	3	0	3	0	0	1	1	4	0	5	1		
	Udder edema	15	0.79	0	3	11	1	2	1	0	12	3	6	6		
	Udder impetigo	20	1.05	0	4	14	2	**7**	3	1	9	0	**20**	0		
	Udder wound	11	0.58	0	3	6	2	4	2	1	4	5	3	3		
	Tail wound	15	0.79	0	4	11	0	1	5	2	7	2	8	5		
	Wound	**102**	**5.36**	34	16	30	22	6	33	23	40	16	**64**	22		
	Maggot wound	22	1.16	6	3	9	4	2	4	4	12	7	4	11		
Diseases of	Aspiration pneumonia	15	0.79	15	0	0	0	1	7	1	**6**	0	**15**	0	94	4.94

Respiratory System	Pneumonia	**78**	**4.10**	**62**	11	4	1	**3**	**30**	**12**	**33**	2	**53**	**23**		
	Pulmonary emphysema	1	0.05	1	0	0	0	0	0	0	1	0	0	1		
Diseases of Reproductive System	Mastitis	**101**	**5.31**	0	18	**73**	10	5	**35**	**19**	**42**	**19**	**54**	**28**	**569**	29.90
	Torsion of uterus	1	0.05	0	0	1	0	0	0	0	1	0	0	1		
	Abortion	26	1.37	0	10	14	2	**10**	4	1	**11**	4	11	11		
	Dystocia	3	0.16	0	3	0	0	0	0	1	2	0	2	1		
	Retention of Placenta	**108**	**5.68**	0	**33**	**62**	13	18	**43**	15	**32**	17	**41**	**50**		
	Prolapse of uterus	26	1.37	0	11	11	4	7	8	0	11	11	10	5		
	Postpartum bleeding	7	0.37	0	2	5	0	1	1	2	3	2	4	1		
	Postpartum debility	10	0.53	0	2	7	1	0	1	2	7	6	4	0		
	Metritis	**114**	**5.99**	0	**45**	**48**	21	21	**38**	17	**38**	**20**	**64**	**30**		
	Cervicitis	36	1.89	0	27	6	3	8	10	7	11	8	12	16		
	Pyometra	27	1.42	0	14	11	2	7	4	1	15	7	9	11		
	Cystic ovary	22	1.16	0	19	0	3	3	3	4	12	9	0	13		
	Anoestrus	46	2.42	0	**35**	9	2	3	12	6	**25**	**21**	**6**	**19**		
	Repeat breeding	11	0.58	0	8	2	1	3	0	3	5	1	2	8		
	Vaginitis	21	1.10	0	13	6	2	3	5	2	11	1	8	12		
	Vulvitis	2	0.11	0	2	0	0	1	0	0	1	1	0	1		
	Premature birth	1	0.05	1	0	0	0	0	0	0	1	0	1	0		
	Stillbirth	1	0.05	0	0	0	1	0	1	0	0	0	1	0		
	Orchitis	2	0.11	0	2	0	0	0	0	0	2	0	2	0		
	Posthitis	4	0.21	1	3	0	0	0	1	1	2	1	3	0		
Deficiency and Metabolic diseases	Debility	**160**	**8.41**	**70**	23	**60**	7	6	42	17	**95**	19	**111**	**30**	173	9.09
	Grass tetany	3	0.16	2	1	0	0	1	1	0	1	1	0	2		
	Milk agalactia	3	0.16	0	3	0	0	0	0	0	3	0	1	2		
	Milk fever	7	0.37	0	1	3	3	1	3	0	3	1	1	5		
Diseases of Eye and ear	Conjunctivitis	25	1.31	16	3	4	2	1	7	4	13	4	9	12	31	1.63
	Otitis	6	0.32	3	1	**2**	0	1	3	1	1	3	2	1		
Infectious diseases	Actinomycosis	2	0.11	1	0	0	1	0	2	0	0	0	2	0	207	10.88
	Brucellosis	1	0.05	0	0	1	0	1	0	0	0	0	1	0		
	Tuberculosis	3	0.16	0	1	2	0	0	**1**	0	**2**	1	**2**	0		

	Foot rot	**68**	**3.57**	5	12	**43**	8	**4**	**18**	**15**	**31**	16	**38**	14		
	Calf scour	1	0.05	1	0	0	0	0	0	0	1	0	1	0		
	Joint ill	14	0.74	**11**	2	1	0	2	4	4	4	2	**7**	5		
	Navel ill	9	0.47	**9**	0	0	0	0	0	0	**9**	1	**8**	0		
	FMD	45	2.36	5	**24**	**16**	0	0	**13**	0	**32**	0	0	**45**		
	Cowpox	11	0.58	0	1	10	0	0	3	3	5	0	**9**	2		
	Ephemeral fever	38	2.00	10	**16**	9	3	1	**11**	**10**	**16**	**34**	3	1		
	Papillomatosis	1	0.05	1	0	0	0	0	1	0	0	1	0	0		
	Dermatophytosis	7	0.37	0	0	7	0	0	3	1	3	1	0	6		
	Babesiosis	5	0.26	1	1	3	0	0	2	1	2	4	0	1		
	Coccidiosis	2	0.11	0	2	0	0	0	0	0	2	2	0	0		
Miscellaneous diseases	Fever	**71**	**3.73**	29	15	24	3	3	**28**	13	**27**	20	**32**	19	79	4.15
	Subnormal temperature	2	0.11	0	0	2	0	0	0	0	2	0	2	0		
	Trauma	3	0.16	1	0	1	1	0	1	0	2	0	3	0		
	Poisoning	1	0.05	0	0	1	0	0	0	0	1	0	1	0		
	Developmental anomalies	2	0.11	2	0	0	0	0	0	0	2	1	1	0		
Total	**74**	**1903**	**100**	**524**	**514**	**711**	**154**	**171**	**617**	**298**	**817**	**390**	**956**	**557**	**1903**	**100.00**

DISCUSSION

Among various groups/ systems of diseases those affecting reproductive system constituted highest occurrence (29.9%). This might be due to dairy herds where reproductive problems were predominant.

In different breed groups, miscellaneous breed (42.9%) was found to be more susceptible to different diseases and local/ indigenous (9.0%) was found less susceptible. The miscellaneous breed group comprised of more types of mixed breeds. Less susceptibility of Local/ indigenous breed might be because of having natural immunity against many diseases or deformity.

Regarding the seasonal trends of diseases, the highest proportion (50.2%) was encountered in rainy season (June to October) because in this season there was inadequate care like grazing and sanitation, and this season consists of five months also.

Regarding age, enteritis was found to be higher A1 (up to two years of age) and rainy season because more calves depended upon milk, milk replacer, and having incapability to digest all kinds of food materials. Overfeeding, sudden change in diet, parasitism, infections and difficult to maintain hygienic condition in rainy season might be the cause.

Debility was seemed to be high in age group A_1 (younger) and A_3 (5-10 years); and was highest in rainy season. Lack of vegetation, malnutrition and other diseases might be the cause of debility.

Lameness was found to be 3.0% in the present study which was very low rate compared with 47.13% reported by Kotresh *et al* (2000). Lameness was high in age group A_3 and A_1. Prevalence of lameness was higher in both LF genotype and rainy season. Rainy season, slippery floor, confinement area might act as predisposing factor. Post complication of parturition might be the cause of lameness in old animals.

The prevalence rate of hump sore was 1.68% in the present study, but higher rate 14.6% was reported by Nooruddin and Dey (1990). Regarding skin diseases, hump sore was found to be higher in age group A_2 and A_3. Prevalence was higher in local and LF genotype, and was higher in summer and rainy season. It might be due to poor farm environment condition or very wet environment.

The prevalence rate of pneumonia (4.1%) in present study was nearly similar to the rate of disease 7% reported by Haque and Samad (1996). Pneumonia was found high both in age group A_1 and rainy season. Younger age, inadequate housing for newborn calves, wet condition in rainy season, climatic change might be the cause for pneumonia because of lack of care.

The prevalence of mastitis was 5.31%. The higher rates 18.6% (Prodhan *et al.*, 1996) and 18.5% (Rahman *et al.*, 1997) had been recorded previously. Mastitis was found to be highest in age group A_3 (5-10 years age) in present study, was supported by Erb and Martin (1980). Higher prevalence of mastitis in miscellaneous breed in current study was resembled with the opinion recorded by Rahman *et al* (1997). Mastitis was found more in rainy season in this study

which was fully supported by Singh *et al* (1996). Local/ indigenous cows had low percentage of mastitis in this study because of having natural resistance against infections/ diseases comparatively.

The prevalence of metritis was 5.99%. Near about similar rates of the prevalence of disease 8.2% (Shamsuddin *et al.,* 1988), much lower rate 0.1% (Chourewar *et al*., 2002), and much higher rate 36% (Markusfeld 1987) were reported by other authors. Metritis was found to be higher in A_3 and A_2 age group, higher in miscellaneous and LF genotype. Rainy season was more favorable for metritis which was coincided with previous study reported by Markusfeld (1984).

The prevalence rate of Retention of Placenta was 5.68%. Similar rate of prevalence of retention of placenta 2-17.8% (Stevenson and Call 1988), and 8.4% (Shukla *et al.,* 1980) were reported. Retention of placenta was found to be highest in age group A_3, was supported by Curtis *et al* (1985) and Alam *et al* (1996). Prevalence of retention of placenta was more in LF and Miscellaneous genotype, was supported by Samad *et al* (1989). Higher prevalence of retention of placenta in winter season in current study was similar to opinion reported by Etherington *et al* (1984). It is possibly due to fatness of cows, nutritional deficiency especially Selenium and Vitamin E, and heavy grain feeding prior to parturition.

The prevalence rate of abortion 1.37% was fully coincided with Chourewar *et al* (2002). Abortion was high in A_3 age group, in miscellaneous and in local breed. Higher prevalence of abortion in rainy and winter season was supported by Markusfeld-Nir (1997). Abortion might be due to faulty management of pregnant cows, slippery floor, unhygienic environment of farm and other infectious diseases.

Anoestrus was found to be highest both in age group A_2 and summer season, and was lowest in local breed. Anoestrus might be resulted from certain pathological conditions for examples ovarian cysts, pyometra and malnutrition etc.

Among bacterial diseases foot rot seemed to be increased according to increasing the age (high in A_3). Higher prevalence of foot rot was found in rainy season and lower in local breed. It might be due to unhygienic condition, muddy nature soil in rainy season.

Joint ill and navel ill were seen more common in age groupA_1 (upto2 years). It might be due to unhygienic condition which favor in the farm.

Among viral diseases FMD was found to be high in A_2 age group. FMD was found high in Miscellaneous and then in LF breed group. Prevalence of FMD was in winter season in the present study, was supported by Chakraborty *et al* (1979). It might be due to movement of cattle for different purposes.

The prevalence rate of Ephemeral fever 2.0% in the present study was nearly resembled with 2.6% reported by Yeruham *et al* (2007). Ephemeral fever was found to be high in A_2 age group and lowest in A_4 age group. Higher prevalence was found in miscellaneous and LF breed cattle

and was high in summer season. This might be due to closeness and contagious nature of the disease.

CONCLUSION AND RECOMMENDATON

Based on the results of retrospective epidemiologic study of cattle diseases of Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka, the following conclusions were made:

1. Most important ten diseases of CCBDF are enteritis, debility, metritis, retention of placenta, wound, mastitis, pneumonia, indigestion, fever, and foot rot.
2. Occurrences of the diseases were higher in cross breed cattle (miscellaneous and Local x Friesian cross).
3. Cows aged between 5-10 years were more likely to be affected by the diseases.
4. Most of the diseases were reported in rainy season.
5. Reproductive diseases were found more because of dairy farm.
6. Retrospective epidemiologic analysis of diseases for a period of 10 years or more would help to identify risk factors of diseases.
7. Identification of risk factors of diseases would help to initiate efficient control program.

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STUDIES ON NORMAL FERTILITY INDICES AND FACTORS ASSOCIATED WITH BOVINE INFERTILITY IN THE HILLS OF NEPAL

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ABSTRACT

Infertility has been regarded as an important problem of cattle and buffaloes in Nepal, but its actual extent at present is yet unknown under the undefined existing management system. Hence as it is important to define the normal indices of the national herd, some attempts have been made in present study to define these indices for western hill districts. In general, indigenous cattle had the poorest fertility status than the exotic crossbred cattle or buffaloes. The average age at first mating, age at first calving, calving interval and calving to conception period in indigenous cattle were 46, 58, 21.5 and 11 months, respectively, while the respective figures for exotic crossbred cattle were 26.7, 43.5, 13.7 and 6.7 months. Similarly in indigenous buffaloes the age at first mating, age at first calving, calving interval and calving to conception period were 46, 55, 17 and 6.4 months, respectively, while respective figures for the crossbred buffaloes were 44.6, 56.4, 17.7 and 6.5 months. These figures were not significantly different between the buffalo breeds. The distribution pattern of cattle and buffaloes on these parameters indicates that 25% cattle and 10% buffalo population are beyond the normal range of fertility parameters which need urgent attention for improving their fertility status. The problems of repeat breeding (3% and 5.4% in cattle and buffaloes); abortion (less than 1% in both cattle and buffaloes), dystocia (less than 1% in cattle and buffaloes) and other abnormalities (e.g. metritis) were of lesser significance in the population. The contributing factors for the fertility are found to be management and particularly the nutritional management of the animals as well as breed characteristics. Diseases and other clinical problems although are significant for individual farmers and animals, were found to affect the overall fertility at relatively lesser extent. The prevalence of Brucellosis and Leptospirosis were 3 and 8.5% of the total sample tested from animals identified with infertility problem. Response to nutritional supplementation to the identified infertile or sub fertile animals was positive (50% individual successfully conceived), though the results were inconclusive and require further in-depth investigation. The study indicates the need to define the normal fertility indices for our condition and to assess the overall fertility status of the national herd, so that the problem could be dealt in a broader context. It is also important to identify the major factors of infertility and address them accordingly.

INTRODUCTION

Bovine infertility has been defined as a degree of reduced fertility, which results in failure to produce or delay in producing the annual live calf (Arthur *et al*., 1989). In the western countries, the fertility indices of the dairy animals are well defined and dairy husbandry is managed to achieve those targets. Animals falling below those targets are regarded to be with infertility problems and culled or treated to alleviate the problem. Since pregnancy and parturition are necessary for the initiation and maintenance of lactation in the species infertility

problem in a dairy herd affects the efficiency of the system and increases the cost of milk production.

Bovine infertility has been regarded as one of the important problems of dairy animals in Nepal. The significance of this problem has been increased in recent years with the beginning of commercial dairy farming and an increase in population of high yielding dairy cattle around the peri-urban areas of the country. However, the actual status of infertility in the national bovine herd is as yet unknown. Although infertility of dairy cattle in the peri urban areas indicates the problem but it does not represent the actual national situation. The problem is more complicated as the normal fertility indices of farm animals of different breeds under different environments and management systems have not yet been defined and the actual proportion of infertile or sub fertile animals are unidentified. Hence, it is important to define the normal fertility indices of the national herd, so that the animals falling below this level could be corrected and the population with high fertility status could be utilized for selection and improvement.

Bovine infertility is caused by many factors, which includes infection of reproductive tracts, hormonal imbalance, poor health and inadequate supply of micronutrients (Arthur *et al.,* 1989). As the factors responsible for infertility are multifold, it is important to understand the problem in the overall population and identify the causative factors associated with this problem. Infertility in bovines may be manifested in terms of delayed puberty and first calving, long calving intervals, low pregnancy rates, low conception rates, high rates of abortion, dystocia and low rates of giving birth to healthy calves or failure to produce annual live calves (Arthur *et al*., 1989).

A study conducted in different villages of western hill districts showed that 17% cattle and 20% buffaloes are with reproductive disorder or infertility problems (Rasali *et al*., 1998). In addition, high abortion rate has also been recorded in some pockets of these districts (LAC, 1985). The reports from veterinary hospitals and clinicians in other parts of the country also indicate that the problem is widespread but has been highlighted more in areas with greater concentration of exotic or exotic cross bred animals.

At the national level, some attempts have been made to alleviate the problem through organization of treatment camps and the results so far from these camps have been encouraging. It must however, be emphasized that these campaigns are focussed to treat the animals identified as infertile by the farmers. In this situation, most of the attention is given to the high yielding animals, which actually comprises a very small proportion of total population of lactating animals in the country. There has been some attempts even on unconventional approach as moxo-cautarisation to alleviate the problem (Kafle, 2001) and investigations have also been initiated to identify the causative factors associated with infertility (Dyson *et al*., 2000).

As fertility of the animals will be reflected on the management of the animals, it would be necessary to identify the normal fertility parameters of national herd, because the fertility of the animals reared in the difficult hill management cannot be compared for European management conditions. Hence, it is important to define the normal fertility indices of the national herd as

well as for the concerned environment so that the animals below this status could be identified and corrected and the most efficient animals could be identified and selected from the population.

It is well documented that infections of reproductive tracts, hormonal imbalance, poor health and inadequate supply of micronutrients are the major causes of bovine infertility (Arthur *et al.*, 1989). It is thus important to investigate the role of these factors on bovine infertility under our conditions, so that their relative importance could be assessed and appropriate strategies could be adopted accordingly. Hence, in the present investigation, the roles of two important diseases associated with bovine fertility were evaluated by screening the prevalence of Brucellosis and Leptospirosis in the animals identified as infertile. In addition, the responses of energy/protein and mineral supplementation was assessed in infertile buffaloes and cows at Taranagar village of Gorkha district and existing fertility status of cattle and buffaloes was evaluated by conducting village surveys on the fertility status of cattle and buffaloes in the western hill districts of the country.

MATERIAL AND METHODS

Village Surveys

Determination of the existing fertility status of bovines in the villages was carried out by survey using structured questionnaire in the villages of various districts of Western Development Region. Individual households of the villages were visited to collect the retrospective fertility data on individual buffaloes and cows reared by the farmers. The body condition of the animals at the time of the visit was also assessed and recorded. Thus, the fertility data of 1504 animals from 13 sites were recorded and analyzed for assessing the fertility status of cattle and buffaloes in the hill villages of western Nepal. SPSS computer software package was used for determining the frequencies and percentile distribution of various fertility indices, while General Linear Model Procedure in SAS package was used for determining the effects of different factors on those fertility indices.

Screening for Brucellosis

A total of 200 serum samples were collected from animals suffering with various infertility problems from different areas (Table 1). The samples were labelled and stored at –20°c until the examination.

Table 1 Sampling area and number of samples collected

Area	Total number of animals		Number of serum samples collected
	Cows	Buffaloes	
Kaski	26	28	54
Gorkha	42	6	48
Syangja	18	17	35
Parbat	0	25	25
Rupandehi	16	0	16
Tanhun	2	9	11
Kapilbastu	0	6	6
IAAS farm, Chitwan	5	0	5
Total	109	91	200

Screening procedure

Two methods, Rose Bengal Plate Agglutination Test (RBPT) and Enzyme Linked Immunosorbent Assay (ELISA) described by Alton (1990) and Carrus and Hum (1998) respectively were adopted to screen the samples. Universal laboratory safety precautions were employed for handling the samples, antigen and equipment used.

Rose Bengal Plate Agglutination Test (RBPT)

Antigen used in RBPT was obtained from the Central Veterinary Laboratory, Weybridge, UK. Antigen and serum samples were kept at room temperature for half an hour before use and 25µl of the test serum were placed at proper place in the agglutination test plate. Equal volume of antigen was added in the test sample and the plate was mixed gently by swirling motion of hand for 4 minutes. Any amount of agglutination present in the sample was considered as positive.

Enzyme Linked Immunosorbant Assay (ELISA)

Antigen, antisera, and assay procedure used in the assay were obtained from Regional Veterinary Laboratory, Elizabeth Macarthur Agricultural Institute, New South Wales, Australia. ELISA was performed as follows:

- *B. abortus* freeze dried antigen JR/2 was reconstituted in sterile phosphate buffer saline and diluted to 1: 7000 dilution.
- Then 100µl of the diluted antigen was transferred in each well of the Linbrow micro titre plate. The plate was wrapped and incubated over night at 4°c.
- Forty test samples were tested in one plate.
- Test serum samples diluted @1/200 in diluting buffer were used in the assay on each plate using rows A –H wells in 1–10 columns.
- Positive control sera diluted @1/5000 and negative control serum diluted @ 1/200 in diluting buffer were used in the assay on each plate using column 11 and 12 respectively.
- Antigen coated plates were washed 5 times with washing buffer immediately prior to the addition of serum. Excess washing buffer was removed by tapping plates in absorbent pad.
- Then 100µl sample and controls were added to the above as described positions in the plate. Microtiter plate was covered by lid and incubated at 37°c for 45 minutes on a plate shaker.

