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Editorial

Nepal is an agricultural mountainous country where more than 65% people depend on the agriculture for their livelihood. Livestock is an integral part of Nepalese farming system thus play substantial role in the national economy. The contribution of livestock to society comes from quality food for human such as poultry and dairy; for increasing/ maintaining the soil fertility, draft power for agricultural operations, tanneries and several other industries such as carpet, wool, and meat etc. Agriculture contributes about 27% to the GDP out of which livestock sector alone shares about 12%.

Several research institutes, academia and extension agencies are conducting research to solve the problems faced by the livestock industry thereby contributing to improve the production and productivity of livestock and ensure the sustainability of the rural livelihood. To publicize these research, NVJ Editorial team presents the 35th issue of Nepalese Veterinary Journal (NVJ). The NVJ journal includes scientific papers from different entities on core as well as relevant research on livestock and poultry development using recent advancement in sciences. In this issue, selected scientific papers presented on the special event jointly organized for the 50th Anniversary of NVA and Nepal Veterinary Conference 2018 (VetCon'18) are included.

This issue includes manuscripts from Animal and Veterinary Sciences inclusive of different disciplines such as animal breeding, genetics and reproduction, animal health, socio-economic market studies, animal welfare, and policies. Articles included in this issue have used advanced scientific technologies. For example, in dairy cows, hormonal treatments are commonly implemented for acyclicity, silent heat and endometritis. After detecting the causes of the infertility, hormonal treatments can be used successfully to treat fertility disorders. Nevertheless, in order to enhance the reproductive performance at the herd level, a sustainable improvement of the general conditions such as housing, feeding, animal health, management are prerequisite. In addition, techniques to improve the semen quality of bucks and turkey are also included. Clean meat production and improving meat quality of ostrich are also included in this issue. Interesting articles such as role of music to improve production and using GPS and smartphone to control Rabies are worth reading and be practically implemented in the field.

I would like to extend my heartfelt thanks to respected reviewers and members of the editorial team for their untiring efforts on editing and reshaping the papers for publication. In addition, the assistance of Ms. Pramina Shrestha in type setting is highly appreciated. The publication team is hopeful and look forward for the everlasting support of our professional colleagues and well-wishers for their articles in future publications, which will help for wider dissemination of technologies and information to our clients and the farming community.

Neena Amatya Gorkhali (PhD, Animal Breeding, Genetics and Reproduction)

Editor-in-chief

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Effectiveness of Different Hormonal Treatment Protocols on Treatment of Postpartum Anoestrus in Cross-Bred Dairy Cows

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ABSTRACT

Postpartum anoestrus (PPA) is one of the major factors causing prolonged calving interval & that leading to decreased annual milk production. Hormonal treatments can be effective tools for treatment of PPA when nutritional & environmental management are good. So, objective of this study was to know the comparative effectiveness of different hormonal protocols on treatment of PPA in cross-bred dairy cows. Used protocols were: single dose of GnRH, Ovsynch with TRIU-B and single dose of PGF₂α injection which were used in three different groups, each containing ten animals. GnRH and Ovsynch with TRIU-B were used in postpartum true anoestrus cattle with inactive ovaries while PGF₂α was used in postpartum silent oestrus condition having palpable corpus luteum and the condition were identified through rectal palpation and history record. Cows treated with Ovsynch+TRIU-B showed higher oestrus expression rate within one week (80%) than cows treated with GnRH (10%) and PGF₂α (50%). Ovulation rate was also comparatively higher in Ovsynch+TRIU-B group (100%) than GnRH (90%) and PGF₂α (70%). But the pregnancy rate, checked after 45 days of first AI through rectal palpation, was comparatively higher in GnRH group (40%) than in Ovsynch+TRIU-B (30%) and PGF₂α (20%). Among treated cows, pregnancy rate was found to be significantly higher (P=0.02) in cows with ≥100 days duration of postpartum anoestrus (53.84%) than in cows with 100 days duration (0%). In conclusion, ovsynch with intravaginal progesterone (p4) releasing device was more effective at inducing oestrus and synchronizing ovulation than the single GnRH injection. However, single GnRH injection was as effective as ovsynch with intravaginal p4 releasing device for pregnancy outcomes in anoestrous cross-bred dairy cows in a commercial dairy farm where formulated concentrate ration is daily fed.

Keywords: hormonal protocols; oestrous synchronization; pregnancy rates; silent estrus; true anoestrus

INTRODUCTION

In Nepal infertility/anoestrus problem in crossbred and exotic cattle has been reported to be the most prioritized problem in dairy pocket areas in the country (Jha, 2000). Fertility and breeding efficiency of dairy animals play a pivotal role in dairy economics (Peter *et al.*, 2009). Cows are regarded as physiologically anoestrous for a few days following parturition, whereas, lack of oestrus after 60 days postpartum is considered pathological anoestrus or extended postpartum anoestrus (Islam *et al.*, 2013). In general, a calving interval of about 1 year is considered economically optimal for dairy cows (Schmidt, 1989). To achieve such a calving interval, a postpartum cow has to resume ovarian activity, to be observed in heat, to be mated, and to conceive within 85 days after calving (Lamming, 1981). It has been suggested that in high-yielding dairy herds, there is increased incidence of anoestrous (Berger *et al.*, 1981). However, factors such as limited energy intake, lower body reserves, hormonal imbalance and postpartum diseases can also delay the return to cyclicity (Opsomer *et al.*, 2000). As it is known that follicle maturation, ovulation or ovarian cyclicity are energy as well as hormonal dependent process, negative energy balance and hormonal insufficiency frequently cause inactive ovaries i.e. postpartum true anoestrus for long time period. Similarly, sometime although there is ovulation timely after calving, the animal can't express heat; called as postpartum silent oestrus (Opsomer *et al.*, 2000). In commercial farms where they use commercial feeds to overcome energy deficit, in this condition hormonal imbalance and high lactation/ production stress may cause temporary cessation of hypothalamus-hypophysial axis leading to inactive ovaries or sub-active ovaries. In such cases treatment with appropriate hormonal protocols can be effective tools to resume the cyclicity in postpartum anoestrus dairy cows or make them conceived. In Nepalese context, there was very few research works have been performed in dairy cattle reproduction; there are no well documented reports on treatment of postpartum anoestrus cows using hormonal protocols. Therefore, this study was designated to evaluate the efficacy of different oestrus synchronization protocols using hormones to overcome this postpartum anoestrus problem in dairy cattle of Nepal.

MATERIALS AND METHODS

This study was conducted in a commercial dairy farm located in chitwan district and 105 postpartum cows were selected as study samples, that had not shown oestrous behaviour upto 60 days postpartum and had not mated yet, using their history record. Among all the postpartum anoestrus cattle, some were selected randomly and examined by trans-rectal palpation of genital organs. They were classified as true anoestrus (having no palpable CL and small follicles i.e. < 5mm) and silent oestrus

(having palpable CL and large follicles). Among true anoestrus cattle 10 were treated with single GnRH injection (day 0 Gynarich inj. 5ml i.e. 20 μ g Buserelin acetate I/M was given, waited for oestrous expression and AI was done) and 10 were treated with Ovsynch with TRIU-B/progesterone impregnated intravaginal device (day 0 Gynarich 5ml I/M and vaginal insertion of TRIU-B for 7 day, at day 7 Lutalyse inj 5ml I/M i.e. 25mg Dinoprost tromethamine was given and TRIU-B was removed, at day 9 Gynarich 5 ml was given and at day 10 FTAI was done). Among silent oestrus cattle 10 were treated with single PGF $_{2\alpha}$ injection (day 0 lutalyse 5ml I/M was given and waited for oestrus expression and AI was done). The research activities are shown in detail in figure 1.

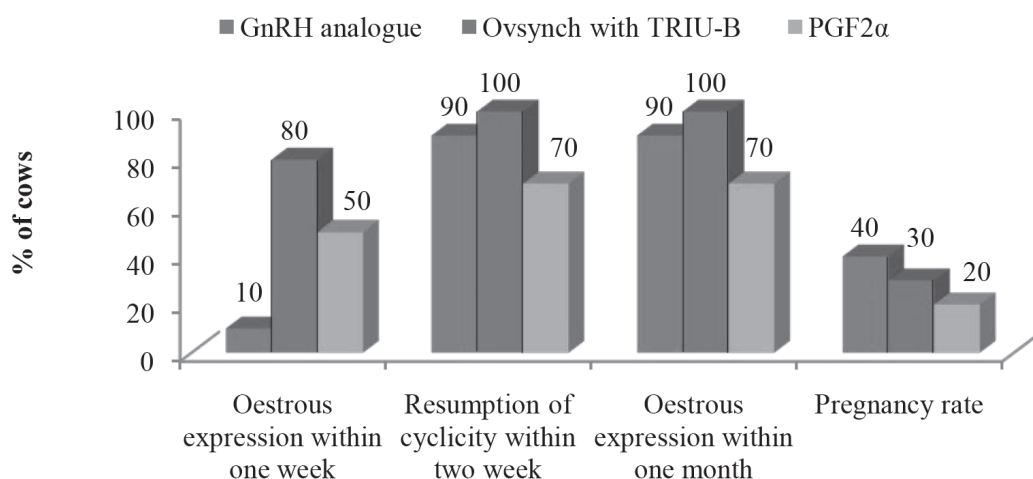


Fig. 1: Comparative Effectiveness of different hormonal treatment protocols on postpartum anoestrus.

Chi-square test and Fisher's exact test in IBM SPSS Statistics version 20 were utilized to know the success rate of different treatment protocols on postpartum anoestrus. Similarly, MS-EXCEL-2007 was used for data entry and making different diagrams.

RESULTS AND DISSCUSSION

Effect of different hormonal treatment protocols on postpartum anoestrus

The oestrous expression rate within one week was found to be significantly higher in Ovsynch-TRIU-B treated cows (80%vs 10%) than in single GnRH inj. treated cows ($P=0.003$). Resumption of cyclicity or ovulation rate within two week and oestrous expression rate within one month was found to be very similar between cows treated with GnRH, TRIU-B and PGF $_{2\alpha}$ i.e. 90%, 100% and 70% respectively. Pregnancy rate was found to be comparatively higher in GnRH treated cows than

Ovsynch+TRIU-B treated and PGF₂ α treated cows i.e. 40%, 30%, 20% respectively. The detailed data was presented in Table 1.

Table 1: Effect of different treatment protocols on postpartum anoestrus.