- Test plate was washed 5 times in washing buffer as described above.
- Diluted conjugate (1/ 4000 dilution) was added @ 100µl per well and incubated at 37°c for 1 hour without shaking.
- Test plate was washed 5 times in washing buffer as described above.
- ABTS Substrate was added @ 100µl in each well. Plate was covered by lid and shaken on a plate shaker until the optical density reached to 1.2 for the strong positive and 0.2 for negative control.
- The measurements of antibodies in the test serum in the ELISA were calculated in comparison to strong positive and negative controls. Following formula was used for this calculation.

$$\text{ELISA Unit} = \frac{\text{Mean OD sample - Mean OD negative control}}{\text{Mean OD of strong positive - Mean OD negative control}} \times 100$$

- >50 ELISA units were considered as positive.

Screening for Leptospirosis

Microscopic Agglutination Test (MAT) as described by Norris (1998) was used to screen the samples. Test serum sample were diluted in normal saline at dilution of 1: 25. Positive and negative control sera were also diluted similarly. A 25µl volume of diluted and well-mixed test samples was transferred to appropriate wells of micro titer plate and equal volume of well-mixed antigen was added in each well. Similarly, positive and negative control test sera were also prepared in appropriate wells for comparing the agglutination produced by the test samples in the MAT. The initial dilution of the test and control serum was 1:25 and with the addition of antigen @ 25µl in each diluted serum in the microtiter plate, final dilution of 1:50 was obtained. The plate was covered by plate lid and the contents of each well were mixed by micro plate shaker. Test plate was incubated at 30°c for 90 minutes. Then each well of the plate was read under dark ground microscope to detect the agglutination. Leptospira hardjo (bovis) formalinised killed antigen, positive and negative control sera used in the study were obtained from Central Veterinary Laboratory, Weybridge, UK. Agglutinated mass equal to or not less than 50% as compared to the mass observed in positive control well was considered positive for Leptospira antibodies.

Evaluation of supplementary nutrition on bovine infertility

This study was conducted at Taranagar village of Gorkha district. At the beginning of the study, the total breedable population of cows and buffaloes in the village was recorded by door-to-door survey of the village and the number of animals with poor reproductive performance were identified. It was found that about 53% of the animals in the village have poor fertility mainly due to anoestrus/sub estrus and repeat breeding problems. Among the identified animals, 71 animals identified with anoestrus/ sub estrus problem were divided in to the following groups:

- Animals (n = 18) supplemented with concentrate feed @ 1.5 kg/cow per day and @ 3.0 kg/buffalo/day for 45 days (n = 18).
- Animals supplemented with concentrate feed @ 1.5 kg/cow per day + mineral mixture (Agrimin:Glaxo India) @ 30 gm/day and concentrate feed @ 3.0 kg/buffalo/day + mineral mixture (Agrimin: Glaxo India) @ 30 gm/day for 45 days -18 animals.

- Animals (n = 17) supplemented with concentrate feed @ 1.5 kg/cow per day + mineral mixture (Agrimin: Glaxo India) @ 30 gm/day + anthelmintics (Fasinex @ 12 mg/kg body weight once) and concentrate feed @ 3.0 kg/buffalo/day and mineral mixture (Agrimin:Glaxo India) @ 30 gm/day for 45 days + anthelmintics (Oxyclozanide @ 10 mg/kg body weight once).
- Animals (n = 18) without any supplementation except (Vitamin B complex @ 10 ml/day as placebo) - 18 animals.

Numbers of animals displaying oestrus symptoms within 45 days after the termination of supplementation were recorded for data analysis. Similarly, numbers of animals conceived were also recorded.

RESULTS

Fertility Status of cattle and buffaloes in the western hills of Nepal

Age at First Mating in cattle and buffaloes

The average age at first mating of the cattle was found to be 46.3±15.6 months. The age at first mating was significantly different (<0.001) between the breeds (Table 2). The crossbred (Jersey x local and Holstein x local) were found to mature earlier (26.7±2.1 months) than the local cattle (49.2±1.2 months).

Table 2 Summary of some of the reproductive parameters of cattle

SN	Factors	Age at first mating (months)	Age at first calving (months)	Calving interval (months)	Calving to mating period (months)
	Overall mean	46.3±15.6 (156)	57.8±16.5 (59)	21.5±9.22 (118)	10.97±8.4 (162)
1	Breed				
	Local	49.2±1.2 (135)	58.9±2.24 (55)	22.0±0.9 (110)	11.6±0.7 (140)
	Exotic/cross	26.7±2.1 (20)	43.5±1.5 (4)	13.7±1.5 (7)	6.7±1.3 (21)
	P Value	0.0001	0.06	0.019	0.012
2	Management System				
	Stall feeding	42.8±1.7 (80)	49.5±2.6 (14)	19.2±0.7 (66)	9.0±0.6 (97)
	Grazing	50.0±1.8 (75)	60.4±2.6 (45)	24.5±1.6 (51)	13.8±1.3 (64)
	p Value	0.11	0.43	0.11	0.045

Note: Numbers in parenthesis are number of observations; overall mean ± SD; Factor mean ± SE

The age at first mating was not significantly affected by the management system (p>0.05). However, cattle kept under stall-feeding management matured earlier (42.8±1.7 months) than the animals kept under free grazing system (50.0±1.8 months). Within the indigenous cattle, the age at first mating was 48 months in stall fed management and 50 months in grazing management system (Table 3).

Table 3: Average age (months) at first mating of cattle under different management system

S.N.	Breed	Management System	
		Stall Feeding	Grazing
1	Local	48	50
2	Exotic/ Cross	26.7	-

The average age at first mating in buffaloes was observed to be 45.8±11.9 months (Table 4). There was no significant difference between breeds (Local vs. Murrah cross) for this reproductive trait ($p>0.05$). Indigenous buffaloes mature at the age of 46 months while the exotic or crossbred mature at the age of 44.5 months (age at first mating).

The management system had no significant effect on the age at first mating of the buffaloes, however, buffaloes kept under stall feeding system mated slightly earlier (45.5 months) than those kept under free grazing system (47.4 months). There was a significant interaction between breed and management systems ($p<0.0001$). The age at first mating in indigenous buffaloes was 46 months in both stall-fed and grazing managements whereas the age at first mating in the crossbred or exotic buffaloes was 43 months under stall fed management and 69 months under grazing management (Table 5).

Table 4: Summary of some of the reproductive parameters of buffaloes

SN	Factors	Age at first mating (months)	Age at first calving (months)	Calving interval (months)	Calving to mating period (months)
	Overall mean	45.8±11.9 (649)	55.0±13 (241)	17.0±5.4 (792)	6.4±5.4 (1076)
1	Breed				
	Local	45.9±0.5 (564)	54.8±0.9 (199)	16.9±0.2 (725)	6.4±0.2 (978)
	Exotic/cross	44.6±1.5 (85)	56.4±2.5 (42)	17.7±0.4 (67)	6.5±0.5 (98)
	Significance	NS	NS (0.439)	NS (0.233)	NS (0.807)
2	Management System				
	Stall feeding	45.5±0.5 (565)	53.8±0.8 (205)	16.9±0.2 (679)	6.4±0.2 (956)
	Grazing	47.4±1.5 (84)	61.9±2.9 (36)	17.6±0.5 (112)	6.8±0.5 (119)
	Significance	NS	** (0.005)	NS (0.703)	NS (0.746)
3	Interactions				
	Breed*Mgt Sys	0.0001	0.0075	NS	

Table 5: The average age (months) at first mating in buffaloes under different management system

SN	Breed	Management System	
		Stall feeding	Grazing
1	Local	45.9	46.0
2	Exotic/ Cross	43.0	69.6

The survey revealed that, only 5% of the indigenous cattle population mated earlier than 3 years of age and 5% of the local cattle mated as late as 6 years or more. Similarly, 25% of the indigenous population was found to mature at the age of 5 years or more. Whereas, in case of exotic/ crossbred cattle, 5% population were found to show oestrous at an early age of one year and only 5% of the exotic or crossbred population were found to mature as late as 4 years.

The frequency distribution pattern of age at first mating in buffaloes revealed that, only 5% of the indigenous buffaloes mature as early as 3 years of age while 75% of the population had their first mating within 4 years of age. The remaining 25% of indigenous buffaloes had their first mating after 4 years of age. Similarly, 5% of the exotic buffalo population had first mating within 25 months of age and 25% of the population after the age of 4 years. During the survey, it was also revealed that 95% of the farmers considered the age at first service in their cattle were normal and only 5% considered it as late. While in buffaloes, 97% of the farmers believed that their animals mature at normal age and only 3% of farmers believed that the maturity in their buffaloes is late.

Mating season

Cattle were found to come in heat throughout the year. No uniform pattern of oestrus season was observed in cattle. Comparatively high proportions of cattle were found to come in heat during the month of October/November (13.6%), May/June (12.1%) and November/December (11.4%). Mating season in buffaloes was mostly concentrated between August and December, i.e. 80% of mating occurring during this period.

Age at First Calving in cattle and buffaloes

The average age at first calving in cattle was found to be 57.8±16.5 months and the effect of breed was not significant ($p>0.05$) for this reproductive trait. The average age at first calving for local cattle was 58.9±2.2 months while that of exotic cross was 43.5±1.5 months (Table 2). However, this difference is not significant, possibly due to the smaller sample size of exotic cross animals included in the survey.

The management system also did not have significant effect on the age at first calving ($p>0.05$). However, the cattle raised under stall-fed management calved earlier (49.5 ± 2.6 months) as compared to those raised under free grazing management system (60.4 ± 2.6 months) (Table 2). Among indigenous cattle, the average age at first calving were 51.9 months and 60.4 months in stall-fed and grazing management system, respectively (Table 6).

Table 6: Average age (months) at first calving in cattle under different management systems

SN	Breed	Management System	
		Stall feeding	**Grazing**
1	Local	51.9	60.4
2	Exotic/ Cross	43.5	-

The overall age at first calving of buffaloes in the surveyed areas was found to be 55±13 months. The age at first calving was not affected by breed ($p>0.05$), whereas, it was significantly different in different management system ($p=0.005$). Similarly, there was a significant interaction between breed and management systems ($p=0.0075$) (Table 4). The first calving in indigenous buffaloes was found to be at the age of 55 months while Murrah crossbred calved at the age of 56 months. Hence, the indigenous buffaloes are not inferior compared to Murrah crosses for this reproductive trait. Buffaloes kept under stall-feeding system had their first calving at the age of 54 months while those kept under grazing system calved at the age of 62 months (Table 4). The age at first calving of different breeds (local and crossbred) of buffaloes at different management system is presented in Table 7.

Table 7: The average age (months) at first calving in buffaloes under different management systems

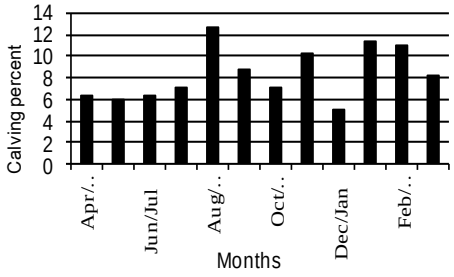
SN	Breed	Management System	
		Stall feeding	**Grazing**
1	Local	53.8	59.7
2	Exotic/ Cross	53.9	80.0

The distribution pattern of cattle population under different management systems gives a slightly different picture. Under stall fed management system, 3 quarter of the population calved for the first time within the age of 57 months, while under grazing system, the first calving age increased to 70 months for the same proportion of the cattle population.

In the survey area, 5% of the indigenous buffaloes were found to calve for the first time by the age of 40 months, while the same proportion of crossbred buffaloes had the first calving by the age of 35 months. Similarly, one fourth of the indigenous buffalo population had their first calving above the age of 59 months. There was not a marked variation in the percentile distribution between indigenous and exotic or crossbred buffaloes.

Calving Season

There was no specific calving season in cattle. Calving was spread throughout the year (Fig 1). In the survey area, the calving proportion was highest in the month of August/September (12.7) followed by January/February (11.3%), February/March (11.0%) and November/December (10.3%).



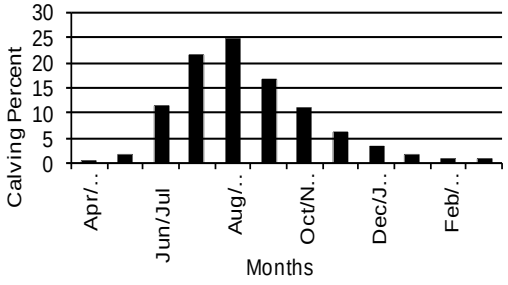


Figure 1: Calving season in cattle

Figure 2: Calving season in buffaloes

Generally, buffaloes were found to calve throughout the year (fig. 1-2). However, the calving proportion was higher during June/July (11.3%), July/August (21.4%), August/September (24.6%), September/October (16.7%) and October/November (10.9%), which accounted for about 85% of the total calving (Fig 2).

Calving Interval in cattle and buffaloes

The average calving interval in cattle was 21.5±9.22 months. The calving interval was significantly different between breeds ($p<0.05$). The indigenous cattle were found to calve once in every two years (22.0±0.9 months) while the crossbred cattle were found to calve almost every year (13.7±1.5 months), which is of significant characteristic of these animals (Table 2).

Calving intervals in cattle kept under stall-fed management was found to be 19 months, while those kept under grazing system was 24.5 months, however, this difference was statistically non significant.

The average calving interval in buffaloes in the surveyed area was 17±5.4 months. The calving interval was not significantly different between the breeds ($p>0.05$) nor there were significant differences between the management systems. The average calving intervals in indigenous buffaloes were 16.9 months whereas those for exotic or crossbred were 17.7 months. The calving interval of buffaloes kept under stall-fed system was 16.9 months whereas those under grazing system were 17.6 months (Table 4).

The frequency distribution of cattle population across the survey area revealed that only less than 5% of the indigenous cattle population calve every year while $3/4^{th}$ of the population calve at an interval of more than 2 years. While in case of exotic/crossbred cattle, none of them were found to exceed 14 months between successive calving.

The distribution pattern of calving interval in buffaloes revealed that 50% of the buffaloes calve once in a year (14 months in indigenous and 15 months in crossbred). It was also revealed that 25% of the population calve once in every two years (22 months or above).

Repeat Breeding

While considering the retrospective breeding records the repeat breeding cases were found to be 3.0% of the total recorded cases of 1122 calving. In the surveyed areas, the repeat breeding

cases were higher in buffaloes than in cattle. Out of total 4527 retrospective calving records in buffaloes, a total of 5.4% repeat breeding cases were observed. A few individuals were found to be the chronic repeat breeder (repeated for 4 or more than four times).

Abortion

The proportion of abortion both in cattle and buffaloes were generally low in the western hills. Among the 1122 recorded calving in cattle, abortion for once was recorded in 0.7% and thrice in 0.1% of the cases. Similarly, in buffaloes, a total of 27 abortion cases for once and 1 case for thrice were recorded out of total 4527 retrospective calving records. Thus, abortion and associated diseases were also not a major infertility problem in cattle and buffaloes in the western hills.

Dystocia and other problems associated with calving

The dystocia cases recorded in the surveyed cattle and buffalo population were also few and not considered as a major reproductive problem. Only 2 cases (0.3%) in cattle and 7 cases (0.1%) in buffaloes had dystocia in their lifetime. Similarly, vaginal discharge was also not recorded as a major problem in these animals. Only one cattle and 0.4% of buffaloes were found with vaginal discharge in the survey.

Calving to conception interval

The average calving to conception interval in cattle was 11±8.4 months, which was significantly different between breeds ($p=0.012$) and between management systems ($p=0.045$). On average, indigenous cattle were conceived 11.6 months after calving, while crossbred cattle conceived 6.7 months after calving. Similarly, the animals raised under stall-feeding management system were found to be conceived earlier (9 months) than those kept under free grazing system (13.8 months) after calving. The average calving to conception period in buffaloes was found to be 6.4 months, which was not significantly different between breeds ($p=0.801$) and management systems (0.746). For indigenous and crossbred buffaloes, the calving to conception period was 6.4 and 6.5 months respectively. Similarly, calving to conception period in buffaloes kept under stall-fed management system was found to be 6.4 months while for those kept under grazing system was 6.8 months.

Half of the indigenous cattle population were found to be conceived within 11 months after calving, while same proportion of the crossbred cattle population were conceived within 4 months of calving. Similarly in buffaloes, half of both indigenous and crossbred populations were conceived within 4 months of calving, while 25% of the population conceived after one year of calving.

Body Condition

Majority (90%) of the cattle observed during the survey were found to be in the poor body condition. This indicates the poor feeding management of these animals leading to poor reproductive performances. The body condition of the surveyed buffaloes was comparatively better than cattle and reflects the higher importance given to buffaloes than to the local cattle by the farmers.

Screening for diseases associated with bovine infertility

Screening of brucellosis in infertile animals

The prevalence of brucellosis was screened by RBPT and Enzyme Linked Immunosorbent Assay (ELISA) in the identified infertile animals. Among the 91 buffaloes tested for Brucellosis, no cases were recorded by both tests. In cattle, (109 samples), RBPT and ELISA tests detected the prevalence of Brucellosis as 4.6% and 5.5%, respectively indicating the higher detection rate by ELISA than RBPT. Therefore, ELISA results were taken as standard for determining the positive percentage of animal in the present study. In the total samples tested (from buffaloes and cattle), the overall prevalence of *B*rucellosis was found to be 3.0 % of the total screened serum samples. Brucellosis was recorded only in the samples at IAAS farm and in Syangja district and all other samples were free from this disease.

Among the different infertility problems, the significance of brucellosis was found to be important only in cattle abortion, in which 46% of the aborted cases had antibodies to *B. abortus*. No other infertility problem had *Brucella* antibodies in the tested serum samples.

Screening of Leptospirosis in infertile animals

Presence of *Leptospira hardjo* antibodies in MAT results of infertile cows and buffaloes in different areas showed that overall presence of *L. hardjo* antibodies was about 8. 5% of the total tested serum samples. The prevalence was higher in cows (11.0%) than in buffaloes (5.5%). Antibodies to *L. hardjo* were detected in samples from Gorkha, Kaski, Kapilbastu, Rupendehi and Syangja but not from Parbat, Tanahun and Rampur farm. Highest prevalence (5% of the total samples and 21% of the area samples) was recorded in Gorkha district followed by Kapilbastu and Kaski districts.

The presence of *L. hardjo* antibodies in the tested samples indicates that leptospirosis could also be a contributing agent in bovine infertility problem. Analysis on the presence of *L hardjo* antibodies in animals with various infertility problems revealed that *L hardjo* antibodies were present in both cows and buffaloes with repeat breeding, abortion and anoestrous conditions.

Table 8: Prevalence of *L. hardjo a*ntibodies in different infertility problems

Problems	Cows	Percent positive to *L. Hardjo*	Buffaloes	Percent positive to *L. hardjo*	Total serum samples	Overall positive percent
Anoestrus	30	6 (20%)	18	0	48	12.5
Abortion	13	0	13	2 (15.4%)	26	7.7
Repeat breeders	36	4 (11.1%)	6	3 (50%)	42	16.6
Others	30	2 (6.6%)	54	0	84	2.4
Total	43	12	60	5	200	8.5

Responses to supplementary nutrition on bovine infertility

Supplementation of concentrate feed, minerals and anthelmintic treatment against fasciolosis were effective in alleviating infertility problems of native cattle and buffaloes as about 50% of the supplemented animals responded positively to supplementation. However, the differences between the groups were non-significant as about 44 percent of the animals under placebo also

displayed the oestrus (Table 9). Of the total animals displaying oestrous after supplementation, 78% of the individuals conceived successfully.

Table 9 Response to supplementation in infertility cases

Treatment groups	Number of animals	Number of animals displaying oestrus	Percent of animals displaying oestrus	Number of animals conceived	Percent of animals conceived
Concentrate feed	17	9	53	7	41.2
Concentrate feed + Mineral	17	6	35.3	6	35.3
Concentrate feed + Mineral +Anthelmintic	16	9	56.3	6	37.5
Vit B complex	18	8	44.4	6	33.3

No specific factor leading to infertility could be identified; however, this finding does suggest that bovine infertility is the outcome of complex interlinking factors to which role of nutrition is important.