Parameters	Treatment group				Total (n=30)
	True anoestrus		P value	Silent oestrus	
	GnRH group (n=10)	Ovsynch with TRIU-B (n=10)		PGF ₂ α Group (n=10)	
Oestrus expression within 1 wk (%)	1 (10)	8 (80)	0.003	5 (50)	14 (46.7)
Resumption of ovarian cyclicity within 2 wk (%)	9 (90)	10 (100)	0.5	7 (70)	26 (86.7)
Oestrus expression within 1 month and AI done (%)	9 (90)	10 (100)	0.5	7 (70)	26 (86.7)
Pregnancy rate after first AI (%)	4 (40)	3 (30)	0.5	2 (20)	9 (30)

GnRH=Gonadotropin Releasing Hormone; TRIU-B = Progesterone Impregnated Intravaginal Device manufactured by Virbac company; PGF₂ α = Prostaglandin F₂ α or its analogue; P value = Degree of association between GnRH group and Ovsynch+TRIU-B group; wk= week; AI=Artificial Insemination

In case of GnRH, Islam et al (2013) found 75% oestrous expression rate and Shah and Nakao (2010) found 100% oestrous expression rate with single injection of GnRH in dairy cows and in buffalo respectively which are significantly higher than this finding (10%). Similarly, Islam *et al.*, (2013) and Wiltbank et al., (1997) found 76.5% and 85% ovulation rate within one month with single injection of GnRH which are very similar to this finding (90%).

Table 2: Effect of duration of postpartum anoestrus on treatment response against postpartum true anoestrus in dairy cows.

Parameters	Categories	Total no.	Oestrus expression within 15 days	Oestrus expression within 30 days	Conception rate after first AI
Duration of anoestrous	100 days	7	5 (71.43%)	6 (85.71%)	0 (0%)
	≥ 100 days	13	9(69.23%)	13 (100%)	7 (53.84%)
P value			0.66	0.35	0.02

Similarly, Islam *et al.*, (2013) found 64.7% and Shah and Nakao (2010) found 90.9% conception rate with single injection of GnRH in dairy cows and buffaloes respectively which are comparatively higher than this finding (40%). Thus, present study has demonstrated that a single injection of GnRH analogue had positive effect on induction of oestrus and conception in anoestrous cows. GnRH and its agonist act on ovarian follicular development and CL formation indirectly via the induced release of pituitary LH and FSH. Administration of GnRH causes the large follicles to ovulate and induces emergence of a new follicular wave within 3 to 4 days after treatment at any stage of the oestrous cycle in cattle.

In case of PGF₂α, very similar findings was obtained by Rasaili *et al.*, (2002) who found 60% oestrous expression rate and 20% conception rate with first injection of PGF₂α in stall-fed dairy herd in Lumle. But Islam *et al.*, (2013) and Shah and Nakao (2010) found comparatively higher oestrous expression rate i.e. 73.9% and 100% respectively. Similarly, they also found higher conception rate i.e. 52.1% and 86.4% respectively with single injection of PGF₂α in dairy cows and buffaloes respectively. PGF₂α and its analogue act on CL, formed on ovary which cause temporary cessation of oestrous cycle. So lysis of CL cause beginning of new oestrous cycle within 1 week.

In case of Ovsynch with TRIU-B protocol, same findings was obtained by Ramakrishnan *et al.*, (2012) i.e. 100% oestrous expression rate and 33.3% conception rate in first time AI with Ovsynch CIDR protocol. Similar result was also found by Zabeel *et al.*, (2009) and Bhoraniya *et al.*, (2012) in buffaloes and Kankrej cows, respectively i.e. 100% oestrous expression rate. In contrary, Tauck *et al.*, (2007) and Cevik *et al.*, (2010) found 43% to 83% oestrous expression rate which was lower than this finding. Similarly, Martinez *et al.*, (1998) found 60%, Cevik *et al.*, (2010) found 53.3% and Tauck *et al.*, (2007) found 69% conception rate with Ovsynch CIDR protocol in dairy cows which are higher than this finding. Better oestrus response

and conception rate in TRIU-B group could be due to progesterone priming through TRIU-B, establishment of proper endocrine harmony/ synchrony and FTAI. While difference in conception rate in different findings might be due to quality of semen, experience of technician etc.

Duration of anoestrous and treatment response against postpartum true anoestrus in dairy cows

It was found that cows with more than 100 days postpartum duration showed comparatively higher oestrous expression rate (100%vs 85.71%) and significantly higher conception rate (53.84%vs 0%) within 30 days of treatment than cows with less than 100 days postpartum duration. The detailed was given in table 2. It might be due to overcome/resumption of negative energy balance and reproductive cyclicity after 100 days of postpartum, so that hormones have worked properly and conception was not lost.

CONCLUSION

This study found no significantly difference in Conception rate on treatment of postpartum anoestrus with these three hormonal protocols (GnRH, PGF₂ α and Ovsynch with TRIU-B), however GnRH and PGF₂ α injection can be used efficiently in small herd size where oestrous detection is not a problem and it is less costly also while Ovsynch with TRIU-B was found to be better for large commercial farms where oestrous detection always seems a problem. Similarly, success rate of hormonal protocols was found to be more in > 100 days postpartum cows than < 100 days postpartum. So, postpartum reproductive rest up to 3 months is required to increase oestrous expression rate as well as conception rate.

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REFERENCES

- Berger BJ, Shanks RD, Freeman AE, Laben RC (1981). Genetic aspects of milk yield and reproductive performance. *J Dairy Sci* 1981; **64**:114–22.
- Bhoraniya, H.L., Dhami, A.J., Naikoo, M., Parmar, B.C. and Sarvaiya, N.P (2012). Effect of estrus synchronization protocols on plasma progesterone profile and fertility in postpartum anestrous Kankrej cows. *Trop. Anim. Health Prod.*, **44**(3): online, DOI 10.1007/s11250-011-0057-1.
- Cevik, M., Selcuk, M. and Dogan, S. (2010). Comparison of pregnancy rates after timed artificial insemination in Ovsynch, Heatsynch and CIDR-based synchronization protocol in dairy cows. *Kafkas. Univ. Vet. Fak. Derg.*; **16**(1): 85-89.
- Jha, V.C. (2000) Study on infectious causes of infertility in crossbred & exotic cattle in Nepal. Annual Report (1999/2000), Animal Health Research Division, Tripureswor: 19-23.
- Lamming GE, Wathes DC, and Peters AR (1981). Endocrine patterns of the post-partum cow. *J Reprod Fertil* 1981; **30**: 155-70.
- M. R. Islam, N. S. Juyena^{1*}, M. M. U. Bhuiyan, M. M. Rahman and R. N. Ferdousy, (2013). Treatment outcomes in postpartum anoestrus cows Guided by transrectal ultrasonography. *Progress. Agric.* **24**(1 & 2): 93 – 100, 2013.
- Martinez, M.F., Kastelic, J.P., Adams, G.P., Janzen, E., Olson, W. and Mapletoft, R.J. (1998). Alternative methods of synchronizing estrus and ovulation for fixed time insemination in cattle. *Theriogenology*, **50**: 350.
- Opsomer G, Grohn YT, Hertl J, Coryn M, Deluyker H, de Kruif A (2000). Risk factors for post-partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology* 2000; **53**:841–57.
- Peter, A.T., Vos, P.L. and Ambrose, D.J. (2009). Postpartum anoestrus in dairy cattle. *Theriogenology*, **71**: 1333–1342.
- Ramakrishnan, A., Dhami A.J., Naikoo, M., Parmar, B.C., And Divekar, B.S., (2012). Estrus induction and fertility response in postpartum Anestrus gir cows. *Indian Journal of Animal Reproduction* **33** (1): June 2012
- Rasali, D.P. *et al.*, (2002). An attempt to synchronize oestrus in on-station and on-farm local cows for mass insemination in the western mid hills. *Nepalese Vet.*

- Journal* – 2002, Nepal Veterinary Association (NVA). Pages; 34-42.
- Sah and Nakao, (2010). A clinical study of anoestrous buffaloes in southern Nepal. *Journal of Reproduction and Development*, Vol. **56**, No. 2, 2010.
- Schmidt GH, (1989). Effect of length of calving intervals on income over feed and variable costs. *J Dairy Sci* 1989; **72**: 1605-11.
- Tauck, S.A., Wilkinson, J.R.C., Olsen, J.R. and Berardinelli, J.G. (2007). Comparison of using 7- or 14-d CIDR treatments in an estrous synchronization protocol that included PGF₂α, timed AI and GnRH in primiparous, suckled beef cows. *Proc. Western Section, Am. Soc. Anim. Sci.*, **58**: 271-273.
- Wiltbank, M. C. 1997. How information on hormonal regulation of the ovary has improved understanding of the timed breeding programs. *In*: Proceed. Annual Meeting of Society for Theriogenology, Montereal, Canada. 83-97.
- Zabeel, S.M., Hegab, A.O., Montasser, A.E. and El-Sheikh, H. (2009). Reproductive performance of anestrous buffaloes treated with CIDR.

Effect of Age and Frequency of Collection in Quality of Jersey Bulls Semen at National Livestock Breeding Center, Nepal

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ABSTRACT

The present study was undertaken to determine the effect of age and frequency of collection on quality parameters of Jersey bull semen at National Livestock Breeding Center (NLBC), Pokhara, Kaski district. Nine Jersey bulls were selected randomly from NLBC. To obtain the effect of age, six bulls of three different age interval (3-4, 5-7 and 8-9 years) were selected randomly. Remaining three bulls were used to determine the effect of frequency of collection on semen quality. Studies were conducted for three months (Nov/Dec to Dec/Jan 2017). Semen quality was analyzed using CASA system and SMILE software. ANOVA and paired t- tests were applied to find out level of significance. All the statistical analyses were done using SPSS 17.0. Sperm concentration (0.551 ± 0.004 to $1.084 \pm 0.014 \times 10^9$), post- thaw motility (42.44 ± 0.11 to 51.72 ± 0.03 %), live sperm (45.62 ± 0.13 to 51.23 ± 0.15 %), and abnormal sperm (11.57 ± 0.12 to 19.59 ± 0.24 %) were significantly affected by the age of the bulls. Semen quality was found superior in the bulls belonging to 5-7 years of age intervals. On the other hand, volume (3.98 ± 0.09 to 6.72 ± 0.06 ml), initial motility (72.68 ± 0.02 to 78.64 ± 0.15 %) and pre-filling motility (71.34 ± 0.17 to 77.62 ± 0.02 %) did not differ significantly among different age intervals. Significantly, higher value for semen volume (3.98 ± 0.12 to 6.02 ± 0.14 ml), sperm concentration (0.572 ± 0.119 to 1.095 ± 0.015) and post- thaw motility (40.34 ± 0.21 to 48.54 ± 0.06 %) was obtained on second collection. However, initial motility (70.58 ± 0.06 to 75.64 ± 0.13 %), pre-filling motility (68.11 ± 0.12 to 71.62 ± 0.02 %), live sperm (53.27 ± 0.54 to 56.17 ± 0.02 %) and abnormal sperm (12.31 ± 0.12 to 14.3 ± 0.47 %) did not differ significantly between the frequencies of collection. Different quality parameters of semen like volume, concentration, initial motility, pre-filling motility, post thaw motility, live and normal spermatozoa percentage were higher in 5-7 years old bulls and in second collection of semen.