DISCUSSION

The age at first mating, first calving, calving intervals and calving to conception period are affected by many factors as breed, management system and as these indices determine the lifetime productivity of the animals, defining and setting the targets on fertility indices is important to alleviate bovine infertility.

The overall mean age at first mating in exotic cattle was significantly less than the native cows, which might reflect the inherent genetic characteristics of exotic cattle. However, it might also suggest that exotic cattle were better cared by the farmers, which would also have contributed for reducing the age at first mating in exotic cattle. It was also observed that animals kept under stall-feeding management matured earlier than grazed animals indicating the better feeding conditions under stall feeding management contributing to earlier mating of the animals. The age at first mating of different exotic 50% crossbred (Holstein X local, Jersey X local, Brown Swiss X Local and Ayrshire X Local) on the station have been reported to be 24-28 months (Shrestha *et al*., 1996), which is similar to the present findings (26 months) on farmers' conditions.

In the present context, due to the late age at first mating in indigenous cattle, the normal fertility indices (if accepted as the mean value of the parameter in the population + 1 SD, which would include 65% population under the normal distribution), for this parameter could be regarded as 63 months (mean + 1 SD). While for exotic crossbred cattle, it could be 36 months. Thus the population above this age of first mating should be considered as the population with problem of infertility. In this study, it was also revealed that nearly 25% of both indigenous and exotic crossbred cattle were found to come under this category.

In buffaloes, there was no significant difference for age at first mating between local and Murrah cross buffaloes. The normal indices for the age at first mating in buffaloes could be

considered as 58 months under same assumption and the animals not coming in heat by this age should be regarded with infertility problem, which includes nearly 10% of the indigenous buffaloes in the western hills of Nepal. There was a significant effect of management system on this parameter for crossbred buffaloes but not in local buffaloes. This finding might reflect that the nutritional requirements of crossbred buffaloes are higher than local animals and could not be met under grazing management. Hence, better feeding management is required for crossbred buffaloes than the local buffaloes. Thus, from the population distribution, it could thus be regarded that about 25% of cattle and about 10% of the buffaloes require attention to improve their age of first mating.

In the present study, the effect of breed on age at first calving in cattle was not found to be significant even though there was substantial difference between breeds. This result contradicts the findings of Rasali *et al* (1996) in which they reported that age of first calving was significantly influenced by the genotypes. However, in the present study the number of observation available on crossbred was too small (only 4) and probably this might have disabled to detect breed effect, even though, there were differences in age at first calving between indigenous and crossbred cattle. The age at first calving of crossbred cattle in this study (43 months) were similar to those reported by Rasali *et al* (1996) for 25-49% Jersey crossbred (44.6 months) but were higher than those reported by Shrestha *et al* (1996) for various exotic 50% crossbred cattle (33-38 months)

Similarly, there was no difference between the age at first calving between the local and Murrah cross animals and the superior effect of stall fed management was clearly reflected by earlier first calving of both local and Murrah cross buffaloes under this management. However, there was no breed superiority of Murrah cross buffaloes over the local breed for this trait. The average age at first mating of indigenous buffaloes in this report is slightly higher than that reported by Rasali (1998).

While considering the normal indices for age at first calving, local cows calving within 75 months (mean+1 SD) could be regarded as normal and above this age as the problem animals. This value for the exotic cattle would be 48 months and the animals above this age should be treated as problem animals. Similarly, the normal value for the age at first calving for local buffaloes could be 67 months and for Murrah cross buffaloes 72 months under the hill environment of western Nepal.

The significantly shorter calving interval of exotic cross breed cattle than the local cattle was possibly due to the influence of breed characteristics, however, this trait might also be influenced by better feeding management of these animals. The present findings contradict with the findings of Rasali *et al* (1996) who reported that there was no significant difference between genotypes (indigenous vs. various blood level of Jersey) in the calving interval. They reported that, calving interval in indigenous cattle was 528 days, while it was 553 days for 25-49% Jersey crossbred and 513 days for 50-74% Jersey crossbred. The calving interval reported by Shrestha *et al* (1996) in 50% Holstein, 50% Jersey and 50% Ayrshire cattle were 458, 442 and 493 days respectively was similar to present findings. In buffaloes, this difference was not significant between the Murrah cross and local buffaloes; possibly because, in both breeds of

buffaloes are better cared by the farmers. The average calving interval in indigenous buffaloes in this report is slightly lower than those reported by Rasali (1998).

The normal indices for calving intervals derived from this survey, would be 31 months for local and 17 months for exotic crossbreed cattle, while this value would be 22 months for local buffaloes and 23 months for Murrah cross buffaloes. It was also revealed that about 50% buffalo population with calving interval of 12-16 months and in the rest, calving interval ranged from 16 to 36 months. Perhaps there might be farmers practice for not breeding their buffaloes early in the lactation and seasonality of heat in buffaloes prolong the calving interval for up to 2 years or more. It is also possible that silent heat in tethered buffaloes was undetected by the farmers resulting increase in calving to conception period and the calving intervals.

The calving to conception period was unusually late in local cattle but was of similar length in exotic cattle and buffaloes in both breeds. This factor was responsible to influence the whole fertility and production performance of local cattle. The unusually long calving to mating period (11 months) in local cattle indicated that these animals have to wait for the next year to come in heat after calving. Two possibilities might be responsible for this delay. The first could be the poor care and management of these animals, which inhibit the exhibition of postpartum heat in the animals. The second factor might be the inhibitory responses of prolactin, which is released during the initiation of lactation induced by suckling calves. Based on the same assumption (population mean+1SD), the normal value for this parameter (calving to conception period) would be 18 months for local and 12 months for exotic cattle and 12 months for both local and Murrah cross buffaloes. The population with longer calving to conception intervals than this would need to be categorised as infertile population.

The role of other clinical problems, e.g. abortion, repeat breeding, vaginal discharge and uterine infection were not so significant in the overall infertility problem of cattle and buffaloes. Hence although, these problems affect the individuals, their overall contribution in the population in bovine infertility problem might not be so serious. In this study, overall prevalence of Brucellosis in infertile animals was found to 3%, which is similar to that reported by Pradhan (1996) but significantly lower than 14.2% reported by Joshi (1976) in Kathmandu valley.

The role Brucellosis and Leptospirosis in the infertile population of cows and buffaloes (identified by the farmers), was 3% and 8.5% of the tested cases. *Brucella* antibodies were recorded in 46% of the aborted cows, while none of the buffaloes have *Brucella* antibodies. The distribution of *Leptospira* antibodies was in wider areas and in different reproductive problems, indicating that the infection might be widespread and attention should be given to this infection. As both these diseases are zoonotic and there are reports from the local hospitals of their human cases, control strategy of these diseases should be considered at national level.

The responses to nutrient supplementation and treatment against *Fasciola* infection showed some positive indications in alleviating the infertility problem of animals. Although the results are inconclusive in the present study, displaying of oestrus and successful conception by about 50% of the supplemented animals supports the importance of proper nutrition of animals in alleviating infertility problem of bovines. There is however a need to investigate the role of specific nutrient and/or mineral/micro mineral in causing bovine infertility in different

environment and management system of the country. Analyzing all these traits in the population, it is evident that bovine infertility is the outcome of complex factors in which feeding management, breed and health care contribute for the outcome with some role of specific clinical problems; although, effect of individual cases, would not influence the overall infertility situation significantly.

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COMPARATIVE STUDY ON GROWTH PERFORMANCE IN MALE GOATS FED WITH NORMAL AND QUALITY PROTEIN MAIZE IN WESTERN HILLS OF NEAPL

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ABSTRACT

Goat is recognized as poor man's cow in most of the countries and also major source of cash income for most poor in Nepal. Quality protein maize (QPM) is a hybrid maize that contains approximately 9-12% crude protein (CP) with two essential amino acids lysine and tryptophan, and is high in energy and low in fiber. A study was carried out in growing male Khari goats at Regional Agricultural Research Station (RARS), Lumle for 90 days to compare the growth performance of goats fed with QPM and normal maize. Twelve male goats of average 5 months old with average body weight of 9.74 kg were divided into two groups having six animals in each group by using Complete Randomized Design (CRD). Two types of concentrate mixtures were prepared for animals with 16% CP level. Goats of Treatment 1 were provided ad libitum amount of fodder and concentrate mixture prepared from QPM whereas goats of Treatment 2 received concentrate mixture made from normal maize. Goats of both groups were fed with concentrate mixture @ 1% of their body weight once daily in individual plastic vessel and fodder in ad libitum amount twice a daily. Experiment revealed that highest daily intake of fodder and concentrate mixture was observed in Treatment 1 (1.68 ± 0.31 kg and 121.4 ± 1.10g, respectively) than in Treatment 2 (1.52 ± 0.29 kg and 113.2 ± 1.40 g, respectively) which resulted in higher total dry matter intake in Treatment 1 (53.37 kg) than in Treatment 2 (48.4 kg). The feed conversion ratio (FCR) was higher for Treatment 1 (12.92:1kg) than for Treatment 2 (12.1:1 kg). Similarly, total weight gain was highest in Treatment 1 (7.19 kg) than that of Treatment 2 (7.09 kg). The fodder and concentrate mixture intake, total dry matter intake and FCR were non significant (P>0.05) between diet groups. Average daily gain of experimental animals was almost similar for both groups (45g and 44 g for Treatment 1 and 2, respectively).

Key words: Quality protein maize, goats, feeding, Nepal

INTRODUCTION

Goat is recognized as poor man's cow among marginal farmers' in most of the countries and also a major source of cash generation for most of the rural poor people in Nepal. The goat is a multipurpose animal that provides meat, fiber, hide and manure. The goat population of Nepal is estimated to be 88, 44,175 that producing 50,315 Metric tons (Mt) meat per annum. Of 88, 44,175 goats, a total of 13,36,891 (15.11% of total goat population) goats are found in western hilly districts producing 5280 Mt meat (10.49% of total goat meat production) per annum (MOAC, 2009/10). In spite of the importance of goat in the farming system, very few efforts have been made to improve their productivity. Kharel and Neopane (1997) assumed that out of

total population of goats in the country, 56.2% are Khari goats which are highly prolific with 3 kidding in two years with 59% twining ability.

The current goat meat production capacity of the country is not been able to meet the demand thus country imported live goats (3, 91,184 heads) of worth NRs 39, 19, 72,327 during the fiscal year 2009/2010 from India (MOAC, 2009/10). However, this figure does not cover the illegal entry of live animals from the free borders between Nepal and India. Similarly, on the occasion of festivals like Dashain and Tihar, Chyangra is being imported from China in huge quantity. Therefore, it can be assumed that there is immense gap of need and supply of goat meat in Nepal. Moreover, a demand driven livestock revolution is underway in Asia and it is very likely that demand for meat and other animal products may almost double by 2020. This in turn will increase demand of cereals for feeding livestock. The demand for some cereals such as maize will increase more rapidly, and will perhaps overtake demand for rice and wheat in the next two decades (Vasal, 2002).

Maize is well accepted as major feed ingredient and a primary source of energy supplement that can contribute up to 30 % protein, 60 % energy and 90 % starch in an animal's diet (Dado, 1999). About 70-80 percent of maize production is used as a feed ingredient in the world. Although normal maize contains between 8 and 9% protein, the quantity of two essential amino acids, lysine and tryptophan is negligible. QPM is a hybrid maize variety developed by CIMMYT, rich in essential amino acids like lysine and tryptophan (Prasanna *et al.*, 2001) which can reduce the dietary inclusions of protein - rich ingredients such as fish meal and synthetic lysine and thereby resulting in savings on the cost of feed and making animal products more affordable (Okai *et al*., 2005; Vasal, 2006). Researchers (Ortega *et al*., 1986; Sproule *et al*., 1988; Osei *et al*., 1999) have compared the chemical composition of QPM with normal maize. Till today, no studies have been done to determine the response of QPM on growth performance of goat in Nepal.

Therefore, replacement of normal maize by QPM is the most effective and attractive measure to meet quality protein needs and to raise human nutritional status. Hence, this study was carried out to compare the growth performance of growing male goats fed QPM and normal maize based diets.

METHODOLOGY

Experimental Animals

This experiment was carried out on growing male Khari goats at Regional Agricultural Research Station, Lumle, Kaski from 23 March to 13 July 2011 (067/12/10 to 068/3/19 B.S.). Twelve male goats of average 5 months old with average body weight of 9.74 kg were divided into two groups having six animals in each group by using Complete Randomized Design (CRD). They were drenched with albendazole against internal parasites before assigning in experiment.

Concentrate Mixture Composition

Feed ingredients such as soybean cake, rice bran, normal maize, minerals and salt were procured in markets of Kathmandu and Lalitpur, while QPM was bought from farmers of

Arghakhanchi district and from National Maize Research Program, Rampur, Chitwan. Two types of concentrate mixture were formulated for experimental animals having 16% crude protein level as given in Table 1.

Table 1: Composition of concentrate mixture

Ingredients	**T1**		**T2**	
	Part	**Crude Protein, %**	**Part**	**Crude Protein, %**
Normal maize	0	0	22	1.98
Quality protein Maize (QPM)	22	1.98	0	0
Rice bran	58	6.38	58	6.38
SB cake	17	7.65	17	7.65
Mineral	2	0	2	0
Salt	1	0	1	0
Total	100	16.01	100	16.01

Experimental Diet of the Animal

The dry matter requirement of goats was calculated based on 4 kg per 100 kg body weight. Following diets were formulated to the experimental animals (Table 2).

Table 2: Experimental diets of the animals

S.N	**Treatment**	**Experimental diets**
1	Treatment 1 (QPM)	Forest mixed fodder (adlib) + Concentrate mixture @ 1% of body weight
2	Treatment 2 (Normal maize)	Forest mixed fodder (adlib) + Concentrate mixture @ 1% of body weight

Feeding Regime

Concentrate mixture was provided to the experimental animals of both group individually in plastic vessel once a day in the morning whereas *adlib* amount of fodder were provided twice a day. Quantity of concentrate mixture given daily to the animals weighed daily and refusal was weighed in next morning. Drinking water was provided thrice a day in adequate amount.

Chemical Analysis

The samples of feed ingredients and the fodder were sent to the Animal Nutrition Division, Khumaltar, Lalitpur for proximate analysis. Representative samples were analyzed for Dry matter (DM), Crude Protein (CP), Crude Fibre (CF), Ether Extract (EE) and Ash contents (TA). The DM was determined by oven drying at 100°c for 24 hrs. Crude protein of the samples was determined using the Kjeldahl method. Ether extract was determined using Soxhlet apparatus. Ash content was determined by ashing at 550°c in a muffle furnace for 16 hrs (AOAC, 1980). Crude fiber of the samples was determined using the Van Soest method (Goering, H.K. and Van Soest, 1970).

Recording of growth measurement

The trial period consisted 90 days after an adaptation period of 7 days. Total feed intake by goats was recorded daily for the entire experimental periods. The body weight gain of individual animal was measured and recorded fortnightly in the morning before feeding.

Data Analysis

Data of feed intake and body weight gain were analyzed by student '**t**' test for every measurement using computer statistical package Minitab 2003, versions 13.20.

RESULTS AND DISCUSSION

Chemical Composition of Feedstuffs

The result of chemical analysis is given in Table 3.

Table 3: Chemical composition of different feedstuffs (% DM basis)

Ingredient	**DM**	**OM**	**TA**	**CP**	**CF**	**EE**
Normal maize	86.0	98.1	1.9	8.92	1.93	4.48
QPM	87.5	98.4	1.6	9.11	2.14	5.12
Rice bran	83.69	95.51	4.49	11.52	12.61	5.1
Soybean cake	92.04	92.7	7.3	45.07	2.5	0.7
Mixed forest fodder	27.80	91.64	8.36	11.91	NA	NA

Feed Intake

Average daily intake of concentrate mixture and fodder by goats during experimental periods is given in Table 4.

Table 4: Feed intake of experimental animals/day/animal

Feedstuffs	**(Mean ± SE)**	
	Treatment 1	**Treatment 2**
Forest mixed fodder, kg	1.68±0.31	1.52±0.29
Concentrate mixture, g	121.4±1.10	113.2±1.40
Total Dry Matter intake / animal, kg	53.37	48.4
Feed Conversion Ratio (FCR), kg	12.92:1	12.1:1

Table 4 revealed that highest intake of fodder and concentrate mixture per day was observed in Treatment 1 (1.68±0.31kg and 121.4±1.10 g, respectively) than in Treatment 2 (1.52±0.29 kg and 113.2±1.40 g, respectively) that resulted to higher total dry matter intake in Treatment 1 (53.37 kg) than in Treatment 2 (48.4 kg). The feed conversion ratio (FCR) was higher for Treatment 1 (12.92:1kg) than Treatment 2 (12.1:1 kg). The fodder and concentrate mixture intake, total dry matter intake and FCR were not significantly different ($P>0.05$) between two groups.

Growth Performance

The growth performance of experimental goats is given in Table 5 and Figure 1.

Table 5: Growth performance of goats

Parameter	(Mean ± SE)	
	Treatment 1	**Treatment 2**
Initial Body weight, kg	9.75±1.0	9.63±1.10
Initial metabolic weight, kg	5.51	5.46
Final Body weight, kg	13.88±0.94	13.63±1.20
Final metabolic weight, kg	7.19	7.09
Total weight, gain, kg	4.13	4.0
Average daily gain, g	45	44

Table 5 showed that the average initial body weight of experimental goats was 9.75 ± 1.0 kg and 9.63 ± 1.1kg in Treatment 1 and 2 and attained the final body weight of 13.88 ± 0.94 kg and 13.63 ± 1.20 kg, respectively during 90 days of experiment. Both initial and final body weights was not significant (P>0.05) between two groups. Total weight gain was slightly but non significantly higher in Treatment 1 (7.19 kg) than that of Treatment 2 (7.09 kg). Average daily gain of experimental animals was almost similar for both groups (45g and 44 g for Treatment 1 and 2, respectively), although, it varied from 19 to 100 g for Treatment 1 and from 21 to 60 g for Treatment 2.

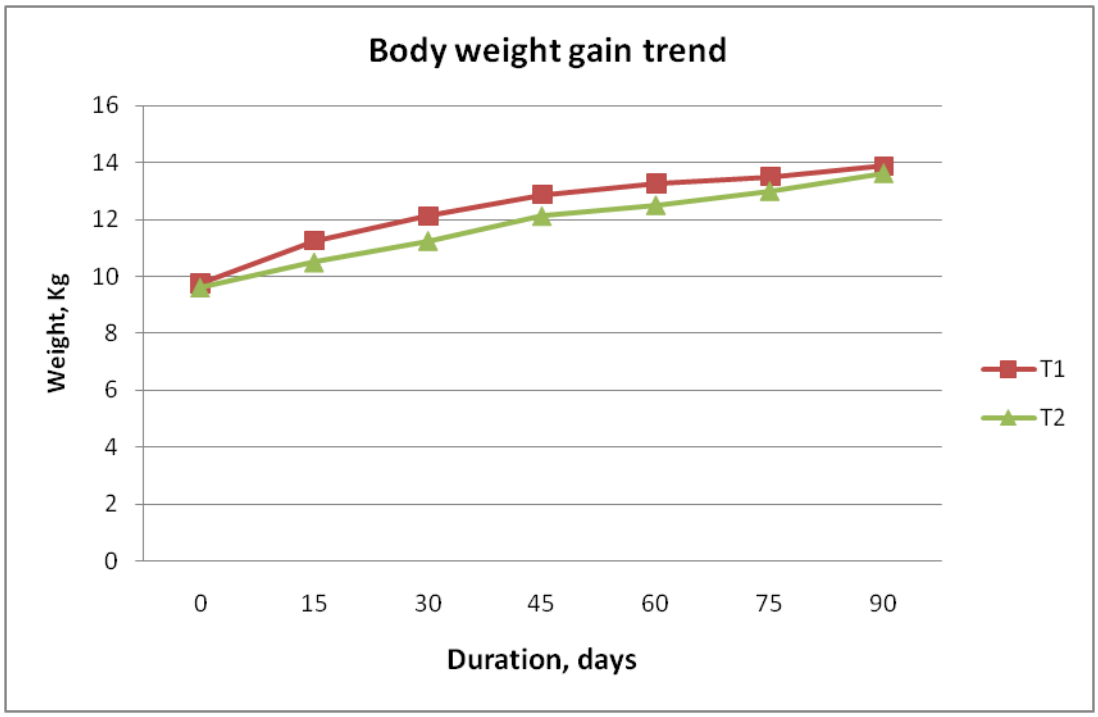

Figure 3: Body weight gain trend of goats during experiment period

DISCUSSION

This study was carried out with objective of comparing the growth performance of male Khari goats fed with normal maize against quality protein maize incorporated concentrate mixture.

Results of growth trial suggested that there was no significant difference in growth rate of animals fed with QPM and normal maize. It might be due to that goats have a ruminant stomach and as such, can produce these amino acids (lysine and tryptophan) through bacterial growth. Anon (2004) also reported that the effect of replacing normal maize by QPM had no beneficial effect on intake, digestibility, growth rate and feed efficiency of growing Black Bengal goat in Bangladeshi condition.

Similarly, Mekonnen *et al.,* (2009) reported that there was no significant difference in the dry matter and crude protein intakes between the Ethiopian highlands Arsi lambs fed with QPM and those fed with the normal maize. Furthermore, they noted that these were significantly increased in animals receiving whole plant silage and the stover form compared to those feeding with earless silage ($P<0.05$). Moreover, the dry matter intake per metabolic body weight per day was significantly higher for the stover form diet followed by the whole plant silage distribution compared to the earless silage diet ($P<0.001$). On the other hand, the growth performances evaluated by the final (empty) body weights and the (empty) body weight gains were maximal when lambs received whole plant silage from the QPM or the normal maize. The body weight gains registered in QPM fed groups were slightly higher (but not significantly) than those of the corresponding normal maize.

CONCLUSION

Results of growth trial suggested that there was no significant difference in growth rate of animals fed with QPM and normal maize. It might be due to that goats have a ruminant stomach and as such, can produce these amino acids (lysine and tryptophan) through bacterial growth. Therefore, it is concluded that feeding of QPM to goats is not beneficial and economically viable than monogastric animals like pigs and poultry.

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SERO-PREVALENCE OF PORCINE CYSTICERCOSIS AND HUMAN TAENIASIS/CYSTICERCOSIS IN NEPAL

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ABSTRACT

The problem of cysticercosis is now considered arising after introducing intensive pig farming system in the rural and peri-urban settings of farming during past decade under poverty elevation programme of the government. A cross- disciplinary approach method was used to tackle the problem. Surveys conducted simultaneously in humans and animals from the same environment to correlate data on an epidemiological level would enable a comprehensive and accurate approach to study the transmission and the burden. So far, 100 pigs' serum samples and 18 human serum samples were collected and tested by Enzyme-linked Immunosorbent Transfer Blot Assay (EITB) techniques. About 320 pig carcasses were examined for Taenia cysts of which 23 (69.69%) pigs were found positive. Out of 18 human sera tested 8 (44.44%) have shown immunoblot positive bands for taeniasis. This study is still in progress as a first phase of the project.

Key words: Taeniasis, immunoblot, porcine, cysticercosis

Background Information about Pig Farming in Nepal

Pig farming is increasing in Nepal day by day with the annual population growth rate of 4.55% while that of other livestock is 1.12% for cattle, 1.93% for buffaloes, 2.03% for goats, –0.82% for sheep and 4.7% for poultry (CLDP, 2003). The distribution of pig population in different ecological belt of country is 9.47% in mountain, 55.58% in hill and 34.95% in Terai (CBS, 2006b). Per capita meat consumption pattern in Nepal is 8.7kg/per person/year (TLDP, 2003). Pork contributes about 7.32% of total share in Nepal and while buff shares about 64.68%, chevon 19.41% and poultry 7.18% (CBS, 2006a). In the global scenario, consumption of pork is the highest (38%) followed by chicken (30%) and beef (25%) (Bhattarai, 2005). Although pig is widely distributed in all eco-regions of the country, its population is the highest (52.68 %) in the mid-hills and the lowest in the high-hills.

Epidemiological Background of Taeniasis/Cysticercosis in Nepal

Cysticercosis, a tapeworm (*Taenia solium*) transmitted to man by pigs is a major preventable cause of epilepsy in developing countries. Neurocysticercosis is regularly diagnosed in India and Nepal, where the disease has some unique epidemiological and clinical features. Data on transmission patterns, the burden of disease and the economical impact it causes are inexistent in South Asia.

The intermediate host (pig) is infected by ingestion of eggs or gravid segments excreted in the human faeces and the eggs transform in to cysticerci in the muscles of the host within a period of three weeks to two months (White, 1997). The life cycle is completed when man ingests infected "measly" pork. Human Cysticercosis develops with accidental ingestion of eggs in contaminated food or by faeco-oral route (Soulsby, 1982).

Worldwide, more than four million people harbour the porcine tapeworm (Schantz, 1989) and 50 million individuals are infected with cystic stage (Schantz *et al.*, 1993). An estimated 50,000 people die from cysticercosis each year because of central nervous system (CNS) or cardiac complications (Ghadishah & Burn, 2006). Neurocysticercosis (NCC) is the commonest neuroparasitic infection in humans (Arasu *et al.*, 2005) and the statistic figure shows NCC is the commonest cause of seizure in Nepal.

In Asian countries it varies from 0.02% to 32.5% and the Taeniasis is 0.11% to 50% (Rajshekhar *et al.*, 2003). Porcine cysticercosis in China has been reported with variable percentages indifferent areas (Rajshekhar *et al.*, 2003). In China, the infection in pigs is highly variable ranging from 0.84 to 15% and in some areas as high as 40% (Rajshekhar *et al.*, 2003). Cysticercosis in a pig farming community in India was found to be 26% and the human taeniosis 38% (Prasad *et al.*, 2002). In Nepal, few reports are available about porcine cysticercosis, human taeniosis and cysticercosis. Joshi *et al* (2004a) reported 14.28% of porcine cysticercosis in meat inspection in Kathmandu in 1997 and by tongue palpation 32% pigs were positive for cysticercosis at the Magar ethnic community of the Syangja District in 2000. The slaughter prevalence of porcine cysticercosis in Kathmandu valley is found as 0.99% (Sapkota, 2005). Joshi *et al* (2004a) reported 14.28% of porcine cysticercosis in meat inspection in Kathmandu in 1997. The prevalence rate of porcine cysticercosis in Chitwan valley has been found as 6.66% (Rana and Dhakal, 2005).

Bista (2005) reported the prevalence of human cysticercosis ranges from 0.002-0.1% in general population. Kathmandu population has higher prevalence (two third) followed by Dharan as compared to population residing to other parts of country. In another study, Agrawal (2005) reported 66 cases of Neurocysticercosis (NCC) at Neurology Service Unit of T.U. Teaching Hospital. Young people of 20-30 years of age were mostly affected: 77.2% presented with seizures of one or other type, 40.9% had weakness of the limbs and 18% presented with headache alone and 9% had signs of increased intracranial pressure. Out of 23,402 biopsies, 62 cases have been detected as cysticercosis in the last 5 years in Patan Hospital. Most (82%) of the patients were presented with solitary skin nodules, another 10% with nodules in the oral mucosa, and 8% in the breast (Amatya & Kimula, 1999). The prevalence of human taeniosis in the Sarki and Magar communities of the Syangja District was found at 47.7% (Gaire, 2000).

Joshi *et al (*2006) conducted a survey of porcine cysticercosis where by 200 pigs were subjected to lingual examination, antibody detection by ELISA and Post-mortem carcass examination with 10.5%, 22.5% and 20.5% found positive, suggesting lingual examination method for detecting porcine cysticercosis is easy, inexpensive and could be utilized as a surveillance tool in developing countries like Nepal where technical resources and technological capacity are very limited.

Dorny *et al* (2004) in a study of 868 slaughtered pigs at Lusaka (Zambia) used a Bayesian approach to estimate values for the prevalence and diagnostic test characteristic of porcine cysticercosis by combining results of four imperfect tests. The estimated prevalence of porcine cysticercosis was 0.642 (95% confidence interval 0.54–0.91) and the performances of the tests were (sensitivity (se)–specificity (sp)): tongue inspection (se 0.210–sp 1.000), meat inspection (se 0.221–sp 1.000), Ab-ELISA (se 0.358–sp 0.917), Ag-ELISA (se 0.867–sp 0.947).

Prasad *et al* (2002) reported 38% taeniasis in human and 26% cysticercosis in pig in a study comprising of 72 members of pig farming communities and 50 slaughtered pigs at Uttar Pradesh, India out of which 9.7% members of that community had reported seizures due to Neurocysticercosis.

OBJECTIVES

1. To determine the prevalence of *T. solium* Cysticercosis in slaughtered pig in Sunsari and Kathmandu valley by sociological diagnosis.
2. To detect the predilection site of the Cyst in the pig carcass by gross examination.
3. To determine the prevalence of human cysticercosis by serology and retrospective study of the data from human hospital in Kathmandu valley.
4. To study risk factors of taeniasis/cysticercosis, in particular pig rearing areas.

METHODOLOGY

Study area

This study was conducted in Sunsari district in the Eastern and Kathmandu valley in Central region of Nepal. The head, carcass and viscera were thoroughly examined visually as per the OIE guideline (OIE, 2004). Meat inspection was done by visual inspection of the carcass and its cut surfaces for the detection of cyst. The muscles of diaphragm, heart, shoulder, thigh and abdomen were thoroughly examined visually; similarly the masseters and the pterygoid muscles were examined on incisions

Collection of Taenia Cyst and Serum Samples of Pig and Human

The positive tissue samples in carcass examination were examined in detail. The cysts were counted on the cut surface and collected in specimen collecting bottles.

Transportation, Storage and Testing of Collected Pig and Human Serum Samples

The collected serum samples were transported to the National Zoonosis and Food Hygiene Research Centre Laboratory, Kathmandu by maintaining cold chain with the help of cool box.

Survey of the Pig Farmers

A questionnaire survey about the pig husbandry was carried out in pig rearing farmers from where the most of the pigs are brought to the slaughtering places.

Survey to Medical Hospitals

In order to find out the prevalence of human cysticercosis a questionnaire survey was carried out from five hospital of Kathmandu valley. They are as T.U. Teaching Hospital, Bir Hospital,

Patan Hospital, Norvic Escorts International Hospital and Nepal Medical College Teaching Hospital, from Kathmandu valley.

RESULTS

Age and Sex wise distribution of Slaughtered pigs

Out of 320 slaughtered pigs, 71% (226) were male and 29% (94) were female. About 62.5% of the pigs are slaughtered between the ages of 7-10 months, followed by 11-14 months (19.06%), 3-6 months (12.5%) and >14 months (5.94%) as presented in Table 1.

Table 1: Age and Sex wise distribution of slaughtered pigs

Particular	Age (month)				Sex	
	3-6	7-10	11-14	>14	Male	Female
Total	40	200	61	19	226	94
Percentage	12.50%	62.50%	19.06%	5.94%	71%	29%

Carcass Examination for Cysticercosis

Out of 320 pigs carcasses examined, only 23 pigs carcasses were found positive for *Cysticercus cellulosae* during meat inspection. This finding indicated that the prevalence of Cysticercosis on meat inspection was 7.19%.

Pig Serum Diagnosis

Age and Sex wise distribution of pigs tested for serology with EITB is presented in Table 2. Out of 320 pigs examined 100 serum samples were diagnosed by EITB method. Out of 100 serum samples, 19 were found positive (infected) for *Cysticercus cellulosae* during EITB diagnostic technique. So, the serological prevalence of infected pig is 19%, which had taken three or more bands.

Table 2: Age and Sex wise distribution of pigs tested for serology EITB

Age group (Months)	Total	Male	Percent	Female	Percent
0-3	1	-	-	1	100
4-6	8	6	75	2	25
7-12	73	52	71.23	21	28.77
13 & above	18	7	38.89	11	61.11
Total	100	65		35	

The assay was a dot blot using 6 purified *T. solium* infection specific antigens (50, 42-38, 24, 18, 14, 13 KDa). The test was on Enzyme-linked Immunosorbent Transfer Blot (EITB) method. It has been taken 3 or more bands (Positive dots) as indicative of cysticercosis which means infection in pigs and 1-2 bands as indicative of exposure which means pigs are exposed to the *Taenia solium* exposure but not carrying infection. Number of bands positive on EITB (Dot blot format) is shown in Figure 1.

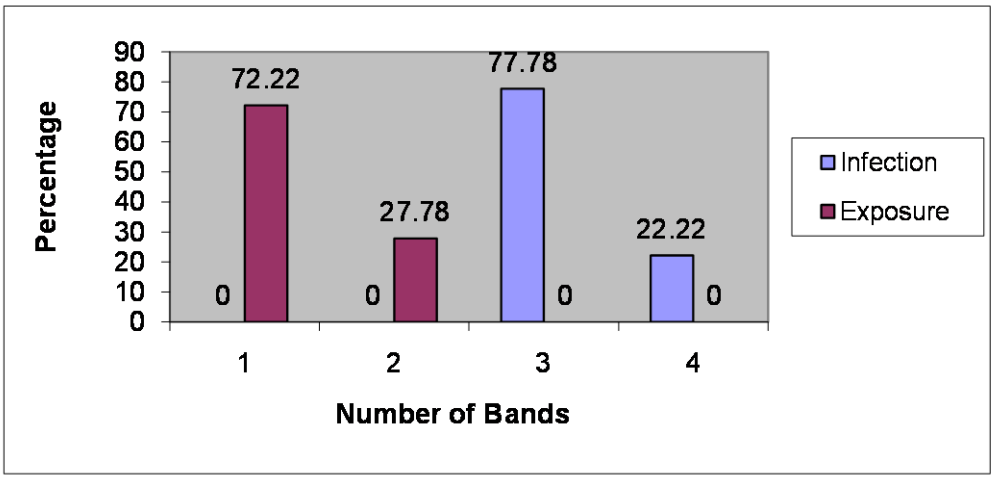

Figure 1: Number of bands positive on EITB (Dot blot format) in pig serum

Human Serum Diagnosis

Immunoblot for cysticercal antibody detection in human serum is presented in Table 3. Out of 18 serum samples tested, 8 were from male and 10 from female samples of which total of 8 samples were (44.44%) positive, 3 from females (37.5%) and 5 from males (62.5%).

Table 3: Profiles of immunoblot results for cysticercal antibody in human serum

Human serum sample	Age	Sex	Result	Diagnostic band
H1	22	M	-	-
H2	4	M	+	13
H3	22	F	-	-
H4	5	M	+	14, 13
H5		F	-	-
H6	6	F	-	-
H7	41	M	-	-
H15	18	F	+	24,14,13
H8	48	F	-	-
H9	6	F	-	-
H10	40	M	-	-
H11	53	M	+	14
H12	60	F	-	-
H13	6	F	-	-
H14 (eye)	22	F	+	14
H16	18	M	+	14
H17	20	F	+	13
H18	25	M	+	39

Total positive = 8 (44.44)
Total negative=10 (55.56)
Note: 7 diagnostic bands 50, (43-39), 24,21,18,14 &13

NCC cases among total admission episodes and total epileptic admission episodes

The distribution of NCC cases in terms of total admission episodes and total epileptic admission episodes is shown in Table 4. NCC per 1,000 admission episodes was the highest (5.27) in Norvic Hospital followed by College of Medical Sciences Teaching hospital and the lowest (0.26) in Patan Hospital. The percentage of NCC in terms of epileptic admission episodes was the highest (39.22), which was followed by the Bir hospital (32.65) and the lowest (5.00) in T.U. Teaching hospital. NCC patients at these 5 hospitals were found at the overall rate of 0.46 per 1,000 admission episodes during the period of 2002-2006 where as the occurrence of NCC was 10.21% of epileptic admission episodes.

Table 4: Neurocysticercosis among the admitted cases in 5 hospitals in the years 2002-2006

SN	Patients	Five Hospitals of Kathmandu Valley					
		Teaching	**Bir**	**Patan**	**Norvic**	**NMC**	**Total**
1	Total admission episodes	85895	50033	94870	8727	28252	267777
2	Total epileptic admission episodes	560	49	321	210	124	1264
3	Total NCC episodes	28	16	25	46	14	129
4	NCC/1000 admission episodes	0.33	0.32	0.26	5.27	0.50	0.46
5	NCC/epileptic admission episodes (%)	5.00	32.65	7.79	21.90	11.29	10.21

Age and Sex wise distribution of NCC Patients

The sex wise distribution of NCC patients is shown in Figure 2. Out of total 841 NCC patients, from seven hospitals, 58% were males whereas 42% were females. The age wise distribution of NCC cases showed the highest (47.09%) in the age group of 15-35, which was followed by the age groups of 0-14 years (29.37%) and the lowest in the age groups of above 35 years (23.54%).

DISCUSSION

Cysts were mostly found in the muscle of diaphragm followed by heart, oesophagus and tongue. The hind limb muscle, forelimb muscle and the masseters muscles were heavily infected with the *Cysticercus cellulosae* followed by abdominal, cervical and intercostals muscles in all positive carcasses. This finding is in agreement with the finding of Boa *et al* (2002) and Sapkota (2005).

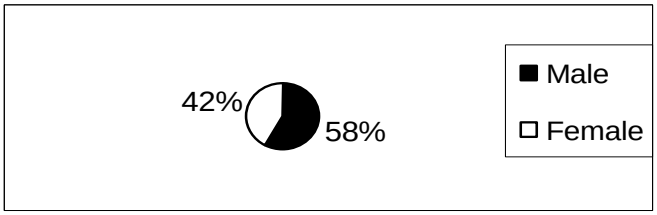

Figure 2: Sex wise distribution of NCC patients

Among 724 seizure cases, 61% were diagnosed as NCC by EITB at Model Hospital, Kathmandu (Pant, 2006). Out of total 200 seizure disorder cases in Shree Birendra Hospital, Chhauni 23% were diagnosed as NCC by CT scan and MRI (Neupane, 2006). In Patan hospital out of 543 seizure disorder patient 7.18% were due to NCC (Chaudhary, 2006). Out of 55 cases of seizure disorder patient at OPD section of Om hospital 43.64% were diagnosed as NCC based on CT scan (Sharma, 2006). This study as well as the review clearly shows that NCC was the major contributory factor in the occurrence of epilepsy in Nepal.

With the sensitivity of EITB as 100% and specificity as 82.65%, this study has indicated that the prevalence of disease 19%, which signifies that more numbers of uninfected pigs were selected for carcass examination..

CONCLUSION

This study shows that cysticercosis is present in pig and human population of Nepal. There are no programs for its eradication, prevention and control. No preventive measures have thus been taken for porcine cysticercosis in the absence of appropriate policy for disease control. The evidence of cysticercosis through this study clearly shows that this chronic infection is a potential zoonotic threat. There is still no provision of hygienic slaughtering and meat inspection practices in Nepal. Slaughterhouse and meat inspection act and regulation should be implemented strictly as soon as possible. The products from cystic pigs are being consumed daily and the possibility of its transmission cannot be neglected. Those carcasses must be frozen for a week before sale.

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PREVALENCE OF CYSTIC ECHINOCOCCOSIS/HYDATIDOSIS IN BUFFALOES SLAUGHTERED IN KATHMANDU

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ABSTRACT

The study was conducted during the year 2010 as an attempt to assess the prevalence of cystic echinococcosis/hydatidosis in buffaloes slaughtered in ward no. 19 and 20 of Kathmandu Metropolitan City (KMC). A total of 164 buffaloes slaughtered were examined for hydatid cysts in liver and lung. Out of 164 buffaloes examined, 29.9% of the buffaloes were found to be infected with hydatidosis. Hydatid infection in males and females were found to be 20% and 37.2% respectively. Among positive cases, 83.3% had infection in lungs, 9.5% had infection in liver and 16.7% had infection in both lungs and liver. The largest cyst collected was 13 cm in diameter while the smallest one was 0.5 cm diameter. Seventy percent of the cysts were fertile and 30% of them were infertile.

Keywords: Echinococcosis/hydatidosis, cyst, fertile, buffaloes

INTRODUCTION

Echinococcus granulosus is a zoonotic cestode that causes hydatid disease in man and animals. The parasite is worldwide in distribution, but endemic in central Asia, Europe, South America, and some countries of Africa. In Nepal, hydatid disease is of considerable economic and public health significance, yet there has been little work undertaken on the epidemiology of the parasite. In Asia, China is the most endemic country, where the number of existing cases in 2000 was estimated to be 600,000 to 1.3 million, and the population at risk was believed to be 60 million (Ito *et al.*, 2003). The occurrence of this parasite is also higher in the Indian sub-continent and the Middle East.