Keywords: Age; frequency; semen; quality; NLBC

INTRODUCTION

Livestock plays a vital role to the Nepalese economy and contributes about 11% to the total national gross domestic product (GDP) and about 26.8% to national agricultural gross domestic products (AGDP) (MoAD, 2016). Dairying is the most important sub-sector contributing about 63% to the total Livestock Gross Domestic Products (LGDP) which is about 8% of total national GDP. It provides a daily source of income and has been well recognized as a means for poverty reduction across the developing world. In Nepal, the demand for milk and milk products is increasing. There is a need of high yielding breeds of dairy animals in order to meet the increased demand of milk. Genetic improvement is one of the prioritized activities of Government of Nepal to improve the productivity of the Nepalese cattle herd. Artificial Insemination (AI) is the cheapest way of improving the genetic potentiality in the indigenous cattle using the semen of exotic breed bull. AI may be defined as the collection of semen from a healthy proven bull by artificial means exteriorly, evaluating it for quality, extending it in a suitable extender and preserving the same in suitable temperature. This extended and processed semen is to be inseminated to an estrus cow during standing heat in mid cervix with the help of a sterilized mechanical device. AI was the first great biotechnology applied to improve reproduction and genetic advancement of farm animals. AI is not only important in genetic improvement but also beneficial for the control of venereal diseases, economical than the natural service, safe in eliminating injury during natural service and availing geographical restrictions (Bearden *et al.*, 2004; Lemma & Shemsu, 2015).

The process of cryopreservation and later on thawing for AI are known to adversely affect the survival of the sperm even under the best technique available today. Factors from selection technique of the bulls to semen processing and storage are believed to have different level of influence on the quality of semen produced and its fertility during application (Lemma & Shemsu, 2015). The influencing factors for the success of AI program are quality and fertility of semen, skilled technician, an increase of AI coverage, and the farmer's awareness about the advantage of AI. The fertilizing capacity of semen depends on quantity (ejaculated volume) and quality (semen morphology, live concentration and motility) (Shamim *et al.*, 2013). It was reported that total sperm per ejaculate increased with age of the bull up to 7.5 years and then decreased (Al-Hakim, 1986). Vilakazi (2003) has suggested that the breed, age and season and their interactions are important source of variation in the sperm morphology and ultimately the production of best quality semen.

It is obligatory to perform the evaluation of semen, either fresh or frozen thawed to

predict its fertility indices. Semen volume, concentration of spermatozoa, proportion of dead and abnormal spermatozoa, and motility of spermatozoa are recognized as important indices of semen quality and significantly correlated with freeze-ability and/or fertility of bovine semen (Fiaz *et al.*, 2010). The evaluation of semen quality and quantity may help in selecting a good quality sire for the breeding purpose.

Sires contribute half of the genetic makeup of the offspring, so commonly called 'bull is half of the herd'. When artificial insemination (AI) is applied; each bull can serve thousands of female per year. The success of AI depends on the ability to screen for semen with high fertilization potential (Holt *et al.*, 2007). Therefore, is extremely important to do the selection of breeding bull to improve their offspring (Kumar *et al.*, 2015).

Reproductive efficiency of dairy cattle can be improved through utilization of highly fertile bull bearing excellent semen quality. The effective screening methods for ejaculates prior to processing are necessary for improving reproductive performance in bulls. The evaluation scheme of the jersey bulls, used for AI either does not exist or if any, poorly evaluated. All such bulls should be evaluated with the efficiency of conception of semen, which is used in AI program. As it has already been 40 years of application of AI in the country, it is necessary to find out the quality parameters of bull semen used for AI. Considering all above facts, the present research was undertaken to evaluate semen quality and quantity in relation to age and semen collection interval of different Jersey bulls in the National livestock Breeding Center (NLBC), Pokhara, Nepal.

MATERIALS AND METHODS

Research Site: National Livestock Breeding Center, Pokhara.

Experimental Animals

In this study 9 jersey bulls were used. According to the bull register, age of the bulls ranged from 3 to 9 years, body weight ranged from 390 to 1070 kg, BCS (1 to 5 range) ranged from 4.0 to 4.5 and scrotal circumference ranged from 35 to 39 cm.

Semen Collection

Usually semen collection was performed twice a week at NLBC. Two ejaculates were collected from each bull during each collection session and only the first ejaculate was used in this experiment. Collection was always performed in the morning between 7 and 9 AM. To determine the influence of collection interval, 3 consecutive collections from 6 selected bulls were done on Monday and Thursday. Semen was

collected using artificial vagina (AV) method following standard procedure at homo sexual mount. The bulls were allowed at least two false mounts before collection of semen. Immediately after collection, the collecting tube was detached from the AV, taken to the laboratory and placed at 37°C in water bath until evaluation and processing of semen.

Evaluation of Semen

The qualitative and quantitative examination of fresh and frozen semen was carried out through different methods as per protocol of NLBC mentioned in “Standard Operation Practice”. First Draft uses the integration of smile software for data recording and CASA system for semen evaluation. Different quality parameters were used for the evaluation of the semen as mentioned in the Table-1.

Preservation of Semen

The egg yolk-tris-fructose-citric acid-glycerol was used as an extender. Briefly, a stock solution was prepared by dissolving tris (297.6 mmol), fructose (82.6 mmol), citric acid (105.3 mmol), penicillin G-sodium (1000 I.U/ml) and streptomycin sulphate (1 mg/ml) in glass distilled water. Fresh yolk was added with the buffer at a concentration of 20% (v/v). The volume of extender was divided into two equal parts. Thereafter, glycerol was added to one part of the extender at two times of the desired concentration (12.8%). The other part of the diluents was used to make the initial dilution of semen that contained two times of the desired concentration of spermatozoa. The initial dilution of semen was made at +37°C. Three equal parts of initially diluted semen and double concentration glycerol- containing extender were mixed together at four steps during a 2 to 3 hours cooling process, the dilution steps were at +18, +12, +8 and +4° C. Finally, the diluted semen contained 6.4% (v/v) glycerol and 20x10 motile spermatozoa per 0.25 ml French straw (one insemination 6 dose). Individual insemination doses were sucked in 0.25 ml French straws and the open ends of the straws were sealed by using automatic filling and sealing machine. After loading, the straws were left for equilibration at +4° C for 4 hours. The cooling and equilibration operation were done in a cold handling cabinet. After equilibration, the freezing operation was conducted in deep semen freezer to cool the semen from +4 to -140° C during a period of 7 min and then the straws were directly plunged into liquid nitrogen (-196° C). Only semen that showed equal to or more than 50% post-thaw motility was preserved for AI.

Thawing of Semen

To check the post-thaw motility and taking sample for formal-saline fixation, the frozen semen straws were thawed at +37° C warm water for 30-40 sec at 24 hours post freezing.

Experimental Approaches

Experiment 1. In this experiment, the influence of age on semen quality with respect to volume color, density, initial motility, pre-freezing motility, post thaw motility, sperm concentration, semen doses produced, number of sperms per dose of semen were determined. A total of 9 jersey bulls of 3 age groups were selected in this experiment. Group-1 included bulls of 3- and 4-years group- 2 included bulls of 5, 6, 7 years and group-3 included bulls of 8 and 9 years old (n=3). Semen from these bulls was collected on Monday and Thursday.

Experiment 2. In this experiment, the influence of collection interval on semen quality with respect to volume color, density, initial motility, pre-freezing motility, post thaw motility, sperm concentration, semen doses produced, number of sperms per dose of semen were determined. Three Jersey bulls of 3 to 9 years old were selected for this study. All bulls were collected at 30 minutes 'interval for evaluation of semen for three consecutive times.

Data analysis techniques

All data were presented as mean $\pm$ standard error of mean (SE). One-way analysis of variance (ANOVA) followed by least significant difference (LSD) was done to find out significant differences among the different age variables. Pair t-test was done to find out the significant differences between the two categories of collection interval of semen. All the statistical analyses were done using SPSS 17.0. The difference between groups was regarded as significant when the P value was less than 0.05 ($P < 0.05$).

The following fixed effect model was used to express each observation of semen quality produced from breeding Jersey bulls.

$$Y_{ijkno} = \mu + a_i + b_j + c_k + g_n + h_o + e_{ijkno}$$

Where, μ is the overall mean

a_i is the effect of i^{th} year ($i = 1, 2, 3, 4, 5$)

b_j is the effect of j^{th} bull ($j = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11$)

ck is the effect of k^{th} interval of semen collection ($k=1, 2, 3$)

gn is the effect of n^{th} density of fresh semen ($n=1, 2, 3$)

ho is the effect of o^{th} mass activity of fresh semen ($o=1, 2, 3$)

eijklmno is the random element (error mean) assumed to be normally and independently distributed among the sampled population.

RESULTS

Quality Parameters of bull semen production

Quality parameter of bull semen includes physical quality (macroscopic quality) and microscopic quality. Physical quality parameters of bull semen included semen volume and density of semen. Similarly, microscopic quality parameters of bull semen included initial motility, sperm concentration, pre-filling motility, post thaw motility and abnormality of sperms. In this study, microscopic quality parameters of bull semen were initial motility, sperm concentration, pre-filling motility and post thaw motility.

Table 1: Quality parameters for the evaluation of bull semen

Parameters	Descriptions
Semen Volume	Volume of semen in ml ejaculated by bull/ ejaculation
Semen Density	Density of semen (1-3 scale as fair, dense and highly dense)
Mass activity of sperms	Mass movement of sperms in mass or group
Initial motility of sperms	Initial motility % of individual sperms of fresh semen
Concentration of sperms	Billion (109/ml) of sperms/ml of semen
Pre-filling motility % of sperms	Motility % of sperms after mixing with diluters and before filling into the straws.
Post thaw motility % of sperms	Motility % of sperms after freezing of semen by post thawing process of it.
Semen doses produced	Doses of semen straw prepared by per ejaculation of semen.

Influence of Age of Bulls on Semen Parameters

The effect of age of bulls on semen quality is presented in Table 2. The highest volume of semen, motility, concentration, post thaw motility and live sperm were obtained from 5-7 years' age group (6.72 ± 0.06 ml, $78.64 \pm 0.15\%$, $1.084 \pm 0.014 \times 10^9$ /ml, $51.72 \pm 0.03\%$ and 55.48 ± 0.05 , respectively) and lowest from 8-9 years' age group (3.98 ± 0.09 ml, $72.68 \pm 0.02\%$, $0.551 \pm 0.004 \times 10^9$ /ml, $42.44 \pm 0.11\%$ and $45.62 \pm 0.13\%$, respectively) as shown in figure 1. Lowest value of abnormal sperm was obtained from bull of same age interval. However, the differences in semen volume, semen density, fresh semen motility and pre-filling motility did not differ significantly among different age groups of bulls. Whereas sperm concentration, total spermatozoa per ejaculate, post-thaw motility, live sperm and abnormal sperm were significantly different ($P > 0.05$) among different age groups of bulls.

Table 2: Effect of Age on Semen Quality of Bulls

Quality parameters	Age (years)			Level of Significance
	3-4	5-7	8-9	
Volume (ml) in LS Mean $\pm$ SE	5.12 ± 0.15	6.72 ± 0.06	5.98 ± 0.09	NS
Density (3 scale)	2	3	1	NS
Concentration (10^9 /ml)	0.942 ± 0.017	1.084 ± 0.014	0.551 ± 0.004	*
Initial Motility % (LS Mean $\pm$ SE)	72.54 ± 0.11	78.64 ± 0.15	72.68 ± 0.02	NS
Pre-filling Motility % (LS Mean $\pm$ SE)	71.64 ± 0.13	77.62 ± 0.02	71.34 ± 0.17	NS
Post thaw Motility % (LS Mean $\pm$ SE)	45.61 ± 0.06	51.72 ± 0.03	42.44 ± 0.11	*
Live sperm % (LS Mean $\pm$ SE)	51.23 ± 0.15	55.48 ± 0.05	45.62 ± 0.13	*
Abnormal sperm % (LS Mean $\pm$ SE)	14.67 ± 0.06	11.57 ± 0.12	19.59 ± 0.24	*

Density: 1= fairly dense, 2= dense, 3= highly dense. * Significant at 5% ($P < 0.05$); NS: Non-significant

Influence of collection interval on semen parameters

Influence of collection intervals (first, second and third ejaculate) of semen on semen parameters are presented in Table 3. The significantly ($P>0.05$) higher volume (6.02 ± 0.14 ml) of semen was found in the first collection then that of second and third collections (figure 2). The semen concentration ($1.095\pm0.015 \times 10^9/\text{ml}$), post thaw motility and $50.74\pm0.14\%$ were obtained significantly ($P>0.05$) higher in the Second collection then that of First and third collections. Whereas density, initial motility, pre-filling motility, abnormal sperm and live sperm were obtained higher in the Second collection though did not differ significantly from other collection intervals.

Table 3: Effect of Semen Quality among Number of Collections

Quality parameters	Collections			Level of Significance
	First	Second	Third	
Volume (ml) in LS Mean $\pm$ SE	6.02 ± 0.14	5.72 ± 0.22	2.98 ± 0.12	*
Density (3 scale)	2	3	1	NS
Concentration ($10^9/\text{ml}$)	1.072 ± 0.017	1.095 ± 0.015	0.572 ± 0.119	*
Initial Motility % (LS Mean $\pm$ SE)	74.54 ± 0.15	75.64 ± 0.13	70.58 ± 0.06	NS
Pre-filling Motility % (LS Mean $\pm$ SE)	70.64 ± 0.13	71.62 ± 0.02	68.11 ± 0.12	NS
Post thaw Motility % (LS Mean $\pm$ SE)	48.54 ± 0.06	50.74 ± 0.14	40.34 ± 0.21	*
Live sperm % (LS Mean $\pm$ SE)	53.27 ± 0.54	56.17 ± 0.02	54.34 ± 0.32	NS
Abnormal sperm % (LS Mean $\pm$ SE)	12.51 ± 0.12	12.71 ± 0.25	14.3 ± 0.47	NS

Density: 1= fairly dense, 2= dense, 3= highly dense. * Significant at 5% ($P<0.05$); NS: Non-Significant.

DISCUSSION

Influence of age of bulls on semen parameters

The overall volume (ml $\pm$ SE) of semen produced by Jersey bulls was found 5.20 ± 0.08 ml. This was similar finding as reported by (JLTA, 2004). The lowest volume of

semen produced by Jersey bulls was observed in age of 3 and 4 year of bull. As they are becoming older, they give larger volume of semen which is also justified by the reports of (Serrano, 1984; Mathevon M, 1998b; Walzl *et al.*, 2004). Similar to the present findings, increased semen volume with age and body weight has been documented elsewhere (Van Denmark & Free, 1970). Serrano (1984) demonstrated that bulls from 2-5 years of age produce larger volumes of ejaculate compared to bulls less than 2 years of age and bulls older than 5 years of age. Increased volume of ejaculate, sperm concentration and semen motility has also been observed with the advance of age of the bulls (Mathevon *et al.*, 1998a). It is also demonstrated that young bulls just coming into service produce as little as 1-2 million sperm per ejaculate, while fully matured bulls produce 10-15 million sperm per ejaculate. Moreover, Kumi-Diaka *et al.*, (1981) indicate that sperm cell concentration is significantly higher in the younger matured bulls of 3-7 years compared to bulls of 7.5-10 years. Accordingly, it has been suggested for attention in semen quality due to variation in age of bulls. This increase in semen production and quality can be explained by the fact that size of the testis is regarded as the main factor determining the total number of sperm production correlates with age of the bulls (Brito *et al.*, 2002b).

Density of mature Jersey bull of age interval 5-7 years is higher than any other age interval. This was similar finding as reported by Muhammed *et al.*, (2000). The result of this study shows the concentration of Jersey bulls' semen of age 5-7 is 1.084 ± 0.014 which is higher than 3-4 age interval (0.942 ± 0.017) and 8-9 age interval (0.55 ± 0.004). This may be due to proper handling of bull, proper management and well-established lab procedure. However, Ahmed *et al.*, (2014) obtained less value for the concentration of Jersey bull, Sahiwal bull and local bulls. This may be due to seasonal variation.

Initial motility, pre-filling motility and post-thaw motility was higher in the bull of age interval 5-7 years. Fiaz *et al.*, (2010) obtained the similar result 73.49 ± 0.8 , 70.44 ± 2.0 and 50.67 ± 0.24 respectively. Ahmed *et al.*, (2014) obtained higher value for these parameters. This result may be due to proper lab protocol, well trained technician. Live sperm count is higher for the bulls of age 5-7 years age interval. There is great difference in the value of live sperm % between the age intervals i.e. 51.23 ± 0.15 , 55.48 ± 0.05 and 45.62 ± 0.13 respectively. Kondracki *et al.*, (2003) obtained similar value for younger and lower value for old bulls.

Abnormal sperm % was observed higher in the bull of age interval 8-9 years than that of adult and young. Ahmad *et al.*, (1986) obtained higher value of abnormal sperm for the young bull and lower for the older bull. This variation may be due to breed differences.

Influence of collection interval on semen parameters

Jersey bulls produced similar volume of semen during first and second ejaculate of semen collection but very low in third collection. Walzl *et al.*, (2004) found slightly higher volume of first (5.76 ± 2.56) than second (5.13 ± 2.22) and third (5.28 ± 2.34) ejaculation. However, Everett & Bean (1982) and Everett *et al.*, (1978) found first ejaculates resulted significantly higher volume of semen than other ejaculation. This study has also shown the similar result that as the number of ejaculates increases, volume of semen reduces.

The density of semen usually correlated with sperm concentration so highly dense semen might have higher volume of semen. In Nepal, the average volume in highly dense and dense semen of Jersey having higher value than the same value of HF in Bangladesh as referred by (Sarder *et al.*, 2000). Highest density was obtained at the second collection than the first and third collection. This may be due to the more stimulation of the male accessory reproductive organs, which produce more semen as the gland get more stimulated than the first ejaculation (Castillo *et al.*, 1987).

The overall sperm concentration ($10^9/\text{ml}$) in semen of Jersey bulls was found to be 1.072 ± 0.017 . When compared to Walt *et al.*, (2004) report that ejaculate of breeding bulls contained sperm concentration ($10^9/\text{ml}$) slightly higher for first (1.26 ± 0.41), similar for second (1.08 ± 0.41) and lower for third collection (0.95 ± 0.41) on same day than present finding. Ahmad *et al.*, (2003) reported lower overall concentration ($10^9/\text{ml}$) of sperms ejaculated from Sahiwal bull in Pakistan (0.98 ± 0.01) than present finding. This result may be due to more stimulation of bull for second ejaculation with the stimulation of the accessory reproductive organs. However, Vilakazi *et al.*, (2000) obtained higher value for the first collection this may be due to proper handling, proper stimulation of bull prior to the semen collection.

Initial and prefilling motility was found almost similar in three consecutive collections. Initial motility for the first, second and third collection is 74.54 ± 0.15 , 75.64 ± 0.13 and 70.58 ± 0.06 respectively. Similarly, pre-filling motility for first, second and third collection are 70.64 ± 0.13 , 71.62 ± 0.02 and 68.11 ± 0.12 respectively, this result may be due to same breed same season and same age interval of bulls. JLTA (2004) reported in its publication the similar ranges of initial motility of sperms ejaculated in bull semen ranges from 70-95%. Rekwot *et al.*, (1987) also reported similar (to present finding) initial motility of sperm in exotic and crossbred breeding bulls (74.65% during dry and 76.57% during rainy season).

Post thaw motility of sperms in frozen semen of Jersey bull was found 52% at Nepal (NLBC, 2009) and Adhikari (2008) reported slightly higher than present finding. The

deterioration in post thaw motility% of bull semen may be due to semen production laboratory practice, qualified technicians and pressure on more doses of semen production.

Post thaw semen motility was found to be 50.24%, which is higher than the minimum motility percentage of DLS recommendation (40%). In a study, Singh *et al.*, (2000) found 49.5% motility of spermatozoa just after thawing while Rao & Rao (1979) and Rao (1996) found 53.8% motility. Blom (1950) mentioned that motility below 50% was often associated with low conception rate or poor fertility. So, to compensate this, NLBC fills more no. of sperm cell per dose to achieve better conception rate. Our finding 50.24% is almost similar with Blom (1950) whereas it is higher than the motility of 43.1% as found by Malla (2005) and 45.29% as found by Shrestha (2007). This data also exceeds the data from the NLBC register in which the post thaw mean sperm motility per at 0 hour of freezing found to be only 48%. This might be due to limited and most recent samples we used. These data indicate NLBC has been gradually improving the quality of frozen semen, which is also supported by the increase of the conception rate over the years.