Occurrences of new cases of human infection are often found to be high in the areas where intimate relationship exists between man, dogs and livestock, e.g. among the sheep farming communities (Torgerson, 2003). In these communities, dogs commonly get infected by feeding on the infected sheep viscera, which often contains sheep strain larva.

In dog, adult worm of *E. granulosus* does not cause much inconvenience but the larval hydatid cysts in intermediate hosts including humans may require medical attention. *E. granulosus* occurs practically worldwide, and more frequently in rural, grazing areas where dogs can ingest organs from infected animals in most countries of the South Asian region. Schantz (1996) evaluated that in South Asian Countries namely India, Pakistan, Afghanistan, Nepal, Bhutan,

Sri Lanka and Bangladesh, buffaloes, cattle, sheep, goats, pigs, and camels serve as intermediate hosts. Both pastoral and urban cycle of this disease exist in Nepal.

Joshi (1985) studied the prevalence of human hydatidosis in Kathmandu from July to December, 1983. Data for a period over five years was collected from Bir hospital, Kanti Hospital and Shanta Bhawan Hospital. Out of 27,188 surgical cases during that period, 76 cases were for hydatidosis. Out of 76 cases, 46 were females and 30 were males. There were 57 cases with cyst in the liver and 19 with cyst in lungs. A total of 59 patients were cured and 17 died due to anaphylactic shock. From 1985 to 1990, 19 (0.2%) and 20 (0.1%) cases of human hydatidosis were operated at Bir and Teaching Hospitals, respectively. In Kanti Children's Hospital, 2(0.3%) hydatidosis cases were handled during 1985 to 1995 (Joshi, 1985 a, b, c).

Joshi *et al* (1996) further reported human hydatid cases from different hospitals. During the year 1985-1993, 32 hydatid cases (0.2% of major operated cases) were operated in Bir Hospital. Most of the patients were harboring cysts in the liver; out of these 32 patients, three died due to anaphylactic shock. Similarly, hospital records of Tribhuvan University Teaching Hospital showed 25 hydatid patients (0.08% of major operated cases) during 1985-1993. Eight cases of hydatidosis (0.03% of major operated cases) were reported by Kanti Hospital, while 10 patients of hydatidosis were recorded at Western Regional Hospital, Pokhara during the same period. During 1990-1994, eight surgical hydatid cysts were recorded at United Mission Hospital, Patan.

Joshi (1991) also documented the occurrence of echinococcosis cyst in domestic livestock. His study found that 5% (153/3065) of water buffaloes, 3% (55/1783) of goats, 8% (12/150) of sheep and 7% (10/143) of pigs were infected with echinococcosis. Baronet *et al* (1996) studied incidence and risk factors or infection by *E. granulosus* in the domestic and street dogs of Kathmandu using ELISA Coproantigen test as a screening method. The highest prevalence (5/88=5.7%) was seen in domestic dogs. Joshi (1996) reported 18% buffaloes, 9% sheep, 4% goats and 9% pigs positive for hydatid cyst in another study conducted among 18,805 slaughtered animals during 1993-1995. Livers and lungs were found equally affected and in some animals, both liver and lungs were found positive (Joshi *et al.*, 1996).

This study was aimed at assessing the prevalence of cystic echinococcosis/hydatidosis in buffaloes slaughtered in Kathmandu valley.

MATERIALS AND METHODS

Study area

Slaughtering slab/houses of Ward Numbers 19 and 20 of Kathmandu Metropolitan City were chosen for the study where a total of 15 to 20 buffaloes are being slaughtered daily.

Study population

During the study period, 164 slaughtered buffaloes were examined which represents an average of 10 slaughtered buffaloes examined per day. It was not possible to examine more carcasses because some buffaloes were slaughtered in the same time at different places. The actual ages of the buffaloes were unknown. Therefore, the buffaloes were divided into two categories, adult

and young by general inspection before slaughtering. The buffaloes were also divided into two categories, male and female. Among buffaloes slaughtered, the number of adult male, adult female, young male and young female were 50, 88, 20, 6 respectively.

Inspection and data collection

The slaughter houses were daily visited in the morning from 22th June to 14th July 2010. The buffaloes slaughtered were inspected for hydatid cysts. Liver and lungs of each buffaloes were inspected with care.

For each animal, the following information was recorded:

- Sex and age categories
- Presence or absence of the hydatid cyst
- Localization of the hydatid cysts

Hydatid cysts from positive cases were collected with tissue of associated organ. A total of 90 cysts were collected during the study period of which 13 were from liver and 77 from lungs. Laboratory analysis was done to determine the fertility status of hydatid cyst, range of size of hydatid cysts, and quantity of cystic fluid.

RESULTS

Out of 164 buffaloes examined, 29.9% (49/164) were positive for hydatid cysts. Among female buffaloes, 37.2% (35/94) were positive for hydatid cyst, 20% (14/70) male were positive for the same. The results are shown on the Figure 1.

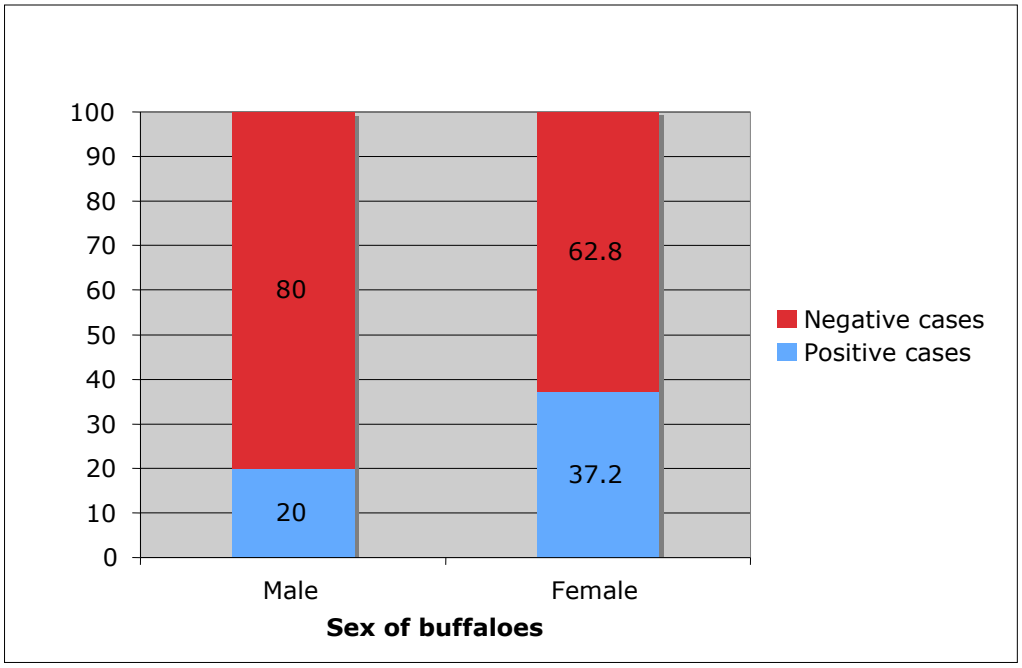

Figure 1. Prevalence of Echinococcosis in male and female buffaloes

Age wise prevalence of Echinococcosis in buffalo

Among male buffaloes, 0% (0/20) of the young and 28.0% (14/50) of the adult were positive for hydatid cyst. Among females, 33.3% (2/6) of the young and 37.5% (33/88) of the adults were positive for the same. The results are shown in the Table 1.

Table 1. Age and sex-wise examined of echinococcosis in buffaloes

Sex of buffaloes	Age category	Total examined	Number of positive cases	Percent (%)
Male	Young	20	0	0
	Adult	50	14	28.0
Female	Young	6	2	33.3
	Adult	88	33	37.5
Total		164	49	100.0

Organ wise prevalence of Echinococcosis in buffalo

Out of 49 positive cases, 83.3% of the animals were showing infection in lungs, 9.5% in liver and 16.7% in both lungs and liver. However, these percentages have been calculated with a total of 41 positive cases among the 49 listed. Indeed, in 8 cases, the lungs or the liver of the animal were impossible to check. In these 8 cases, both lungs and liver were infected (Figure 2).

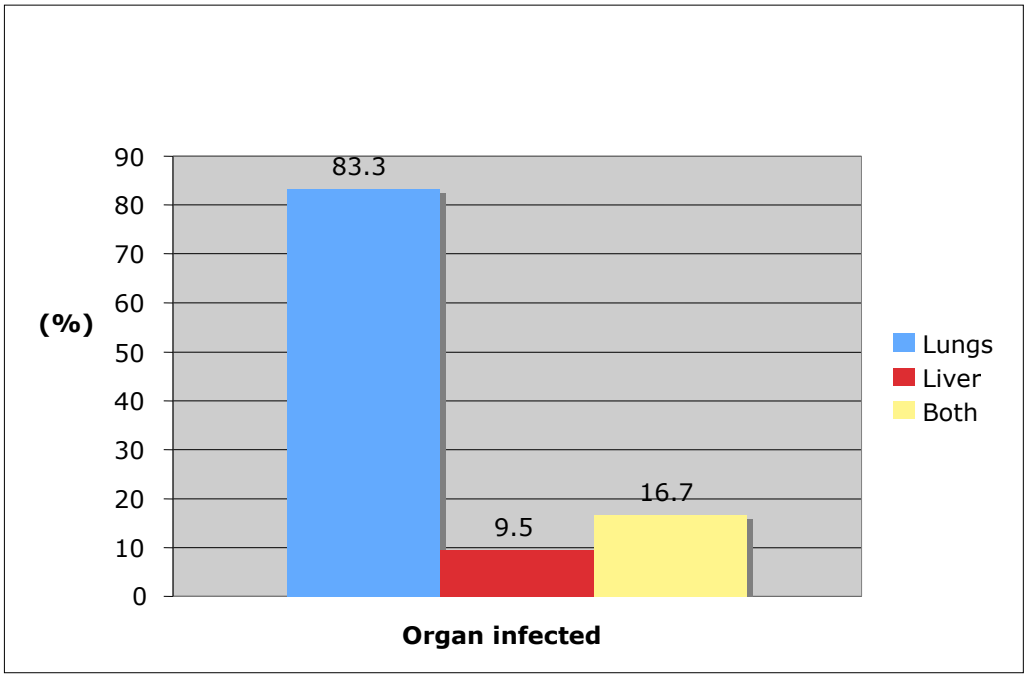

Figure 2. Organ wise prevalence of echinococcosis in buffalo

Organ wise prevalence of echinococcosis in different age categories of the buffaloes is shown in the following Figure 3.

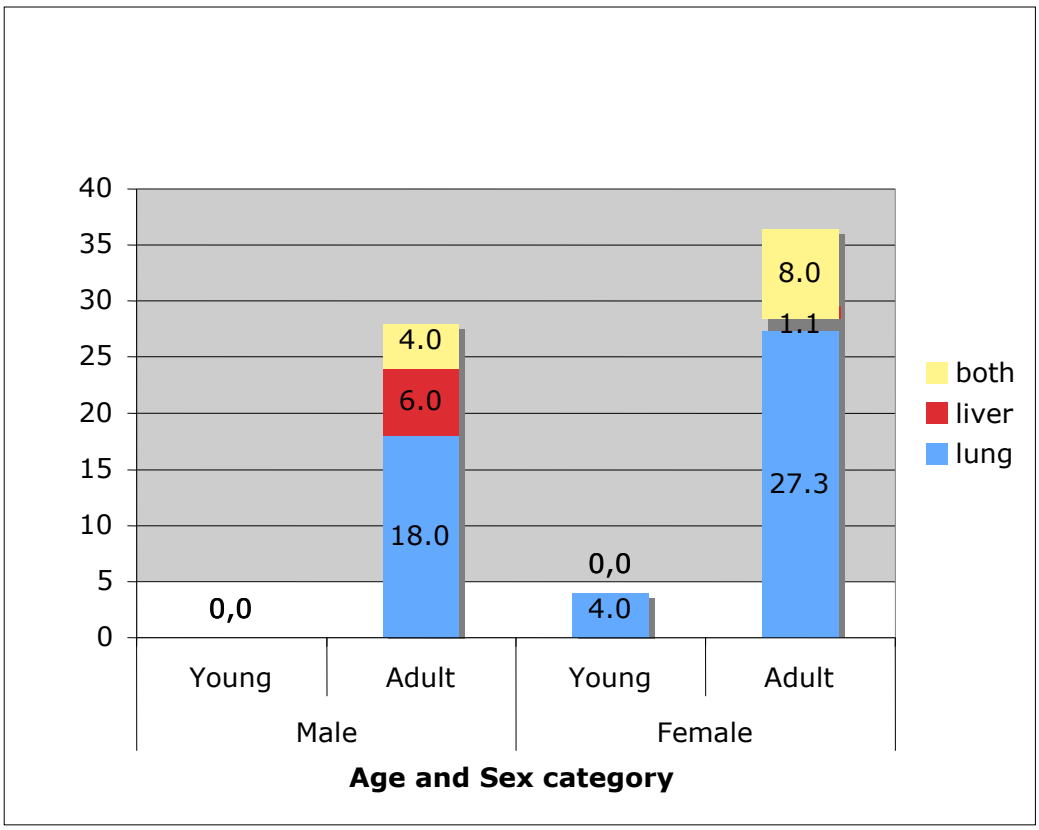

Figure 3. Organ-wise prevalence of echinococcosis in different age group of buffalo

Result of laboratory analysis

Laboratory analysis of 90 cysts at National Zoonoses and Food Hygiene Research Center revealed that the diameter of the cysts ranged from 0.5 cm to 13 cm as shown in Table 2.

Table 2. Size of the cysts in lungs and liver

Organs	Minimal size (cm)	Maximal size (cm)	Average (cm)
Lungs	0.5	13	3.9
Liver	0.8	9.1	3.5

Volume of fluid in the cysts

Maximum and minimum amount of fluid in the hydatid cysts were 278.5 ml and 0 ml, respectively (Table 3).

Table 3. The volume of the cystic fluids in the lungs and liver

Organs	Minimal volume (ml)	Maximal volume (ml)	Average (ml)
Lungs	0	278.5	29.3
Liver	0	277.5	44.1

Fertility status of the cysts

The cysts were categorized as fertile or infertile on the basis of the presence or absence of protoscolices, daughter cysts and brood capsule. Out of 90 cysts collected, 70% of them were fertile and 30% infertile (Figure 4).

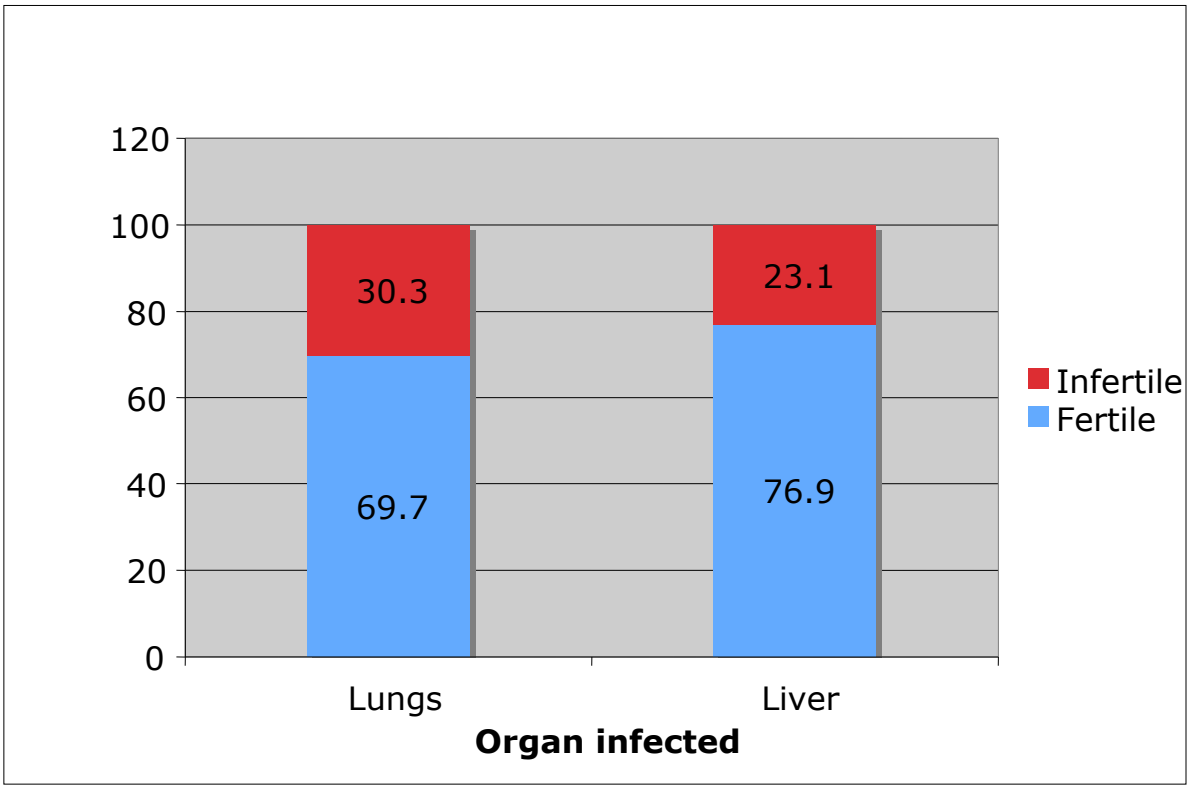

Figure 4. Fertility status of the cysts from lungs and liver

DISCUSSION

The present study found higher incidence (29.9%) of echinococcosis in buffaloes slaughtered at Ward Numbers 19 and 20 of Kathmandu Metropolitan City as compared to that from Kirtipur Municipality of Kathmandu Valley. In a separate study conducted by Maharjan *et al* (2010) in Kirtipur Municipality, 475 buffaloes, 50 pigs, 50 goats were observed for the presence of hydatid cyst out of 575 slaughtered animals. Altogether 54 (11.37%) buffaloes, 4 (8.00%) goats and 1 (2.00%) pig were found infested with hydatidosis. Out of total 475 samples from slaughtered buffaloes, females (12.65%, 41/324) were found infected with hydatidosis more often than males (8.61%, 13/151) but the difference was not significant ($p = 0.19$). Among males, 3.88% (4/103) young and 18.75% (9/48) adult were found infected. Among females, 4.17% (2/48) young and 14.13% (39/276) adult carcass were positive for hydatidosis. Younger animals were found infected significantly more often than adult buffaloes ($p< 0.01$). The organ wise infection rate was highest for lungs with 61.11% (n=33) cysts followed by, 22.22% (n=12) for liver, 12.96% (n=7) for both liver and lungs and 3.70% (n=2) for spleen.

The sheep-dog-sheep cycle is seen to be occurring most abundantly throughout the world, however, in the countries like Nepal (Joshi *et al.*, 1997a) and Poland (Pawjowski and Stefaniak, 2003), the parasite cycles between pig and dog. Moreover, life cycle patterns involving other ungulates and domestic dogs are also important economically (Torgerson, 2003). Wild animals are involved in sylvatic cycles in different parts of the world although, their zoonotic importance is generally small as compared to the domestic cycles.

CONCLUSION

This was an attempt made to assess the prevalence of cystic echinococcosis/hydatidosis in buffaloes slaughtered in Ward Numbers 19 and 20 of Kathmandu Metropolitan City (KMC). Total 164 buffaloes were examined for hydatid cyst in liver and lungs. Among positive cases 83.3% had infection in lungs, 9.5% had infection in liver and 16.7% infection both liver and lungs. This study shows that there is a huge amount of economic loss due to cyst in liver and lungs which is not useable for consumers to eat and also butcher cannot sell it at market price. Since control and eradication program requires long-term projects and good deal of money, the developing countries are not in the position to get rid of this parasite as easily the rich countries got till now.

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THE RELATIONSHIP BETWEEN BLOOD PARAMETER AND MYCOBACTERIUM CULTURE STATUS IN CAPTIVE ELEPHANTS OF NEPAL

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ABSTRACT

A study to investigate the prevalence of tuberculosis among 108 Asian captive elephants (Elephas maximux) of Chitwan National Park and its buffer zone was conducted. The association of blood parameters with Mycobacterium culture status was also examined by the Chi square test and Relative Risks (RR) analysis.