Live sperm and abnormal sperm count almost remain similar in the first, second and third collection. This may be due to the same breed, age interval, season, managemental practices and proper laboratory procedure for semen evaluation. However, Kandracki *et al.*, (2014) obtained more dead sperm in the first collection which may be due to the sperm residue that may be previously present in the male tract before semen collection.

CONCLUSION

The highest volume of semen, motility, concentration, post thaw motility, live sperm and lower abnormal sperm were obtained from 5-7 years' age group of Jersey bulls and lowest from 8-9 years' age group. The sperm concentration, post-thaw motility, live sperm and abnormal sperm were significantly different among different age groups of bulls. The significantly higher volume of semen in the first collection whereas semen concentration and post thaw motility were obtained significantly higher in the Second collection from other collection intervals.

Based on the above findings, following recommendations could be made:

- Older Jersey bulls of more than seven years of age do not produce a good quality semen.
- Semen from third collection in the collection day is not of the good quality to be processed for AI program.

REFERENCES

- Adhikari, R. (2008). Study on NLBC produced buffalo semen quality and its relationship with conception. *Internship Report*, Institute of Agriculture and Animal Science, Rampur, Chitwan, **5**(1):8-12
- Ahmad, K., Ahmad Maqbool, K., & Ahmad, M. (1986). Cryopreservation of buffalo spermatozoa in Tris. *Pakistan Veterinary Journal* , **6**(1): 1-3.
- Al-Hakim, M. (1986). A comparative study on biochemical constituents of semen of Jersey and Jersey Sahiwal bulls. *Indian Journal of Animal Science* , **57**: 1180-1186.
- Bearden, H. J., Fuquay, J. W., & Willard, S. T. (2004). *Applied Animal Reproduction* (6 ed.). Pearson Education.
- Brito, L., Silva, A., Rodriguez, L., Vieira, F., Deragon, A., & Kastelic, J. (2002b). Effect of environmental factors., age and genotype on sperm production and semen quality of B. indicus and B. taurus AI bull in Brazil. *Theriogenology* , **70**: 181-190.
- Castillo, E., Tizol, G., Alvarez, J., Perez, M., & Baez, R. (1987). Reduction of protein concentrates level in ratio for Holstein sires. *Cuban Journal of Agricultural Science* , **21**: 247-251.
- Everett, R., & Bean, B. (1982). Environmental influences on semen output. *Journal of Dairy science* , **65**: 1303-1310.
- Everett, R., Bean, B., & Foote, R. (1978). Sources of variation of semen output. *Journal of Dairy Science* , **61**: 90-95.
- Fiaz, M., Usmani, R. H., Abdullah, M., & Ahmad, T. (2010). Evaluation of semen quality of Holstein Friesian and Jersey bulls maintained under subtropical environment. *Pakistan Veterinary Journal*. **30**(2): 75-78.
- Holt, W. V., O'Brien, J., & Abaigar, T. (2007). Applications and interpretation of computer-assisted sperm analyses and sperm sorting methods in assisted breeding and comparative research. *Reproduction, Fertility, and Development*. **19**(6): 709-18.
- JLTA. (2004). Japan Livestock Technology Association. <http://jlta.lin.gr.jp/english/>
- Kondracki, S., Wysokińska, A., Iwanina, M., Banaszewska, D., & Sitarz, D. (2003). Effect of sperm concentration in an ejaculate on morphometric traits of spermatozoa in Duroc boars. *Polish Journal of Veterinary Science* , **14**(1): 35-40.
- Kumar, U. G., Patil, M. S., Lakde, C. K., Bonde, S. W., & Ramteke, B. R. (2015). Assessment

- of semen quality in pure and crossbred Jersey bulls. *Veterinary World* , **8**(10): 1266–1272.
- Kumi-Diaka, J., Nagerathman, V., & Rwuaan, J. (1981). Seasonal, age- related changes in semen quality and testicular morphology of bulls in tropics. *Veterinary Research* , **108**: 13-15.
- Brito, L.F.C., Silva, A.D.E.F., Rodrigues, L.H., Vieira, F.V., Deragon, A.G., & Kastelic, J.P., (2002b). Effect of environmental factors, age and genotype on sperm production and semen quality of B. indicus and B. taurus AI bulls in Brazil. *Animal Reproduction Science*, **70**(3-4): 181-190.
- Lemma, A., & Shemsu, T. (2015). Effect of Age and Breed on Semen Quality and Breeding Soundness Evaluation of Pre-Service Young Bulls. *Journal of Reproduction and Infertility* , **6**(2): 35–40.
- Mathevon, M., Dckker, J.C.M, & Buhr, M.M., (1998b). Environmental, management and genetic factors affecting semen production in French Montbeliard bulls. *Livestock Science* , **55**: 65-77.
- Mathevon, M., Buhr, R.M., & Dckker, J.C.M, (1998a). Environmental, management, and genetic factors affecting semen production in Holstein bulls. *Journal of Dairy Science* , **81**: 3321-333.
- MoAD. (2015). *Nepal Portfolio Performance Review*. Retrieved November 20, 2017, from Ministry of Agricultural Development: http://www.mof.gov.np/uploads/document/file/Agriculture_NPPR-2015_20150913011507.pd
- NLBC. (2009). *Annual progress report 2009/10*. National Livestock Breeding Centre. Pokhara, Nepal.: National Livestock Breeding Centre.
- Rao, T., & Rao, A. (1979). Fertility and its relationship with semen characteristics in crossbred bulls. *Indian Veterinary Journal* , **56**: 33-36.
- Sarder, M., Joarder, O., Ali, M., & Imam, M. (2000). Influence of genetic groups, season and age on their semen characteristic of breeding bull. *Bangladesh Journal of Genetics and Biotechnology* , **1**: 51-57.
- Serrano, A. (1984). Criteria by which to evaluate fertility in bulls in Central America., (p. 65). University of Illinios at Urbana- Champaign, USA.
- Shamim Akhter, M., Abul Kalam Azad, M., Zahidur Rahman, M., & Ashraf, A. (2013). Study on the Quality of Semen of Different Genetic Groups of Bull from Khulna Region of

- Bangladesh. *International Journal of Pharmaceutical and Medical Research* , **1**(1): 19–23.
- Singh, S. P., Pandit, R. K., & Bhadoriya, H. B. (2000). Sexual behaviour and seminal characteristics in Jersey Sahiwal and halfbred bulls. *Indian Journal of Animal Science* , **70**(3): 279- 280.
- Van Denmark, N. and Free, M. (1970). *Temperature effect* (Vol. 3). (J. A.D., G. W.R., & V. D. N.L, Eds.) Academic Press.
- Vilakazi, D. M. (2003). Factors affecting the quality of semen of A.I dairy bulls in South Africa.
- Waltl, B., Schwarzenbacher, H., Perner, C. and Solkner, J. (2004). Environmental and age affects on the semen quality of Austrian Simmental bulls. Slovenia.

Effect of Non-Genetic Factors on Different Genetic Group of Buck's Semen Quality and Quantity at National Livestock Breeding Center, Nepal

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ABSTRACT

This study was conducted to assess the quality and quantity aspects of different genetic group of buck's semen used for Artificial Insemination (AI) at National Livestock Breeding Centre (NLBC), Pokhara. For this, records from different breeds and genetic groups of buck semen collected over 3 years (2014-2016) were analyzed. Parameters such as volume, density, mass motility, initial motility concentration and post thaw motility were analysed for various non-genetic factors like year, season, green based feeding. Data were analyzed by least square method using Harvey 1990 software package. The result showed that different genetic group of bucks ($P < 0.001$) have highly significant effect ($P = 0.00$) on volume of semen. Significant interaction ($P = 0.01, 0.001$) was observed between year and season on volume and density of the semen. Similarly, different genetic group of bucks had significant effect on density ($P = 0.03$). Year showed a significant effect ($P = 0.02$) on initial motility of the buck semen. Similarly, significant interaction between year and season on initial motility percentage ($P = 0.01$) and post thaw motility percentage ($P = 0.00$) was found. Likewise, year has a significant effect ($P = 0.01$) on the Post thaw motility percentage ($P = 0.03$) of the semen. Also, a significant effect of different breed of buck was found on post thaw motility percentage ($P = 0.00$). In addition, the average post thaw motility percentage semen of all the genetic groups of bucks was above 52% which is considered as a good percentage for the success of AI. This result might be due to use of egg yolk free extender which is soya-based protein. Moreover, adoption of new buck semen processing technologies might further improve the semen quality and long term storability along with better management and distribution of these buck

semen by Government of Nepal through livestock unit at different municipalities will improve the conception rate and save huge effort and investment for the importation of buck semen.

Keywords: *Buck; Genetic group, Non-genetic factor; Semen quality*

INTRODUCTION

Nepal's goat population is enormous, being more than 10.17 million in number with an average annual growth rate of 3.67% from 2001/02 to 2014 (MOAD, 2014). Goat contributes 19.80% to the total meat production of the nation and ranks in second position after buffalo (58.31%) with respect to meat production (MoAD, 2013). Though there are 10.25 million goats in the country, but their production is not enough to meet the country demand which encourages large numbers of goats to be imported from bordering countries. Due to indiscriminate breeding and unawareness among the animal owners, the productive and reproductive efficiency of goats' lags behind and need improvement. Establishment of true line breeds with high prolificacy and fast growth are major challenges to be addressed. Selective breeding of goats can be alternating for genetic up gradation.

Artificial insemination (AI) using cryopreserved semen is an important tool for successful implementation of breeding program to establish pure line breed of goat. The success of AI largely depends on quality of cryopreserved semen that in turns determines the conception rate. Male fertility is highly influenced by semen quantity and quality (Mittal *et al.*, 2014). The quality of thawed frozen semen is very important for success of AI. With the objectives of replacing the import of goat from neighboring countries the Directorate of livestock production has initiated the goat mission program to increase the number of AI coverage in goat and to develop a goat resources center in 80 private farms. Understanding the sources of variation in semen quality, early detection of highly fertile bucks and with good quality thawed frozen semen would be of great interest to all livestock breeders (Arredondo *et al.*, 2015). Quality assurance through regular monitoring of semen quality has becomes an important component of freeze-thawing protocol. But there is great challenge during processing and production of buck frozen semen. So, this study will help to evaluate the quality and quantity of semen that are produced at National Livestock Breeding Center (NLBC) and along with non-genetic factor affecting the different parameter of semen.

MATERIALS AND METHODS

Study Site

National Livestock Breeding Center, Pokhara.

Study Design

Longitudinal descriptive study was conducted during the period of July 2016 to January 2017. Three years data of buck semen quality, collected at National Laboratory Breeding Centre (NLBC), Pokhara were included for the analysis.

Data Collection

Data on Season, Year, Feed, and Breed (Jamunapari, Sannen, Ajmeri, and Boer) were collected. Regarding semen quality parameters, volume, density, mass motility, initial motility, concentration, post thaw motility was collected. The recorded data were collected based on the following Standard Operating Procedure (SOP) of NLBC.

Collection of Semen

Semen was collected in the morning between 7- 8 am by Artificial Vaginal (AV) method. The AV consists of outer rubber cylinder, inner rubber line, rubber band, cone and collecting tube. Semen was collected with the help of a teaser animal. All the apparatus used for semen collection was sterilized before collection of semen using autoclave machine. The inner liner temperature of AV was maintained at 42-43° C water by loading at 52-54°C two-third of its volume. The rest of one-third area of water jacket was filled with air. Before collection of semen some sterile non spermicidal gel was applied into the inner side of artificial vagina by glass rod. The semen collected tube was labeled with the identification of the breed and mouth of the tube was sealed with aluminum foil. The semen containing tube was kept in water bath at 37°C for five minutes.

Semen Evaluation

Volume

The volume of ejaculates (ml) was recorded from the graduated collection vials. The color and opacity of the semen and presence of any foreign body, such as pus were checked.

Mass Motility

Mass motility of raw semen was examined by using a drop of raw undiluted semen on a pre-warmed (37°C) slide under a light microscope at 10x and rated based on the

swirling currents.

0 = no motility

+ = Less than 20 percent of the sperm showing progressive motion.

++ = 20-40 percent showing progressive movement but no wave.

+++ = 44-60 percent showing progressive movement but with slow wave.

++++ = 60-80 percent showing progressive movement with rapid wave.

+++++ = 80-100 percent showing progressive movement with rapid wave.

Individual Motility

One drop of semen was mixed with 2 ml of diluter. The composition of Diluter is given in annex. Then the individual motility of diluted semen was examined by using a drop of diluted semen on a pre-warmed (37°C) slide covered with cover slip. Slide was examined under a light microscope at 20x and rated based on the forward movement of the individual sperm.

Concentration of Sperm

The concentration of spermatozoa in semen sample (ten billion/ml) was estimated by using Semen photometer SDM6 of Minitube Company.

Procedure

35 µl of buck semen was diluted with 4ml of normal saline in a cuvette and mixed well with micropipette. Then the cuvette was inserted in sample container of a photometer which was pre-set with the buck semen. Then the reading was shown on the screen.

Dilution of semen

Andromed R, a readymade diluter of Minitube company was used for dilution of semen. Andromed R is a soybean lecithin-based extender that has been suggested as a replacement of egg yolk as animal source diluents. The composition of the extender is presented in annex 2. The volume of diluter required for dilution of semen was calculated by using the following formula. This formula was developed by the NLBC. With the help of auto dispenser required volume of dilution was taken in the beaker and semen was added in it. The beaker was labeled with the identification number and dilution amount. The following formula for calculation of extender was used according to NLBC.

Extender =

Sealing Filling and Printing of Straw

Minitube automated filling sealing and printing machine was used for filling sealing and printing of buck semen on the straw. The genuine CASSOU straw of imv technologies and minitube semen straws were used for this purpose. 0.25 ml of semen was filled in each straw. Buck number, breed, date of semen collection was printed on the straw.

Cooling of Straw

Only filled and sealed straw were used for the cooling and those half-filled and unsealed straw were discarded. The sealed straws were carefully spread over the freezing rack and kept on cooling chamber at 5°C for three hours.

Freezing of Straw

For freezing, the Styrofoam container was filled with liquid nitrogen. The rack carrying semen straws was suspended for 10 min above liquid nitrogen, keeping a gap of 5 cm between the surface of liquid nitrogen and the semen straws. After 10 min, the straws containing semen were submerged into liquid nitrogen. Then the semen straws from each buck were grouped and loaded into a liquid nitrogen canister and stored in at -196°C for approximately 3 months.

Evaluation of Post Thaw Motility

Post thaw motility was examined after the semen straws were loaded in the liquid nitrogen canister. Semen straws were thawed, by dipping them into a water bath at 37°C for 20 s before evaluation. Post thaw motility was observed in microscope in 20 xs and in the Computer Assisted Semen Analysis (CASA).

Data Management and Analysis

The collected data were entered in Microsoft Excel. The variables year, Season, Feed and genetic group were coded numerically and entered. Continuous data volume, concentration, mass motility, initial motility and post thaw motility were entered directly. Data were analyzed using Harvey 1990. Mean, least square mean, standard deviation, coefficient of variation was computed. Inferential statistics were used to compare volume, density, mass motility, initial motility, concentration, post thaw motility among season, year, feed, genetic group. P value ≤ 0.05 was considered significant. Linear regression analysis was done of dependent variable volume, density, concentration, mass motility, individual motility, post thaw motility with predictor year, season, feed and genetic groups.

Model used for analyzing the data

The fixed effect and random effect models of ‘Harvey 1990’ statistical package were used to analyze all the observations of collected data.

Model used for analyzing the semen quality (semen volume, semen density, mass activity, sperm concentration, initial motility, pre-filling motility and post thaw motility)

The following fixed effect model was used to express each observation of semen quality produced from breeding Jersey bulls.

$$Y_{ijklmno} = \mu + a_i + b_j + c_k + d_l + e_{ijkl}$$

Where, μ is the overall mean

a_i is the effect of i^{th} year ($i=2014, 2015, 2016$)

b_j is the effect of j^{th} season of semen collection ($k=\text{Dry, Wet}$)

c_k is the effect of k^{th} genetic group buck ($k=\text{Boer 100\%, Sannen, Boer 50\%, Boer 75\%, Ajmeri, Jamunapari}$)

d_l is the effect of l^{th} grass base feeding ($l=\text{Teosinte, Oat, Napier}$)

e_{ijkl} is the random element (error mean) assumed to be normally and independently distributed among the sampled population

RESULTS AND DISCUSSION

The information of semen parameter (volume, density, mass motility, concentration, initial motility and post thaw motility along with the non-genetic factor (year, season, forage and genetic group) affecting these parameters are presented and discussed below. Also, the summary tables of ANOVA for each parameter are shown respectively.

Physical quality parameters

Semen volume

The overall volume ($\text{ml} \pm \text{SE}$) of semen produced by different genetic group of bucks was found 1.45 ± 0.11 ml (Table 1). This finding was higher than other reports (Sultana et al., 2013) and Hassan *et al.*, (2010). Other studies reported that buck semen volume ranges from 0.58 ± 0.17 to 1.04 ± 0.11 ml.

Effect of year on volume and density of semen

No significant effect ($P > 0.05$) on volume of semen was found with respect to year of semen collection (Table 1). There was no specification trend of semen volume over three years. Highest semen volume was recorded in the year 2014 (1.67 ± 0.23) followed by 2016 (1.43 ± 0.23) and 2015 (1.23 ± 0.07). Like many other non-genetic factors semen quantity is affected by the frequency of semen collection and favorable temperature which might have affected on the volume of semen quantity. Year showed slight variation in semen density. Accordingly, semen density was found (3.20 ± 0.09) in the year 2014 followed by year 2016 (3.08 ± 0.09) and 2015 (3.06 ± 0.03).

Effect of season on volume and density of semen

There was no significant association of season with the volume of the semen. The semen volume was 1.43 ± 0.15 ml in dry season and 1.46 ± 0.15 ml in wet season. The result of the present study similar with the findings of Maina *et al.*, (2006). This finding is also supported by the Aguiar *et al.*, 2013 who found the non-significant association of season with volume of the buck semen. However, this result is contradicted with the Elsheikh and Elhammali (2015), Sultana *et al.*, (2013) who found that the season has a significant effect on semen volume of mature crossbred bucks. This result shows that there might be no variation or little variation on environmental factors like temperature, day length, moisture percentage etc. No seasonal effects were found on semen density. Season showed slight variation on semen density. i.e. mean 3.04 ± 0.06 in dry season and 3.19 ± 0.06 in wet season.

Table 1: Least square means and standard errors for volume (ml) and density of buck semen

Factors	Number (n)	LS Mean $\pm$ SE (Volume)	P- value	LS mean $\pm$ SE (Density)	P- value
Overall mean	241	1.45 ± 0.11		3.11 ± 0.04	
Year			0.15		0.34
2014	32	1.67 ± 0.23		3.20 ± 0.09	
2015	151	1.23 ± 0.07		3.06 ± 0.03	
2016	58	1.43 ± 0.23		3.08 ± 0.09	
Season			0.92		0.11
Dry (Dec- May)	109	1.43 ± 0.15		3.04 ± 0.06	
Wet (June-July)	132	1.46 ± 0.15		3.19 ± 0.06	

Green based forage			0.98		0.14
Teosinte	89	1.47 ± 0.17		3.04 ± 0.07	
Oat	46	1.43 ± 0.18		3.12 ± 0.07	
Napier +Maize	106	1.44 ± 0.11		3.18 ± 0.04	
Genetic group ***			0.00		0.03
Boer 100%	44	1.26 ± 0.28 ^{bc}		2.95 ± 0.11 ^c	
Boer 75%	23	1.58 ± 0.15 ^c		3.09 ± 0.06 ^a	
Boer 50%	29	1.48 ± 0.24 ^b		3.22 ± 0.10 ^a	
Sannen	53	1.18 ± 0.23 ^{ab}		3.35 ± 0.09 ^b	
Ajmeri	39	1.21 ± 0.27 ^c		3.02 ± 0.11 ^{bc}	
Jamunapari	53	1.97 ± 0.12 ^a		3.05 ± 0.05 ^{bc}	
Interactions					
Year x Season			0.01		0.001
Year x Genetic group			0.25		0.43
Season x Genetic group			0.87		0.61
Green based forage x Genetic group			0.45		0.01

* Significant at 5% (P<0.05); ** Significant at 1% (P<0.01); *** Significant at 0.1% (P<0.001); NS: Non-significant