Sample for Mycobacterium culture were collected according to standard procedures. Three trunk wash samples were collected in a one-week period from each elephant on non consecutive days. Approximately 35 ml blood was collected from each elephant via auricular vein and placed into Ethylenediamine Tetra-Acetic Acid (EDTA) tubes for hematology and serum separator tubes for biochemical analysis. Elephant trunk wash samples were cultured for Mycobacterium tuberculosis in National Tuberculosis Centre (NTC), Nepal according to standard protocol. Colony growth was confirmed to be Mycobacterium by acid fast microscopy. PCV was determined by using microhematocrit method and haemoglobin determination was done by cyanmethemoglobin method. Mean Corpuscular Haemoglobin Concentration (MCHC) was calculated from haemoglobin and PCV. Total Leucocytes count (TLC) was done manually using Thoma's WBC counting pipette and Neubauer's chamber. Total Protein and albumin was determined by using auto analyzer (Pointe 180 chemistry analyzer). Chi- square value, P value and RR were calculated using Microsoft Excel and SPSS software to determine the association of each of the blood parameters under study with culture results.

The prevalence of Mycobacterium infection by trunk wash culture was found to be 9.25% and that of Mycobacterium tuberculosis infection was found to be 3.7 % higher than the prevalence (3%) reported in North America. Higher prevalence was found in males (20%) and elephants aged 1-9 years (16.66%). The study did not show significant association based on p value (p> 1.0) between haematological and serum biochemical parameters and Mycobacterium culture status study. However, RR analysis indicated an association (value > 1) between all parameters under study with culture except for TLC, MCHC, Hb, PCV, glucose, total protein, albumin, globulin and A/G ratio, to have a greater probability of varying from normal in TB positive elephants. TB positive elephants were found to have 3.93, 1.47 and 2.85, 11.9 times greater probability of having elevated PCV, glucose, total protein and globulin, respectively. While haemoglobin, MCHC, albumin and A/G ratio were shown to have 1.17, 1.14, 1.54 and 1.77 times greater probability to have decreased levels in TB positive elephants, respectively. There is no significant influence of Mycobacterium infections on blood parameters and serum

biochemical parameters although trends were seen based on relative risk that may be useful in leading to a diagnosis. These findings are consistent with the unpredictable and insidious nature of the disease in elephants. Further investigation into other biochemical parameters and new advanced serological technologies is required for the early diagnosis of the tuberculosis in the elephants.

1. INTRODUCTION

Elephants are the largest land animals on the earth. In 2000, there were estimated to be only 35000 – 50000 wild Asian elephants (*Elephas maximus*) compared to over 600,000 wild African elephants (*Loxodonta Africana* and 16,000 captive Asian elephants, although the current population is thought to be much lower (WWF, 2000). They are listed at Appendix I of the Convention on International Trade in Endangered Species of the Wild Fauna and Flora (CITES) and in the endangered status category of the World Conservation Union (IUCN) (WCMC, 2001). All trade of ivory and other parts of Asian elephants is currently banned. Elephants are considered a "flagship" species because their protective survival will maintain biological diversity and ecological integrity in the environments in which they live (WWF, 2003). Their survival is also critical to people in Asia where they are important in ecotourism, forestry and wildlife management, as well as religion and culture.

Despite their popularity and ongoing conservation efforts, Asian elephants are found in isolated, small population today. There are estimated to be only 40-60 resident wild elephants in Nepal, in addition to 40-140 elephants migrating across the Nepal- India order annually (WWF, 2000). Wild elephants are found in three populations in Nepal: (1) West –Sukla Phanta Wildlife Reserve and Bardia National Park, (2) East-Koshi Tappu Wildlife Reserve, and (3) Central – Bara Forest Reserve District and Parsa Wildlife Reserve, which is within the Chitwan National Park (Johnsingh *et al*., 1999 and Mishra *et al*., 2000). The first two populations are migratory as part of a larger population crossing into India, whereas the third populations confined to Nepal as residents. One third of the Asian elephants remaining on earth are captive (Sukumar, 2000). The vast majority of these 15,000 elephants live in Asia where they are used for work, ceremonies, and ecotourism. Nepal has about 200 captive elephants found in several populations working both in the forests and in ecotourism, giving rides to tourists in and around the Chitwan National Park (CNP) which is vital to the livelihood of many Nepali families. Captive elephants are found in the Elephant Breeding Centre, Hattisar and at numerous private hotels around Chitwan (WCMC, 2001). Most captive elephants give tourist rides and particularly the government elephants graze in the same areas where wild elephants and other wildlife live. They also are kept in open air, a framed shelters resulting in common interactions, such as fighting or breeding between wild and captive elephants. Captive elephants particularly in the private sector are also in contact with domestic livestock.

Although the health status of wild elephants in Nepal is unknown, a previous study examined the health of randomly selected captive elephants in and around the CNP. Based on physical examinations, body condition scores, complete blood counts, serum chemistry, urinalysis and faecal examinations, the captive elephants examined appeared to be rather healthy. They have regular access to veterinary care and are routinely dewormed and vaccinated for rabies and foot and mouth disease. Despite all of this, there have been four deaths within the captive

population in the last three years (Personal Communication). Postmortem examination revealed tuberculosis as the cause of death.

Tuberculosis (TB) has been described in domesticated elephants as long as 2000 years ago (Mikota *et al*., 2000). The most common signs of TB are vague including chronic weight loss, anorexia, and weakness with occasional dyspnoea or coughing. Exercise intolerance may be seen in working elephants. Diagnosis and management of TB has been identified as a research priority by the Elephant Species Survival Plan (Oslon, 2004). Thirty-one cases have been identified in North America since 1994. Of the approximately 250 Asian elephants in North America, 12% have been diagnosed with TB since 1994. Elephants have been infected primarily with *Mycobacterium tuberculosis*, the human strain. Routine intradermal tests used for human and bovine have proven unreliable in elephants; therefore the gold standard adopted by the American zoological community is to culture respiratory secretions collected from "trunk washes" (Mikota *et al*., 2000). TB positive elephants should be considered as potential risk for disease transmission to humans as a study has revealed the same strain of *M. tuberculosis* on molecular analysis of the TB cultures from the TB positive elephants and handler.

With one third of human population infected, TB kills over 2000 people each day in the Southeastern Asia region, representing 40% of the world's TB cases. TB is not only of medical importance but also of economic importance as 80% of TB victims are in their most economically productive years of life and treatment can become expensive or unattainable with the development of multi-drug resistant strains. With the possibility of one infected person infecting 10-15 other people, TB can be devastating to densely populated developing countries (WHO, 2001). The World Health Organization (WHO) started the Directly Observed Therapy Short-course (DOTS) program to help decrease the prevalence of TB. In 2004, out of 246, 09,000 people, new TB cases have been estimated to be 46,714 and new smear positive TB cases have been estimated to be 20,931 (SARRC Tuberculosis Center, 2004).

To date, captive elephants in Asian countries have not been systematically evaluated for TB. The close proximity to human, the high prevalence of TB among humans and intermingling of captive and free-range elephants in some areas make TB a serious threat to this endangered species. As mentioned above, recent studies in the US have shown that TB is passed between captive Asian elephants and their handlers as a zoonotic disease. These results reveal the urgent need for research in the developing countries like Nepal that are home to both captive and wild Asian elephants and a large population of TB infected people.

Intradermal testing in Asian elephants has not had correlation with culture results. Thoracic radiographs in a 6000 to 12000 pound animal are not realistic. A nucleic acid amplification test (NAAT) is currently used in humans to diagnose *M. tuberculosis* complex (the mammalian pathogens), which includes *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. mircroti*. These different species are all included in the reported 95% specificity of NAAT when compared with acid fast sputum smears and culture. This test would not be specific enough for the diagnosis of *M. tuberculosis* in elephants. ELISA test with gamma interferon technique is promising but not currently validated in elephants. Diagnosis of TB in elephants is challenging. As a zoonotic disease and a contagious and fatal disease in elephants, it is extremely important to diagnose and control. Early diagnosis would be very advantageous to minimize disease spread among

people and elephants, manage captive elephant populations and improve the chances for curative treatment of the disease.

This study is aimed to know if basic hematology and serum biochemistry tests can be used to assist in making an early diagnosis of TB in elephants.
These tests are routine, simple and inexpensive to perform. To date, there has not been much study regarding the relationship between hematological and serum biochemical parameters previously reported to differ (Harr *et al*., 2001) between TB positive and clinically normal elephants have not been repeated. Therefore, the present study was aimed to evaluate the health status of captive elephants in Nepal with the following objectives:

- To find out the prevalence of tuberculosis among captive population of elephants in Nepal by culture (the technique currently recommended by the United States Department of Agriculture (USDA).
- To assess the relationship between haematological and serum biochemical parameters and *Mycobacterium* culture status.

MATERIAL AND METHODS

Study site

Chitwan National Park (CNP) established in 1973 and accredited as the World Heritage Site in 1984 was the study site. The park is situated in South Central Nepal, covering 932 square kilometers in the subtropical lowlands of Chitwan, Nawalprasi, Makwanpur and Parsa Districts of Inner Terai. In 1996, an area of 750 square kilometer surrounding the park, consisting of forests and private lands, was declared a buffer zone. It is located at 141 to 196 meters above sea level. The park consists of diversity of ecosystems, including Churia hills, ox bow lakes and the flood plains of the Rapti, Reu and Narayani rivers.

Methodology

The study was conducted from January, 2006 to August, 2006. One hundred and eight captive elephants, 32 inside the CNP and 76 in the park buffer zone comprised the study population. A photo identity and written record was established on each elephant. Microchip numbers were noted for easy follow up of individual elephant.

Trunk wash sampling

Samples for Mycobacterial culture were collected according to standard procedures (Isaza *et al*., 1999) with modification. Three samples were collected in a one-week period from each elephant on non-consecutive days. Water was withheld for 2 hours prior to sampling to reduce dilution and contamination. Each of the elephants were allowed to suck 500-1000 ml of sterile saline from the separate bucket, except in two elephants in which 500 ml sterile saline was instilled into the trunks. The trunk was elevated and the elephant was allowed to exhale into a one-gallon zipper lock plastic bag. The sample was divided and transferred to 50 ml screw top tubes, placed on ice stored at -20°c until analysis.

Blood collection

Approximately 35 ml of blood was collected from each elephant via the auricular vein placed into EDTA tubes for hematology and serum separator tubes for biochemical analysis using 21 gauze winged blood collection sets. Serum was separated within an hour and transferred to cryovials and labeled appropriately to provide samples for biochemical profile.

Mycobacterium Culture

Elephant trunk washes for the isolation of *M. tuberculosis* were processed in National Tuberculosis Centre according to the following standard procedure as explained by Della Latta and Weitzman (1998).

a. The 10 ml of trunk wash was carefully poured into a 50 ml conical centrifuge tube.
b. At the time, 10 ml of sterile distilled water was also poured into another 50 ml conical centrifuge tube; the sample was labeled as "negative control" and was processed in the same as the rest of the samples.
c. The 10 ml of trunk wash was pulse spinned in the 50 ml centrifuge tube to spin down excess sediment. This was accomplished by centrifuging for 1 minute and 40 seconds at 3000 RCF, using the LX- 130 Multipurpose Refrigerated Centrifuge made by Tomy, Japan.
d. The supernatant was slowly poured into a sterile 50 ml conical centrifuge tube, trying not to disturb the sediment.
e. The N-Acetyl-L- Cysteine (NALC) - sodium citrate solution was poured into the equal amount of trunk wash supernatant up to a maximum to 10 ml using a sterile pipette.
f. Vortex for 20 seconds.
g. The trunk wash was allowed to remain in contact with NALC for 10 to 15 minutes.
h. Enough sterile distilled water was added to the NALC/wash solution to fill the centrifuge tube.
i. The water/NALC/wash solution was centrifuged for 20 minutes at 6000 g and 10°c.
j. The supernatant was carefully poured off and discarded.
k. The sediment was re-suspended with 2 ml of sterile distilled water and 1ml of the Johnes antibiotic mixture.
l. Re-suspended mixture was vortexed for 20 seconds.
m. Overnight incubation was vortexed for 20 seconds.
n. Then 0.5 ml the processed sample was pipetted out and inoculated into the Ogawa medium, one sample into two slants.
o. The inoculated solid media was placed on a slant rack and incubated overnight at 37°c.
p. After being incubated on a slant rack, the media tubes were stored in the 37°c incubator in an upright position for 8 weeks.
q. Tubes were read at the first, fourth and eighth week.
r. Reading and recording the results was done and the tubes with no suspicious growth for Mycobacterium were discarded.
s. Then the remaining samples with positive growth were examined by acid fast staining using standard method recommended by Fujiki (2001).
t. A niacin test on all positive cultures was done by "Aniline method" as recommended by Fujiki (2001) for the confirmation of *M. tuberculosis*.

Haematological Analysis

a. Packed Cell Volume (PCV) was determined by microhematocrit method.
b. Haemoglobin (Hb) was determined by Cyanmethoglobin method.
c. Mean corpuscular haemoglobin concentration (MCHC) was calculated by the formula: MCHC = 100 × Hb% in gm/ PCV.
d. Total Leukocyte Count (TLC) was done manually using Thomas WBC counting pipette, and 2% glacial acetic acid as a diluent.

Serum biochemical analysis

a. Quantitative determination of Albumin and Total Protein was done by using an auto analyzer according to the procedure recommended by Kalpan and Szabo (1983).
b. Globulin and A/G ratio was calculated by subtracting albumin from total protein. And A/G ratio was calculated by dividing albumin by globulin.
c. Serum glucose was determined by glucose oxidase, hydrogen peroxide (Trinder) method using the Hitachi 747 machine.

RESULT AND DISCUSSION

Culture and acid fast microscopy

Out of 108 samples cultured for Mycobacterium, growth was found in 10 samples and was verified by acid fast staining. Most of the growth colonies were buff colored.

Niacin Test

Out of 10 acid fast positive samples subjected to niacin test, 4 samples were found positive to Niacin test indicating 4 elephants being infected with *M. tuberculosis*, the human strain.

Prevalence of TB according to age, sex and origin

The present study showed 9.25% prevalence of Mycobacterium infection by culture among samples collected from 108 captive elephants of Nepal (Table 1). Prevalence rate of *M. tuberculosis* infection was found to be 3.7%. This prevalence rate is higher than the prevalence rate (3%) reported in North America by Mikota *et al* (2001). Remaining 5.55% Mycobacterium infections confirmed by cultural characteristics and acid fast staining could be either due to *M. bovis* or due to *M. avium* or due to *M. africanum* or *M. mircroti*. Instead of using medium recommended by NVSL protocol, Ogawa medium was used. This challenges the accurate result.

Mycobacterial culture as the primary method of detecting infected animals has several limitations. It has some biological assumptions. The first assumption is the most infected elephants have respiratory infections. Although the literature suggests that most infected animals shed Mycobacterial organisms into the respiratory tract. There is little data that determines if and when an infected animal will begin shedding. It is unknown what proportion of elephants can carry latent infections that would be mixed with culturing techniques. A third assumption is that the animals that are shedding will pass Mycobacterial organism at least once in three day testing periods. Currently, it is unknown if shedding animals pass organisms periodically or continuously. Finally, the samples collected from the distal trunk are often contaminated with normal bacterial flora and foreign material. It is assumed that these contaminants do not routinely overgrow or mask the growth of the pathogenic Mycobacterium although no studies have tested this assumption. So, interpretation of the culture result is

limited. However, a positive culture is the strong evidence that the animal is shedding Mycobacteria and is infected; negative culture results provide little information as to whether the elephant is infected or not.

Table 1. Prevalence of Mycobacterium infection among elephants of different age, sex and origin

Factor		**No. of elephants**	**TB positive**	**Prevalence Percentage**	**Chi-square**	**Relative Risk**
Age	1-9 years	6	1	16.66		
	9-30 years	17	1	5.88		
	> 30 years	85	8	9.41		
	All ages	108	10	9.25		
Sex						
	Male	15	3	20		
	Female	93	7	7.52		
	Association with culture				1.14 (p=0.28)	2.66
Origin						
	Nepal	15	4	26		
	India	90	6	6.66		
	Thailand	2	0	0		
	Burma	1	0	0		

The higher prevalence (26%) of tuberculosis among captive elephants originating in Nepal could be due to possible transmission of *M. tuberculosis* from elephant handlers as there is high prevalence of tuberculosis among Nepalese people (190 TB cases per 100, 000 Nepali). The incidence rate (46,714 new TB cases per 24,609,000 Nepalese people) is also high (STC, 2004). Transmission of *M. tuberculosis* between elephants and human in captive elephants in North America has been established by DNA finger printing (Michalak *et al*., 2002). Consequently, it is of utmost importance to monitor TB infections in elephants care takers to prevent Mycobacterium negative elephants from being infected and to prevent caretakers from becoming infected from positive elephants. Recommendations to break this cycle should include routine TB testing for all elephants' caretakers and elephants and isolation and treatment of Mycobacterium positive elephants. In addition, the prevalence of Mycobacterium infections in the captive elephants may present a risk for free ranging elephants, as free ranging elephants come in frequent contact with the captive elephants for breeding purposes. A study of Mycobacterium infection status in free ranging elephants may be warranted but would present considerable challenges.

The present study shows that Mycobacterium infection is prevalent in all age groups and both sexes of elephants. A mycobacterium infection is prevalent in the elephants originating in Nepal and India. Approximately 83% of captive elephants in the study population originated from India. In India, 3 cases of tuberculosis in captive Asian elephants from Kziranga National Park, Assam (Chandrashekharan, 1998) and five cases from Kerala have been reported (Gangadharan *et al*., 1996). Therefore, the prevalence of tuberculosis (6.66%) among the elephants originating from India in the present study may be either due to their exposure to TB

infected elephants or elephant handlers during their stay in India or due to their stay in India or due to their exposure to TB infected elephants or people in Nepal. Preliminary testing in 48 elephant handlers working with the study population revealed a 4.16% prevalence rate of *M. tuberculosis* infections.

The RR of 6.6 for association of sex with *M. tuberculosis* indicates that male elephants have 6.6 times more probability to have TB infections than female elephants. However, the association of sex factor with Mycobacterium infection ($p > 0.05$) is insignificant.

Association of various haematological and biochemical parameters with culture

The association of haematological and serum biochemical parameters with mycobacterium culture status was analyzed with Chi-square test and P-value and relative risk (RR) (Tables 2 and 3). Statistical analysis for the association was done using Microsoft Excel and SPSS Computer software. Reference ranges for standard physiological data values (all ages and both sexes combined) for the parameters under study were taken for comparison with the obtained values, from International Species Information System (ISIS).

Table 2. Mean ± SD and Range of haematology and serum biochemistry of 108 elephants

Parameters	**Unit**	**Mean ± SD**	**Range**
Haemoglobin (Hb)	(gm/dl)	11.88±1.91	6.92-16.08
PCV	%	42.61±5.23	31-55
MCHC	Gm/dl	28.19±4.97	14.41-39.77
TLC	/Cu mm	15423.15±5297.75	2750-32600
Serum glucose	Mg/dl	82.40±26.19	24-156
Total protein	Gm/ dl	8.27±.839	5.8-10.7
Albumin	Gm/ dl	2.81±0.37	1.8-3.5
Globulin	Gm/ dl	5.45±0.74	3.6-8.1
A/G ratio		0.69±0.17	0.4-1.4

Table 3 Association of haematological and biochemical parameters with positive TB culture

Parameters	**Chi square value**	**P Value**	**Relative Risks**		
			RR	LB	UP
Haemoglobin (Hb)	0.02	0.88	1.17	0.32	4.22
PCV	0.39	0.52	3.93	0.24	64.55
MCHC	0.28	0.59	1.14	0.28	4.69
TLC	0.34	0.56	0.57	0.17	1.92
Serum glucose	0.93	0.330	1.47	0.46	4.74
Total protein	3.42	0.06	2.85	0.78	10.39
Albumin	0.05	0.81	1.54	0.23	10.48
Globulin	1.13	0.28	11.9	0.72	196.64
A/G ratio	0.07	0.79	1.77	0.48	6.46

Chi-Square value (Table 3) as a measure of association of the mentioned biochemical and hematological parameters with culture was less than 3.84, and the p-value was greater than 0.05, so the association was not found to be significant for each of the parameters under study. However, the p-value is 0.06 for total protein, so its association with culture status is near to significant and warrants further investigation. RR value (Table 3) for all the parameters under

study, except for TLC, is greater than 1. The RR is the measure of the magnitude of the association between two variables i.e. parameters under study with culture. Since RR for globulin is 11.9, mycobacterium culture positive elephants have 11.9 times higher probability to have elevated globulin. Similarly, TB positive elephants have 3.93, 1.47, 2.85 and 11.9 times more probability to have elevated PCV, glucose, total protein and globulin, respectively. While Hb, MCHC, Albumin and A/G ratio have 1.17, 1.14 and 1.54 and 1.77 times more probability to have decreased values.