Effect of genetic group of bucks on volume and density of semen

Highly significant effect (P<0.001) on volume of semen was found with respect to different genetic group of bucks (Table 2). Highest volume of semen was found in Jamunapari breed (1.97 ± 0.10ml) followed by Ajmeri (1.61 ± 0.12ml), Sannen (1.38 ± 0.11ml), Boer cross 50% (1.29 ± 0.13ml), Boer 75% (1.38 ± 0.11ml) and Boer 100% (1.02 ± 0.12). The maximum average volume of semen in Jamunapari was higher than the report of Hassen *et al.*, who reported the average volume of semen 0.9 ± 0.2 ml in Jamunapari buck. Similarly, average volume of 0.57ml of semen in Saanen buck was

reported by Ferreira *et al.*, 2014 in Brazil which was lower than our findings. (Bezerra 2012) reported the average volume of semen ejaculated in pure Boer was 1.1 ± 0.4 ml which is similar to our findings but Suyadi 2012 found 0.74 ± 0.43 ml of semen in pure Boer breed which was lower than our findings. This significant effect ($P < 0.001$) may be due to the genetic potential, age variation among bucks and genetic potentiality and genetically superior buck could produce higher volume of semen. Older bucks produced higher volume of semen than younger bucks. Walzl *et al.*, (2004) found age of bull had highly significant effect on semen volume (higher the age higher the volume of semen). Significance effect ($P = 0.03$) of Genetic group on semen density was found. Highest semen density was found in Sannen (3.35 ± 0.09) followed by Boer 50% (3.22 ± 0.10), Boer 75% (3.09 ± 0.06), Jamunapari (3.05 ± 0.05), Ajmeri (3.02 ± 0.11) and Boer 100% (2.95 ± 0.11).

Effect of grass-based feeding on volume and density of semen

There was no significant effect on the volume of semen with respect to green based forage feed to different genetic group of bucks. Average volume of semen recorded by feeding Teosinte (*Euchlaena maxicana*) was (1.47 ± 0.17 ml) followed by Napier and maize (1.44 ± 0.11) and Oat (*Avena sativa*) (1.43 ± 0.18). Similarly, no significant effect on density of semen was found with respect to green based forage feed to different genetic group. Average density of semen of buck feed to Napier and maize was (3.18 ± 0.04) followed by Oat (*Avena sativa*) (3.12 ± 0.07) and Teosinte (*Euchlaena maxicana*) was (3.04 ± 0.07).

Interaction of different factors on volume of the semen:

Significant effect on semen was found with respective to interaction between year and season. Interaction between other factors (Year x Genetic group, Season x Genetic group, and Feed x Genetic group) had no significant effect on the volume of semen production of different genetic group of bucks. Interaction between year and Season showed highly significant ($P < 0.001$) effect on density of semen. Similarly, interaction between feed and genetic group also showed the significant effect on density of the semen. Interaction between year, season and genetic group showed no significant effect on density of semen.

Mass activity/Mass motility of sperms

The collective movement of sperms or their wave motion is called as mass activity. Mass activity/Mass motility of sperms in fresh semen was found to be variate (Table 2) due to different genetic group and so many non-genetic factors.

Effect of year on mass motility and initial motility of sperm

Mass activity of buck semen was different according to year. Highest mass activity was found in year 2015 followed by year 2016 and year 2014. This shows that mass activity of buck improved as the frequency of the collection or number of ejaculations increased. Significant effect ($P < 0.001$) of year on initial motility percentage of sperm was found (Table 2). Highest initial motility of buck semen was found in year 2016 ($81.86 \pm 1.26\%$) followed by year 2015 ($78.55 \pm 0.39\%$) and year 2014 ($77.38 \pm 1.23\%$).

Effect of season on mass motility and initial motility of sperm

Season had no effect on mass activity of fresh semen. Average mass activity on wet season was 3.52 ± 0.10 followed by dry season 3.46 ± 0.10 which was not statistically significant different ($P = 0.367$). This finding is supported by findings of Mathur *et al.*, (2002) who observed no significant seasonal difference in mass motility and progressive motility. Season had no effect on initial motility of fresh semen. Average initial motility on dry season was $79.41 \pm 0.83\%$ followed by wet season $79.11 \pm 0.83\%$ which was not statistically significant different ($P = 0.80$). This finding is also supported by Brito *et al.*, who reported that ambient temperature, month (season) and humidity did not significantly affect sperm production and semen quality probably because there is a little variation in these variables.

Effect of green based forage on mass motility and initial motility of semen

No significant effect on mass motility and initial motility of semen was found with respect to green based forage feed to different genetic group of bucks. Average mass motility of semen of different buck feed to Napier and maize was (3.61 ± 0.08) followed by Teosinte (*Euchlaena maxicana*) (3.48 ± 0.11) and Oat (*Avena sativa*) (3.38 ± 0.12).

Effect of year on concentration and post thaw motility of sperm

No significant effect of year on concentration ($10^9/\text{ml}$) of sperm was found (Table 3). Highest concentration of sperm of different buck was found in year 2016 (2.43 ± 0.18) followed by year 2015 (2.07 ± 0.05) and year 2014 (2.03 ± 0.18). Significant effect of year on post thaw motility was found (Table 3). Highest post thaw motility percentage was found in the year 2016 (57.99 ± 2.56) followed by 2015 (57.21 ± 0.80) and 2014 (50.35 ± 2.50). This shows that as the age and frequency of semen collection increase there is improvement in the post thaw motility percentage of the sperm.

Table 2: Least square means and standard errors for mass activity and initial motility of buck semen

Factors	Number (n)	LS Mean $\pm$ SE mass motility (%)	P-value	LS Mean $\pm$ SE Initial motility (%)	P-value
Overall mean	241	3.49 $\pm$ 0.07		79.26 $\pm$ 0.59	
Year			0.12		0.02
2014	32	3.32 $\pm$ 0.15		77.38 $\pm$ 1.23	
2015	151	3.64 $\pm$ 0.05		78.55 $\pm$ 0.39	
2016	58	3.51 $\pm$ 0.16		81.86 $\pm$ 1.26	
Season			0.68		0.80
Dry (Dec- May)	109	3.46 $\pm$ 0.10		79.41 $\pm$ 0.83	
Wet (June-Nov)	132	3.52 $\pm$ 0.10		79.11 $\pm$ 0.83	
Green based forage			0.07		0.83
Teosinte	89	3.48 $\pm$ 0.11		79.70 $\pm$ 0.92 ^b	
Oat	46	3.38 $\pm$ 0.12		78.96 $\pm$ 0.96 ^b	
Napier +Maize	106	3.61 $\pm$ 0.08		79.13 $\pm$ 0.63 ^a	
Genetic group			0.10		0.22
Boer 100%	44	3.20 $\pm$ 0.19		80.29 $\pm$ 1.49	
Boer 75%	23	3.27 $\pm$ 0.10		78.84 $\pm$ 0.83	
Boer 50%	29	3.72 $\pm$ 0.16		78.61 $\pm$ 1.28	
Sannen	53	3.47 $\pm$ 0.16		77.59 $\pm$ 1.24	
Ajmeri	39	3.80 $\pm$ 0.18		81.84 $\pm$ 1.47	
Jamunapari	53	3.48 $\pm$ 0.08		78.41 $\pm$ 0.67	

Interactions					
Year x Season			0.10		0.010
Year x Genetic group			0.15		0.14
Season x Genetic group			0.25		0.48
Green based forage x Genetic group			0.48		0.93

* Significant at 5% ($P<0.05$); ** Significant at 1% ($P<0.01$); *** Significant at 0.1% ($P<0.001$); NS: Non-significant

Effect of genetic group on mass motility and initial motility of semen

Mass activity and initial motility of fresh semen was different according to different genetic group of bucks. Highest mass activity was found in Ajmeri (3.80 ± 0.18) followed by Boer 50% (3.72 ± 0.16) Jamunapari (3.48 ± 0.08), Saanen (3.47 ± 0.16), Boer 75% (3.27 ± 0.10) and Boer 100% (3.20 ± 0.19) had relatively low mass activity. Average mass motility of all breeds was above 60%.

Interaction between different factors on mass motility of the semen

No significant effect factors on mass motility of the semen were found with interaction between different factors (Year x Season, Year x Genetic group, Season x Genetic group and Feed x Genetic group). Interaction between year and season showed the significant ($P<0.05$) effect on initial motility of the semen. Whereas no significant effect was found with the interaction between year and season, year and genetic group, season and genetic group, feed and genetic group.

Concentration of sperms

Concentration of sperms in fresh semen along with effect of non-genetic factors are presented (Table 3). The overall sperm concentration ($10^9/\text{ml}$) in semen of different buck was found to be 2.18 ± 0.08 .

Table 3: Least square means and standard errors for concentrations of sperms of buck semen

Factors	Number (n)	LS Mean $\pm$ SE concentration	P-value	LS Mean $\pm$ SE Post thaw motility (%)	P-value
Overall mean	241	2.18 $\pm$ 0.08		55.18 $\pm$ 1.19	
Year			0.18		0.03
2014	32	2.03 $\pm$ 0.18		50.35 $\pm$ 2.50	
2015	151	2.07 $\pm$ 0.05		57.21 $\pm$ 0.80	
2016	58	2.43 $\pm$ 0.18		57.99 $\pm$ 2.56	
Season			0.16		0.35
Dry (Dec-May)	109	2.30 $\pm$ 0.12		54.07 $\pm$ 1.69	
Wet (June-July)	132	2.06 $\pm$ 0.12		56.30 $\pm$ 1.68	
Green based forage			0.81		0.85
Teosinte	89	2.24 $\pm$ 0.13		54.62 $\pm$ 1.87	
Oat	46	2.12 $\pm$ 0.14		55.94 $\pm$ 1.95	
Napier +Maize	106	2.17 $\pm$ 0.09		54.99 $\pm$ 1.28	
Genetic group ***			0.003		0.06
Boer 100%	44	2.24 $\pm$ 0.21 ^a		56.93 $\pm$ 3.02	
Boer 75%	23	1.99 $\pm$ 0.12 ^a		53.27 $\pm$ 1.69	
Boer 50%	29	2.37 $\pm$ 0.18 ^a		55.05 $\pm$ 2.59	
Sannen	53	2.27 $\pm$ 0.18 ^b		52.38 $\pm$ 2.51	
Ajmeri	39	2.43 $\pm$ 0.21 ^a		54.73 $\pm$ 2.97	
Jamunapari	53	1.76 $\pm$ 0.09 ^b		58.75 $\pm$ 1.36	

Interactions					
Year x season			0.24		0.00
Year x genetic group			0.67		0.14
Season x Genetic group			0.96		0.48
Green based forage x Genetic group			0.89		0.93

* Significant at 5% ($P < 0.05$); ** Significant at 1% ($P < 0.01$); *** Significant at 0.1% ($P < 0.001$); NS: Non-significant

Effect of season on concentration and post thaw motility of sperm

Season had no effect on concentration of sperm. Average concentration ($10^9/\text{ml}$) of sperm on dry season was 2.30 ± 0.12 followed by wet season 2.06 ± 0.12 which was not statistically significant different ($P = 0.16$).