Unlike the findings by Harr *et al* (2001) who found significantly lower ($p< 0.05$) MCHC, glucose and A/G ratio in TB positive elephants, the present study did not show this association to be significant ($P> 0.05$). However, the sample size of Harr *et al* was only 5 infected elephants in a population of 20 whereas sample size in present study was 10 out of 108. So, the result from the present study could be more accurate. Other parameters under study such as globulin, albumin, total leukocyte count, PCV and Hb also failed to show significant association with culture status. A similar finding with those latter parameters has been reported by Harr *et al* (2001). However, when calculating RR for these parameters, the measure of size of association is greater than 1 for total protein, PCV, globulin and glucose indicating that there is a probability of those parameters being elevated in Mycobacterium culture positive elephants. RR is also greater than 1 for hemoglobin (Hb), MCHC, albumin and albumin: globulin (A/G) ratio indicating more probability of those values being decreased in culture positive elephants. The relative risk finding for association of MCHC, A/G and glucose with culture results are similar to the findings of Harr *et al* (2001).

The lack of insignificant correlation ($P>0.05$) of haematological and serum biochemical parameters with culture indicates that those parameters may not be predictive of Mycobacterium infections. However, the observed trends may be used to support further investigation for disease with limited confidence. The routine hematology and biochemistry tests considered in the present study are not very useful in predicting TB infection in elephants. This is probably because of the chronic and complex nature of this disease in elephants where clinical manifestations are quite varied and unpredictable. In many cases animals diagnosed at necropsy are suddenly found dead without previous clinical disease. Elephants in this study with positive TB culture results all had body condition scores greater than 10 except for one animal. Few animals had notable clinical disease issues greater than cracked nails. In future studies, additional biochemical parameters, such as plasma protein electrophoresis, hepatic enzymes and electrolytes should be examined for association with TB infection. A differential white blood cell count should also be conducted to determine if particular white blood cell types may be associated with infection, as seen by Harr *et al* (2001). Differential counts in the present study were considered too inconsistent to be used for analysis.

Published reference ranges for captive elephants in Nepal were not available for this study. The reference values used were from the captive North American Population (ISIS) and may not be accurate for the Nepal captive population. Further investigation to determine appropriate reference ranges for this population is warranted and when available, could be used to reassess the findings of the current study.

CONCLUSION

From the present study it is concluded that there is prevalence of tuberculosis in Asian captive elephants in Nepal. *M. tuberculosis*, the human strain found in 3.7% elephants indicates that tuberculosis could have been transmitted to elephants from human beings. Consequently, continued tuberculosis monitoring of elephants and their caretakers is one of the major issues to pursue. Isolation and treatment of infected individuals should also be considered. Recommendations to break this cycle should include routine TB testing for all elephant caretakers and elephants , and isolation and treatment of Mycobacterium infection in the captive elephants may present a risk for free ranging elephants as they may come in frequent contact with the captive elephants for breeding purposes. So, a study of Mycobacterium infection status in free ranging elephants may be warranted although it would present considerable challenges.

From the relative risk analysis (RR>1), males have higher probability of getting Mycobacterial infection. There is no significant influence of Mycobacterium infection on haematological and serum biochemical parameters although trends were seen based on relative risk that may be useful in leading to a diagnosis. These findings are consistent with the unpredictable and insidious nature of this disease in elephants. Accurate and early diagnosis of tuberculosis in elephants remains a challenging prospect. Further investigation into other biochemical parameters and new advanced serologic technologies is pursued aggressively.

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STUDY OF SENSORY CHARACTERISTICS AND QUALITY EVALUATION OF CHICKEN DRIED MEAT (SUKUTI)

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ABSTRACT

A study of process optimization of chicken dried meat "sukuti" was carried out by optimizing the most favorable recipe, temperature and packaging material. The organoleptic qualities by sensory evaluation were studied. All these parameters were studied on 0 day, 30 day, 60 day and 90 day at room temperature (25 ± 3^0C) storage and were analyzed statistically. Out of four recipes, one recipe was selected through sensory evaluation by panelist through offering the score for appearance, flavor, tenderness, juiciness, over all acceptability and odor. Flavor was found to be highly significant (p<0, 01) difference among the recipes. The sensory evaluation showed that the score of appearance, flavor, tenderness, juiciness, over all acceptability and odor were highly significant (p<0.01) with storage periods among 0, 30, 60 and 90 days. However, the organoleptic qualities did not show any significance (p<0.001 or p<0.05) difference among temperature and packages. This study also revealed that 100℃ temperature and aluminum laminate were the most suitable for chicken dried meat though there were no sign of spoilage in any of the samples of all different temperatures and packages. Alumunium laminate packaging was the most suitable for chicken dried meat.

Hence, it is recommended that chicken dried meat can be prepared as light weight preserved protein food with high nutritive value and without any quality deterioration up to 90 days of storage.

INTRODUCTION

Nepal is a land-locked country of diversified geographical distribution ranging the altitude from 60 meter up to the highest point of the world 8848meter. It is located between India and China. The population of Nepal is 23.1 million (population census, 2001). Livestock is an age old occupation of Nepalese people under mixed farming system of agriculture. Agriculture is a largest sector contributing 39% to GDP (MOAC, 2004) and engaging65.66% (Agriculture Diary, 2011) of population. Livestock sector contribute 27% of AGDP (MOAC, 2010).The available data indicates that the annual meat production 250.2 thousand metric ton in which buffalo meat contributes the largest supply (64.88%) followed by goat (20.10%), poultry (7.01%), pig (6.81%), sheep (1.08%) and duck (0.09%).(Agriculture Diary, 2011)

Sukuti (dried meat) is a simple and low cost method of meat preservation. Sukuti is generally prepared from buff meat, goat meat, pork and rarely with wild dear in Nepal. However, nowadays chicken meat and its products are quite popular among urban and rural people. Chicken sukuti could be a generous product among people living in both rural and urban areas as people aim to preserve the chicken meat at the time of surplus production and can utilize in lean period for their best supplementation of protein in their diet. Acceptance of chicken into

the diet of Nepalese households has significantly increased over the last four decades. Rearing of chicken and consumption of chicken and their products are no more confined to certain ethnic group (Bhurtel and Shaha, 2000).

Based on recent trends, there is an increasing demand of meat which suggests that current levels of consumption are 9.4 kg /person /year. This is still less than the 14 kg generally considered necessary for balanced diet (TLDP, 2003).Nutritional composition of chicken meat shows moisture (74 %), protein (18.5%), fat (6%), Ash (0.80%), and energy 125 cal /100g. (Sharma, 1999).

MATERIALS AND METHODS

A study on process optimization and quality evaluation of chicken dried meat (sukuti) was carried out in the meat technology laboratory of Himalayan college of Agriculture sciences and Technology (HICSAT), Central Veterinary Laboratory, Tripureswor and Livestock Product Quality Management Laboratory, HariharBhavan

The Live healthy poultry birds were procured at the age of 42 days by selecting the healthy and active bird. The ante mortem of the live birds was performed and the defective bird was nil at the time of examination. Fresh chicken meat of breast region and leg pieces was procured. The chicken meat with the least subcutaneous fat was taken and kept into freezer for over 6 hours. Proper labeling was done on the spot with specification of date, time, age of the birds, place etc.

The collected meat was thoroughly washed under clean water to remove dirt, hair, blood or any extraneous matters if present. Trimming was carried out to recover lean meat. During trimming, bones, connective fattening tissues, tendons and adhered dirt, skin were separated from the meats. Strict hygienic measures were followed while handling the carcass for deboning and cutting. The lean meat was hard frozen in deep freezer prior to slicing.

Process of Preparation of Chicken Sukuti

After frozen for 8 hours, the chicken meat was brought outside on laboratory table using all the preventive measures to protect the meat from contamination. The tables, cloths, equipments, hands were cleaned and sterilized properly. The entry of outside people was prohibited.

The deboned meat was diced into convenient sizes of pieces (meat slices of 5 mm uniform thickness or sized pieces of about 5 g each.) The meat was sliced with knife on the cutting board or with any other suitable chopping boards. The ready to eat chicken sukuti was prepared from different four recipes to establish the process of the preparation.The receipe no.1 was the mixtures of table salt 14g, chilly powder 7g, meat masala 3g, turmeric 3g , coriander 7g, Vinegar(10%) 10ml with 1 kg meat (Halwai, 2005). The receipe no.2 contains salt 30g, black peeper 15g, cumin 7g, cloves 3g, cinnamon 7g, red chillies 35g, coriander 25g, aniseed 5g, turmeric 3g , preservatives 0.75g ,ginger 100g, garlic 200g with chicken meat 1.5 kg(Ramani, 2004). The third receipe was chicken meat 1kg, red chilli 25g, salt 22g, cumin seed 5g, black peeper 12g (Everest Dry meat,2006). The fourth receipe was the use of 14% salt in chicken meat (FAO,2005)

The spiced meat was marinated and conditioned in refrigerator at 4°c for 24 hours. The conditioned meat was cooked to an internal temperature 69-71°c. The seasoned meat was cooked with its contents and spice gravies. The water was also added whenever it was required.

After cooking the meat pieces were spread in aluminum tray and dried in a baking oven at 100°c for 3 hours. Finally, the dried meat was cooled to a room temperature (25±3°c). The product was then packaged in an aluminum laminate (AL) and low density polyethylene (LDPE) 100 gauge plastic (PL) and stored at room temperature (25±3°c). The product was packaged by using vacuum packaging machine in the laboratory.

Optimization of suitable recipe by sensory evaluation

Two sets of four recipes were analyzed through organoleptic qualities of the dried meat. A team of sensory panelist was invited. This panel comprised of well experienced, having at least graduate education in the field of Food science, Veterinary science and Agricultural science professionals and academicians. A 9 point Hedonic sensory evaluation as well as 10 point scale odor score , described by Pearson (1968) were applied to assess the parameters of eating qualities like appearance, flavor, tenderness , juiciness, over all acceptability and odor by 10 experienced panelists. The panelists were briefed about the objectives of the experiment and the attributes to be evaluated. The samples were coded so as to mask the identity of the samples. Care was taken to provide uniform sized ready to eat chicken dried meat on white porcelain plates for judging. The sensory evaluation was carried in a closed room, with single panelist to be entered and held between 12 noon to 2P.M.

Each Panel was provided with "sensory Evaluation score Card "to mark their observations and preferences in the appropriate box provided against each attributes of particular sample to which it was fitting into. Care should be taken to provide uniform sized ready to eat chicken sukuti on white porcelain plates for judging. Totally 6 samples were provided (2x3) i.e. two treatment (packaged into plastic and Aluminum laminate) and three drying temperatures (80,100and120°c). Best of them were chosen based on the overall acceptability scores awarded by the technical trained sensory panel using and nine point Hedonic scale (9 is extremely desirable and 1 is extremely poor) score card.

The organoleptic acceptability of the stored samples of chicken sukuti on 0, 30,60 and 90 days were judged by a trained sensory panel of 10 members by assessing the odor score and by awarding marks as a 10 point scale, described by Pearson (1968). A descending numerical rating was given to lesser acceptable samples and putrid odor was at the bottom of the score card with one point rating.

Statistical Analysis

The data obtained in this study are subjected to statistical analysis as per the method outlined by Snedecor and Cochran (1994) .The data obtained from sensory evaluation were statistically analyzed for Friedman Chi squared rank sum test at 5% and 1% level of significance (by using 'R' programme software.). TO analyze the parameters of sensory qualities, the significance test was done by Analysis of Variance by using Genstat Release 4.21 fifth edition (PC/Windows XP) .The results were expressed in mean±SE. The statistical analysis was performed by using 'R' programme software, spreadsheet of Microsoft Excel and Genstat fifth

Edition (Service Pack 1).The statistical design was Completely Randomized Design with 2x3x 4 factorial analysis. Minimum three samples were analyzed for each parameter in every applied storage period.

RESULTS AND DISCUSSION

Sensory evaluation is defined as a scientific discipline used to evoke measure, analyze and interpret results of those characteristics of foods, and as they are perceived by the senses of sight, smell, taste, touch and hearing. (Dharam Pal *et al.*, 1995). According to Vasundhara *et al.*, (1988), when total counts are less than 10^8 organism /g , the quantities of Free Fatty Acids have been found to be between 1000 to 1800mg/100g lipid and the product did not posses any off odor but an increase in total counts from 10^8-10^9 drastically brought about irreversible flavor determination which was detectable in the product . Between 10^8 and 10^9, there is an appreciable increase in lipolytic activity which leads to faster accumulation of FFA, as well as proteolysis activity, which leads to accumulation of compounds causing off odor and unacceptability of the product.

Selection of Recipe by Sensory Evaluation

The data obtained through sensory evaluation of four different recipes was analyzed by using R version 2.4.0:2006 The R foundation for Statistical computing. The statistical tool applied was 'Friedman test' which gave chi-squared value for significance (P<0.01).

The chi- squared 'p' value of flavor was 0.009668 which was highly significant (p<0.01).
Median score values of recipes 2 and 3 were the same i.e. 8.5 in the case of flavor attribute of dried meat. The standard deviations of recipes 2 and 3 were 1.3333333 and 1.61932771 respectively. Lesser the value of the standard deviation better is the recipe and hence recipe no.2 became the most suitable recipe for processing of the dried meat.

Table No: 1 Friedman rank sum test for ranking the recipes:

Organoleptic qualities	Friedman Chi-squared	df	p-value
Appearance	5.5125	3	0.1379
Flavor	11.4179	3	0.009668**
Tenderness	3.069	3	0.3811
Juiciness	2.1724	3	0.5374
Overall acceptability	5.8846	3	0.1174
Odor	1.7885	3	0.6174

**- highly significant

Sensory Evaluation:

1. Appearance:

The over all period means in two different packages of AL and PL were 6.833,7.967,7.950 and 7.533 for 0, 30, 60 and 90 days of storage respectively. The over all mean value of different temperatures in AL package were 7.40, 7.875 and 7.575for 80°c, 100°c and 120°c respectively. Similarly, the over all mean of different temperatures in PL package were7.550, 7.575 and

7.450 for 80°c, 100°c and 120°c respectively. The standard error was ±1.182 and the percentage of CV was 16.5%.Grand mean of appearance was 7.571. It increased as the storage period advanced but it decreased after 30 days of storage. For different drying temperatures of 80°c, 100°c and 120°c, the mean of appearance score were found to be 7.475, 7.725and 7.512 respectively. The mean of Appearance score of packages were 7.617 and 7.525for AL and PL respectively.The analysis of variance revealed that Appearance score of the dried meat was significant ($p \leq 0.01$) for source of variation like; Storage periods.

The drying temperature of 100°c was found to have the highest Appearance score of the dried meat among all temperatures applied for optimization of chicken dried meat. By comparing the Appearance score of two different packaged dried meats, it was found that AL had higher Appearance score than that of PL. For any meat food product to enter into the market and become popular, its eating quality characteristics have to be tuned to the needs and preference to the consumers." appearance of the product plays a very crucial role in earning consumer's acceptability.

2. Flavor:

The over all period means in two different packages of AL and PL were 6.617,7.917,7.433 and 7.617 for 0, 30, 60 and 90 days of storage respectively. The over all mean value of different temperatures in AL package were 7.575, 7.550 and 7.450 for 80°c, 100°c and 120°c respectively. Similarly, the over all mean of different temperatures in PL package were7.175, 7.250and 7.375 for 80°c, 100°c and 120°c respectively. The standard error was ±1.029and the percentage of CV was 14.7%.Grand mean of flavor score was 7.396. It increased as the storage period advanced. For different drying temperatures of 80°c, 100°c and 120°c, the mean of flavor score were found to be 7.375, 7.400and 7.412 respectively. The mean of flavor score of packages were 7.525 and 7.267for AL and PL respectively. The analysis of variance revealed that flavor score of the dried meat was significant ($p \leq 0.01$) for source of variation like; Storage periods.

The drying temperature of 120°c was found to have the highest Flavor score of the dried meat among all temperatures applied for optimization of chicken dried meat. By comparing the Flavor score of two different packaged dried meats, it was found that AL had higher Flavor score than that of PL. Flavor is a complex sensation involving odor, taste, texture, temperature and pH of the product. In this study, the mean value of flavor score was of the highest value and maintained up to the 90 days of storage. Though chicken meat having high level of MUFA and low level of tocopherols but the product did not show any sign of off flavor which may be due to the addition of several antioxidant agents in spices and condiments at one hand and dehydration beyond the moisture level 4-5% also helped to prevent the microbial spoilage. The selection of less fat, lean meat for the production of dried meat would reduce the occurrence of rancidity.

3. Tenderness:

The over all period means in two different packages of AL and PL were 6.650, 7.567,7.817 and 7.650 for 0, 30, 60 and 90 days of storage respectively. The over all mean value of different temperatures in AL package were 7.450, 7.625and7.425 for 80°c, 100°c and 120°c respectively. Similarly, the over all mean of different temperatures in PL package were7.350, 7.475and

7.200 for 80°c, 100°c and 120°c respectively. The standard error was ±1.013and the percentage of CV was 14.4%.Grand mean of Tenderness score was 7.421. Initially it increased as the storage period advanced but later on it decreased after 2 months of storage. For different drying temperatures of 80°c, 100°c and 120°c, the mean of Tenderness score were found to be 7.400, 7.550 and7.312 respectively. The mean of Tenderness score of packages were 7.500 and 7.342for AL and PL respectively. The drying temperature of 100°c was found to have the highest Tenderness score of the dried meat among all temperatures applied for optimization of chicken dried meat. By comparing the Tenderness score of two different packaged dried meats, it was found that AL had higher Tenderness score than that of PL. Tenderness is rated by consumers as one of the most important factor of eating quality of meat and meat product. Sensory evaluation is a valuable tool in solving problems involving food acceptability and is useful in product improvement, quality maintenance, new product development and market research.

4. Juiciness

The over all period means in two different packages of AL and PL were 6.583, 7.417, 7.383and 7.583 for 0, 30, 60 and 90 days of storage respectively. The over all mean value of different temperatures in AL package were 7.375, 7.400 and 7.125 for 80°c, 100°c and 120°c respectively. Similarly, the over all mean of different temperatures in PL package were7.175, 7.350 and 7.025 for 80°c, 100°c and 120°c respectively. The standard error was ±0.00and the percentage of CV was 14.9%.Grand mean of Juiciness score was 7.242. Initially it increased as the storage period advanced but later on it decreased after 2 months of storage. For different drying temperatures of 80°c, 100°c and 120°c, the mean of Juiciness score were found to be 7.275, 7.375 and7.075 respectively. The mean of Juiciness score of packages were 7.300and 7.183 for AL and PL respectively. The drying temperature of 100°c was found to have the highest Juiciness score of the dried meat among all temperatures applied for optimization of chicken dried meat. By comparing the score Juiciness of two different packaged dried meat, it was found that AL had higher Juiciness score than that of PL.

Juiciness is an attribute of eating quality which is reflected by water holding capacity of meat. Juiciness includes moistness of the product during initial phase of mastication as well as just prior of swallowing. There was no significant difference in the sensory indices with respect to juiciness. It was proved that the degree of shrinkage on cooking was directly correlated with loss of juiciness to the palate. The first was the impression of wetness during the first few chews and was produced by the rapid release of meat fluid; the second was one of sustained juiciness, due to stimulatory effect of fat on salivation. In this study too, juiciness was given the higher score of grand mean value 7.242, which was the value of highest quality of meat product in terms of juiciness quality of sensory evaluation.

5. Overall Acceptability

The over all period means in two different packages of AL and PL were 6.667, 7.650, 7.767 and 7.650 for 0, 30, 60 and 90 days of storage respectively. The over all mean value of different temperatures in AL package were 7.625, 7.525 and 7.400 for 80°c, 100°c and 120°c respectively. Similarly, the over all mean of different temperatures in PL package were7.275, 7.475 and 7.300 for 80°c, 100°c and 120°c respectively. The standard error was ±0.970and the percentage of CV was 15.1%.Grand mean of over all acceptability score was 7.433. Initially it

increased as the storage period advanced but later on it decreased after 2 months of storage. For different drying temperatures of 80°c, 100°c and 120°c, the mean of over all acceptability score were found to be 7.450, 7.500 and 7.350 respectively. The mean of over all acceptability score of packages were 7.517and 7.350 for AL and PL respectively. The drying temperature of 100°c was found to have the highest over all acceptability score of the dried meat among all temperatures applied for optimization of chicken dried meat. By comparing the score of over all acceptability of two different packaged dried meats, it was found that AL had higher over all acceptability score than that of PL.

The over all general appealingness, palatability characteristics and receptivity of the product were evaluated by over all acceptability ratings. The foregoing attributes together with personal liking of the product might have influenced the assessment of over all acceptability ratings for chicken dried meat. In this study, the grand mean of over all acceptability was 7.433 which were the score of highest sensory quality of dried meat. It is the valuable tool in solving the food acceptability by several researchers.

6. Odor Evaluation

The over all period means in two different packages of AL and PL were 6.967, 7.783, 7.533and 7.417 for 0, 30, 60 and 90 days of storage respectively. The over all mean value of different temperatures in AL package were 7.350, 7.650 and 7.525 for 80°c, 100°c and 120°c respectively. Similarly, the over all mean of different temperatures in PL package were7.325, 7.575 and 7.125 for 80°c, 100°c and 120°c respectively. The standard error was ±1.062 and the percentage of CV was 15.1%.Grand mean of Odor score was 7.425. Initially it increased as the storage period advanced but later on it decreased after 1 month of storage. For different drying temperatures of 80°c, 100°c and 120°c, the mean of odor score were found to be 7.338, 7.612and 7.325 respectively. The mean of odor score of packages were 7.508and 7.342 for AL and PL respectively.

The drying temperature of 100°c was found to have the highest Odor score of the dried meat among all temperatures applied for optimization of chicken dried meat. By comparing the score of Odor score of two different packaged dried meats, it was found that AL had higher Odor score than that of PL.

It has been observed by the researcher that when total counts are less than 10^8 organism /g, the quantities of FFA have been found to be between 1000 to 1800mg/100g lipid and the product did not posses any off odor. In this study, the TPC did not exceed the limitation of spoilage and the development of the rancidity was not observed, hence there was high grand mean score of Odor during sensory evaluation by the panelists up to the storage periods of 3 months.

Correlation Study of sensory Characteristics

Flavor had the highly significant ($p<0.01$) positive correlation with Appearance and Juiciness. Odor also showed the highly significant positive correlation with appearance, flavor and juiciness. Over all acceptability showed the highly significant positive correlated to appearance, flavor, juiciness and odor. Tenderness of the dried meat was highly significant positive correlated with appearance, flavor, juiciness, and odor and over all acceptability. In conclusion, it had been shown that all the sensory indices were highly significant positive correlated with

each other. Due to their high quality score to the chicken dried meat, they established the highly significant positive correlation.

Table No.2. Correlation Matrix:

	Appearance	Flavor	Juiciness	Odor	Over all acceptance	Tenderness
Appearance	1.00					
Flavor	0.596**	1.00				
Juiciness	0.340**	0.465**	1.00			
Odor	0.583**	0.590**	0.319**	1.00		
Overall acceptance	0.586**	0.640**	0.497**	0.640**	1.00	
Tenderness	0.459**	0.475**	0.510**	0.463**	0.602**	1.00

*-Significant ($p<0.05$), **- Highly Significant ($p<0.01$), Numerical not bearing superscripts are Non Significant, 1.00- are complete correlation.

CONCLUSION

The sensory evaluation showed that the score of appearance, flavor, tenderness, juiciness, over all acceptability and odor were highly significant ($p<0.01$) with storage periods among 0, 30, 60 and 90 days. However, the organoleptic qualities did not show any significance ($p<0.001$ or $p<0.05$) difference among temperature and packages.

This study also revealed that 100 °c temperature and aluminum laminate were the most suitable for chicken dried meat though there were no sign of spoilage in any of the samples of all different temperatures and packages. Aluminum laminate packaging was the most suitable for chicken dried meat. This result was concluded on the basis of quality indices up to 90 days of storage.

Hence, it is recommended that chicken dried meat can be prepared as light weight preserved protein food with high nutritive value and without any quality deterioration up to 90 days of storage. It has been also advised to carry out the further investigation and experimental trials for longer storage and its implication of powdered form into children's diet as protein supplementation.

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Short Communication

AN INVESTIGATION OF SUDDEN DEATH SYNDROME IN BROILER CHICKEN IN KATHMANDU VALLEY NEPAL

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ABSTRACT

The incidence of sudden death syndrome (SSD) in broiler birds above 6 weeks of age reportedly increased during July-October 2008-2009 in Kathmandu valley. Birds presented for post-mortem examination at the Central Veterinary Laboratory, Tripureshwor Kathmandu were reportedly found dead on their backs with wings out-stretched. Gross lesions recorded were muscle oedema, pulmonary, renal, hepatic and splenic congestions. Necropsy examination revealed that liver with yellowish streaks and distended gall bladder; blood clot in atrium, haemorrhage in duodenal muscle, whitish yellow pasty fluid in the proventriculus, greenish intact feed particles in gizzard and swollen intestine with excessive mucous. Incidence rate was recorded between 1.5 to 2.5% of the flock. The mean mortality rate due to sudden death syndrome was 1.3 - 9.6%. Penicillium spp., Aspergillus spp. with colony forming unit (CFU) ranging from 56×10^4 *—* 62×10^5 *to uncountable mold counts, E. coli, Streptococcus spp. and Staphylococcus spp. were isolated from the culture samples of liver, lung, spleen and proventriculus. Reduction of mortality was achieved by feed restriction with 5-7% reduction in nutrient density and supplementation of glucose containing electrolyte, liquid toxin binder, immunomodulator, acidifier and antibiotic therapy.*

Key word: Sudden death syndrome (SDS), broiler, Penicillium, CFU, uncountable mold count

BACKGROUND

During August to October 2009, there was a sudden increase in mortality of broilers above 6 week age (Table 1) in Kathmandu valley. There were no premonitory signs. Just before death, birds appeared normal and birds were extending their neck, squawking, wing beating and extending the legs before falling on their back.

Postmortem finding of SDS birds

Gross lesions of dead birds recorded were muscle oedema, congestion of lungs, kidney, liver and spleen. The other abnormalities noted were liver with yellow streaks and distended gall bladder, blood clot in the atrium, haemorrhage in duodenal muscle, whitish yellow pasty fluid in the proventriculus, greenish ingesta in the gizzard and swollen intestine with excessive mucous. All post mortem observations confirmed to the descriptions of the sudden death syndrome made by Ononiwu *et al* (1979).

Table 1. Epidemiology of affected flock with SDS during July-October2009

Observation /Duration	Number of farmers	Population at risk	Morbidity (%)	Mortality (%)	No. of samples examined
July	63	16620	4250 (25.57%)	369 (2.22%)	63
August	51	15450	1235 (7.99%)	232 (1.50%)	51
September	32	10260	848 (8.26%)	157 (1.53%)	32
October	30	15700	2380 (15.16%)	149 (0.94%)	30
Total	176	58030	8713 (15.01%)	907 (1.56%)	176

Laboratory Findings of Tissue samples

A total 176 tissue samples of lung, liver, spleen, proventriculus and gizzard, were collected during postmortem examination and were subjected for both bacterial and mycological culture. Results of microbiological examination are given in Table 2.

Table: 2. Cultural characteristics of tissue samples

No. of samples	Bacterial isolates	Fungi isolates	Positive samples	Negative samples
176	*E. coli*, *Streptococcus spp. and* *Staphylococcus spp*.		35 - -	141 - -
176		*Aspergillus spp. and* *Penicillium spp.*	145 -	31 -

Treatment and preventive measures adopted

All birds remained in flocks were subjected to restricted feed up to 8-10% and fed twice daily. Supplementation with glucose containing electrolyte, liquid toxin binders, immunomodulator and simple broad-spectrum antibiotics and acidifiers were provided in water. Vitamin B complex supplementation was totally withdrawn. All birds remaining in the affected farm responded well to the above treatment and there was a marked improvement in overall condition of the flock.

RESULT AND DISCUSSION

Sudden Death Syndrome (SDS) is an acute heart failure disease that affects mainly fast growing male chickens in good bodily condition. Although a common feature in fast growing birds, the pathogenesis remains unclear (Ononiwu *et al.*, 1979). Cardiac arrhythmias are involved in the pathogenesis of SDS with ventricular arrhythmias (VA) being the most common observation representing premature ventricular contractions and fibrillation (Olkowski and Classen, 1997 & 1998). It has been reported that broilers fed with high Vitamin D3 diet above the recommended levels in an attempt to prevent commonly occurring leg problems were

2.5 fold more likely to succumb to acute heart failure and die of SDS (Nain *et al.*, 2007). SDS was also experimentally induced by feeding diets containing the mycotoxin moniliformin that resulted into cardiac injury with subsequent alterations in cardiac electrical conductance (Reams *et al.*, 1997) suggesting the possible role of chronic mycotoxicosis to the causation of SDS.

The present investigation indicates that broilers in good bodily condition when not harvested timely and remaining in poultry shades for prolonged periods suffer stressful events and even die suddenly. Also, it is possible that increased humidity and hot seasons favors the growth of mold and fungus in stored feeds increasing the risk of birds to mycotoxicosis.

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PROGRESSIVE DISPLACEMENT OF MULTI-HOST TICKS BY SINGLE HOST TICK IN LALITPUR DISTRICT

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ABSTRACT

The clinical investigations were made on ticks collected from domestic livestock and dogs of Lele Village Development Committee (VDC) at Pet Clinicare, Ekantakuna, Lalitpur. This is the first time that the host-parasite relationship has been discussed in relation to the habitat and epidemiological significance. The tick fauna isolated from domestic were Boophilus microplus, Rhipicephalus haemaphysaloides and Ixodid species. In case of dogs, the tick fauna was Rhipicephalus sanguineous and B. microplus. From the study, it is observed that progressive displacement of multi host ticks by single host tick is taking place gradually. B. microplus was by far, the most abundant tick species both in domestic and pet animals .Some reasons for this change from multi-host to single host has been discussed.

INTRODUCTION

Ticks are important as vectors of disease throughout most of the world. They possess a number of qualities which account for their vector potential; they attach firmly, suck blood, feed slowly, and may go unnoticed for a lengthy period of time. Many species are quite resistant to environmental stresses and may live for years. They have few natural enemies and have a wide range of hosts. Pathogens are transmitted to their off spring via transovarial transmission and transstadial transmission. Usually the ticks remain attached to a host for a long time, dropping off occasionally for molting or egg-laying.

There are several types of life cycles which are found in ticks, depending on the number of hosts utilized,.In one host tick, such as *Boophilus microplus*, virtually the entire life cycle may be passed on a single host. The genus *Boophilus* are one- host species, the larvae, nymph, and adult all accuring on cattle or domestic livestock (goats, Buffalo, pigs) and even dogs. They transmit the causative organism of *Babesia & Anaplasma*. *Boophilus* are the most important and common tick attacking livetock.. In a two host tick, the larva attach one host, molts to the nymphal stage whereupon it leaves the host, molts to the adult form, reattaches to a new host, engorges and drops off to oviposit. A tick which requires three or more hosts to complete its life cycle is called a Multihost (plural host) tick. A good example of this type is the *R haemaphysaloides*. The adult form may feed intermittenly on several successive hosts. Dog ticks (Rhipecephalus), as well as other speines are attracted by the scent of animals hence are most numerous along roads, paths, and trails. Engorged ticks that drop from animals using the passageways further increase the cancentration at these sites.
Climatic factors, particularly temperature, are highly important determinants of tick development and activity. The several tick species are extremely variable in their ability to

withstand temperature extremes , some surviving frigid winters as hibernating adult, nymphs or larval, and others surviving high temperatures and arid condition .Some hard tick,such as those in the *Boophilus,* may produce three generations per year under favourable climatic conditions (Harry.D Pratt and Kent S. Littig,1986)In multi host tick depending on the normal seasonal length, the full cycle of development may require one year, two years, or even three years .However, in most cases the high temperatures of spring and summer serve to accelerate tick development and activity.

MATERIALS AND METHODS

Surveys involving cattle, Buffaloes and goats of Lele V.D.C and dogs brought to Pet Clinicare Ekantakuna Lalitpur were undertaken during 2007, 2008 and 2009. The surveys were conducted in all the years during main tick season (April to October). Percent animals harbouring either *Ixodid, Rhipicephalus, Boophilus* or any other tick species or mixed infestation were used to work and the comparative prevalence of the various thick species . Only adult ticks were used from species identification as par the keys developed by various scholars. Sen and Fletcher (1962), Trapido et *al* (1964) IMR Acarology Div. (1995) and Harry D. Pratt and Kent S. Littig (1986).

RESULT AND DISCUSSION

Ticks from cattle, Buffaloes and goats of Lele V.D.C and that of dogs from Pet Clinicare, Ekantakuna Lalitpur were screened for the hard ticks. Number of ticks per animal varied from a few to several hundreds, particularly *Boophilus microplus* on some poorly kept indigenous cattle. The cattle, Buffaloes and goat Ixodid (hard) tick fauna in lele V.D.C. of lalitpur was found to be almost entirely made up of three tick species viz. *Boophilus microplus, Rhiphicephalus hamaphysaloides* and *Ixodid.* (Figure 1). Where as in case of dogs, the tick fauna was the *R. sanguinous* and B *microplus* (Figure 2). From the ticks identified, multi host ticks harbouring animals decreased gradually, cattle from 6 to none, Buffaloes from 2 to none and goat from 13 to 2 respectively. Where as single host tick *(Boophilus*) harbouring animals have increased to cent percent. *Boophilus microplus* was by far the most abundant tick species in this study. The displacement of multhost ticks by single host is progressing every year (Figures 1 & 2).

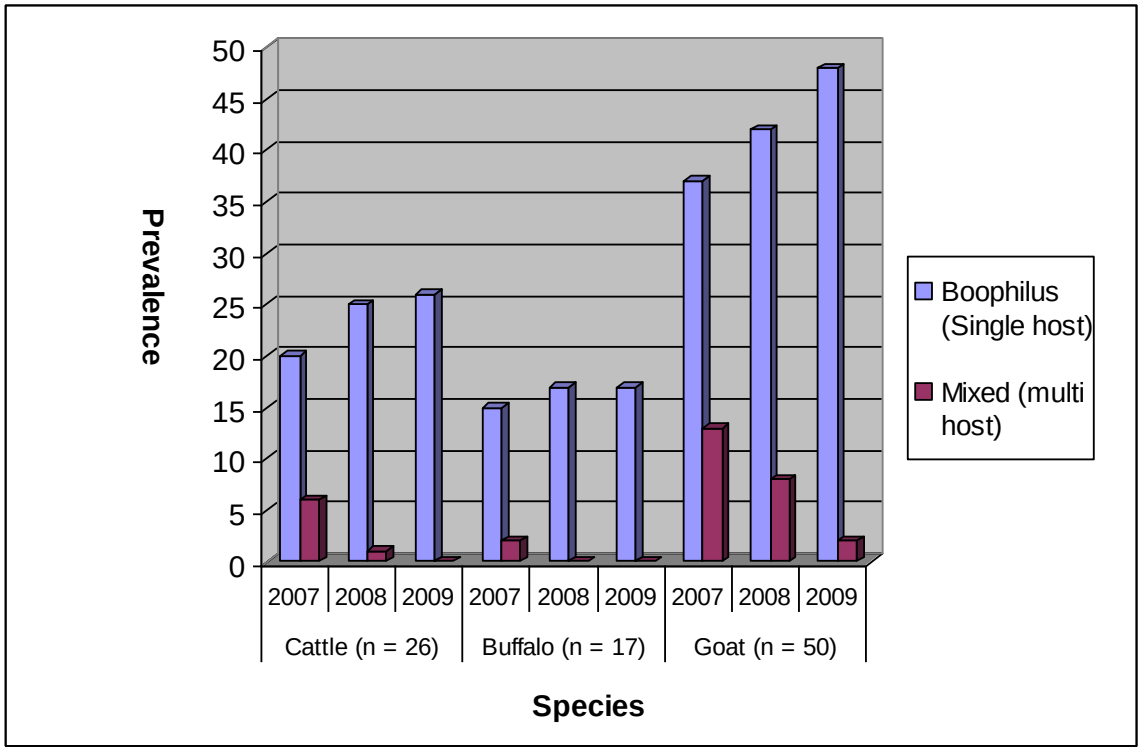

Figure 1. Showing the prevalence of tick population over three year periods in domestic animals

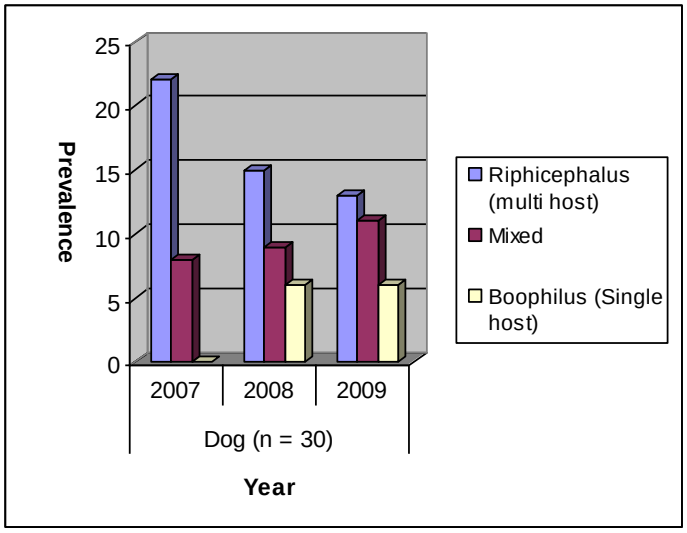

Figure 2. Showing prevalence of tick population over three year periods in dog

Not only in case of domestic livestock, the displacement of multihost ticks are observed in pets too, the brown dog tick (*Rhipicephalus*) harbouring dogs have decreased from the year 2007 to 2009 where as the cattle blue tick (*Boophilus*) harboring dogs have increased from 8 in the year 2007 to 17 in the year 2009 (Figure 2). It is intresting to find out that 6 dogs in the year 2008 and 2009 had only harboured *B. microplus* ticks. Within few years, *B. microplus* has established itself to be the most prevalent tick in Kathmandu valley. This seems to be a major epidemiological change concerning tick borne diseases in the valley. There might be several

possible factors responsible for this change. We observed that a change in the animal habitat (mud plastered wall to cemented brick wall of animal houses) was more detrimental to multi host ticks than single host tick. Further, wherever there was regular spray of acaricides for the control of tick, *Rhipicephalus* & *Ixodid* were wiped out but *Boophilus* was always present in small numbers. This suggests either acaricide resistance in *B. microplus* population or a few of these ticks might escape the sprays since these ticks are found on whole of the body. Increased crossbred cattle population which supports huge populations of *B.microplus* seems to be major factor in increasing this tick exponentially. Due to increasing water supply in villages (Lele) micro climate in animal houses is humid enough to support *B. microplus*, dry summers are unsuitable for *B. microplus*. Alternate wild host (angulates, rhodents) population is decreasing particularly for immature stages of multihost ticks which is proving detrimental to these ticks. Competitive exclusion of multihost ticks by *B. microplus* might be a possible factor since both the species are competing for the same hosts.

CONCLUSION

Maintenance of endemic stability of a vector borne disease depends upon the optimum availability of the host, the vector and the parasite populations in the area. In haemoprotozoan diseases (Theileriosis), where premunity is the mainstay of maintaining the endemic stability, any disturbance in the vector population might break the premune status of the host population rendering them more susceptible. If the multi host ticks are displaced by *B microplus* at the present rate, several disturbances of veterinary importance are likely to occur in the near future. Changes in the endemic stability of haemoprotozoan diseases, increased incidence of Babesiosis and emergence of acaricide resistance, as *B.microplus* is known to develop resistance quickly, might become major concerns in future. In some cases, livestock tick infestations may be reduced by rotation of pastures. No control method replaces dipping spraying, or dusting of cattle, buffaloes, goats, dogs or other hosts**.**

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NOTE FOR CONTRIBUTORS

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