Effect of green based forage on concentration and post thaw motility of sperm

No significant effect of feed was found on the concentration and post thaw motility of sperm. But highest concentration of sperm was found in buck feed with Teosinte (*Euchlaena maxicana*) (2.24 ± 0.13) followed by Napier and maize (2.17 ± 0.09) and Oat (*Avena sativa*) (2.12 ± 0.14).

Effect of genetic group on concentration and post thaw motility of sperm

High significant effect ($P < 0.003$) of genetic group on sperm concentration ($10^9/\text{ml}$) was found (Table 3). The significant differences in sperm concentration among different genetic group of bucks indicate the genetic potentiality and well adaptability of the different breed in the environment. No significant effect ($P > 0.05$) of different genetic group of breeds on post thaw motility was found (Table 3). Highest post thaw motility was found in Jamunapari breed ($58.75 \pm 1.36\%$), followed by Boer 100% ($56.93 \pm 3.02\%$), Boer 50% ($55.05 \pm 2.59\%$), Ajmeri ($54.73 \pm 2.97\%$), Boer 75% ($53.27 \pm 1.69\%$), and Sannen ($52.38 \pm 2.51\%$). Dorado et al. (2010) reported that the post thaw progressive motility to be $33.00 \pm 1.02\%$ while mean post-thaw motility was $56.07 \pm 1.32\%$ in Florida bucks. Mohammed et al. (2012) reported the significant effect of genetic group on Post-thaw semen motility percentage at 0hr in Aradi (42.3) and Damascus (39.6) than cross bucks (38.1) which was lower than

our findings. The average post thaw motility percentage of different genetic group of bucks was above 52 % which is considered as good percentage for the success of the artificial insemination in goat. This satisfaction result might be the use of diluents which is egg yolk free concentrate medium named AndroMed®. Janeet et al; 2005, demonstrate that total and progressive motility (%) in frozen-thawed semen processed in AndroMed ® (54.4 ± 3.2 and 25.5 ± 2.3) were significantly ($P < 0.05$) higher when compared to TRIS-egg yolk extender (45.9 ± 2.0 and 14.9 ± 1.0).

Interaction between different factors on concentration and post thaw motility of the semen

No significant effect on concentration and post thaw motility of the semen was found with interaction between different factors (year and genetic group, Season and genetic group, green based forage and genetic group) but interaction between year and season showed the highly significant effect ($P < 0.001$) on post thaw motility of semen.

CONCLUSION

The quality and quantity of semen production is affected by different non genetic factors. The quality and quantity of semen production varies according to the breed. Except on the initial motility and post thaw motility year had no significant effect on volume density, mass motility and concentration of semen among different genetic group of bucks. Similarly season and Green based forage had no significant effect on the quality and quantity parameter of semen of different genetic group of bucks. Significant effect of different genetic group of buck were found on the parameter like volume, density and concentration of semen but there was no association of this factors on the parameter like mass motility, initial motility and post thaw motility. Average Post thaw motility % of the entire breed maintained at NLBC for semen production was above 52% which might be the result of diluents that is egg yolk free protein and it might have improved the post thaw motility percentage. The demand of Boer, Jamunapari and Ajmeri semen is high in different district of the country. Large volume of semen from government and non-government sector is imported annually for AI in the country. But NLBC Pokhara has already started production and distribution of semen from the exotic buck of goat. Semen quality and quantity produced and used in Nepal is of good quality and meet the international requirement. Therefore, better management and distribution of quality semen by Government at different DLSO will improve the conception rate and save huge effort and investment for the importation of semen.

REFERENCES

- Aguiar, G. V., M. F. Van Tilburg, A. G. V. Catunda, C. K. S. Celes, I. C. S. Lima, A. C. N. Campos, and A. A. Araújo. 2013. Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian Northeast: the season's influence on the cooling of semen. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **65**(1), 6-12pp.
- Arredondo, A. J. G., A. G. Gomez, J. F. Vazquez-Armijo, R. A. Ledezma-Torres, H. Bernal-Barragan and F. Sanchez-Davila. 2015. Status and implementation of reproductive technologies in goats in emerging country. *African Journal of Biotechnology*, **14**(9): 719-727pp.
- Brito, L.F.C., A.E.D.F. Silva, L.H. Rodrigues, F.V. Vieira, L.A.G. Deragon and J.P. Kastelic, 2002. Effects of environmental factors, age and genotype on sperm production and semen quality in *Bos indicus* and *Bos Taurus* A.I bulls in Brazil. *Animal Reproduction Science*, **70**: 181-190pp.
- Dorado, J., I. Rodriguez and M. Hidalgo. 2007. Cryopreservation of goat spermatozoa: Comparison of two freezing extenders based on postthaw sperm quality and fertility rates after artificial insemination. *Theriogenology* **68**: 168–77pp.
- Dorado, J., A. Munoz-Serrano and M. Hidalgo. 2010. The effect of cryopreservation on goat semen characteristics related to sperm freezability. *Animal Reproduction Science* (121): 115p.
- Elhammali, N. S.; Alqurashi, A. M.; Ibrahim, M.; Elsheikh, A. S. 2013. Puberty of crossbred male goat kids. *Journal of American Science*, **9**(4); 95-99pp.
- Elsheikh and Elhammali. 2015. Semen quality of mature crossbred male goats during different seasons. *Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, p-ISSN: 2319-2372. Volume 8, Issue 9 Ver. II (Sep. 2015), 01-05pp.
- Elsheikh, A. S.; Elhammali, N. S.; Ibrahim, M. T.; Alqurashi, A. M. 2013. Influence of season of birth on pubertal characteristics of male goat kids. *JASA*, **2**(2): 108-111pp.
- Mittal, P. K., Anand, M., Madan, A. K., Yadav, S. and Kumar, J. 2014. Antioxidative capacity of vitamin E, vitamin C and their combination in cryopreserved Bhadawari bull semen. *Vet. World*. **7**(12): 1127-1131pp.
- Maina, V. A., Chaudhari SUR, Mshelia, Williams. 2006. Influence of Season on

- Semen Characteristics of Sahel Bucks in Borno State. *Journal of Applied Sciences*, **6**: 353-356pp.
- Mathur, A. K., S. Tyagi and S. P. Singh. 2002. Frieswal bull - an experience of HF with Sahiwal. *J. Livest. Poultry Prod.* **18**(1-2): 21-23pp.
- Mittal, PK, Anand M, Madan AK, Yadav S, Kumar J 2014. Antioxidative capacity of vitamin E, vitamin C and their combination in cryopreserved Bhadawari bull semen. *Veterinary World*, **7**(12): 1127- 1131pp.
- MoAD. 2012. Ministry of Agricultural Development, Agribusiness Promotion and Statistics Division, Agri Statistics Section, Singhdurbar, Kathmandu Nepal. Statistical Information on Nepalese Agriculture. (Accessed 10 February 2016.)
- Mohammad, K. M. 2012. Effect of genetic group, semen diluents and freezing regimens on sperm freezability and goats reproductivity. *Assiut Vet. Med. J.* Vol **58** No. 135p
- Mohammed et al 2014. Effect of genetic group, semen diluents and freezing regimens on sperm freezability and goats reproductivity, *AssiutVet. Med. J.* **58** No. 135 October 2012.
- MoLD. 2016. Ministry of Livestock Development. Commitment paper (Nepali). <http://www.mold.gov.np/uploads/files/pratibaddata.pdf> (Accessed 10 April 2016.).
- Sultana, R.2013. Study on buck evaluation based on semen quality and fertility, *Bang. Journal Animal Science.* **42**(2): 101-108pp.
- Suyadi, A. 2014. Sexual behaviour and Semen Characteristics of Young Male Boer Goats in Tropical Condition: A Case in Indonesia, *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering* **6**: 6p.
- Tardiff A.L., Farrell P.B., Trouern Trend V. and Foote R.H. (1997). Computer assisted sperm analysis for assessing initial semen quality and changes during storage at 5 °C. *J. Dairy Science.* **80**: 1606-1612pp.
- Toe, F., Lahlou-Kassi, A., Mukasa-Mugerwa, E., 1994. Semen characteristics of Ile-de-France rams of different age and physical condition. *Theriogenology* **42**: 321–326pp.

Vilar AC, Barnabe VH, Birgel FH, Barnable RC and Visintin JA (1993). Testis and semen characters in goats reared in a semi-arid area in pariaba state. *Revista Brasileira de Reproducao Animal*, **17**(1- 2):23-32pp.

Vishwanath, R. and S. Shannon .2000. Storage of bovine semen in liquid and frozen state. *Anim Reprod Science*; **62**: 23-53pp.

Status of PPR and Sero-conversion of PPR Vaccines in Goats and Sheep of Midwestern Region of Nepal

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ABSTRACT

Peste des petits ruminant (PPR) is a common, acute, highly contagious and infectious viral epidemic disease of small ruminants which has caused significant economic losses in Nepal. Total of 247 suspected serum samples from 13 outbreaks from 8 districts of the Midwestern region of Nepal in 2015 were confirmed by using antibody ELISA test and in case of dead animals, the tissue samples were further confirmed by using antigen ELISA test. Out of 247 serum samples, 153 (61.9%) samples showed PPR antibody positive. The Ecozone wise distribution of PPR showed that the PPR outbreak were reported the highest in mountain followed by hills and the lowest in Terai. The highest outbreaks in mountain may be due to excessive movements of nomadic herd of sheep and goat, common pasture, low vaccine coverage in mountain whilst in hill region due to geographic difficulty and low sero-conversion of vaccine. Hence, strategic vaccination campaigns, proper biosecurity, movement control, risk analysis and early diagnosis need to be implemented for the control of PPR in the country.

Keywords: *small ruminants, Peste des petits ruminant, ELISA*

INTRODUCTION

Nepal is an agricultural country where 60.4% of the population is based on agriculture for their livelihood. Out of this 80% of the population have livestock farming as an integrated way. Cattle, buffalo, goat and poultry farming is the common animal species. The population of different livestock species in million is as buffalo 5.2, cattle 7.2, goats 11, sheep 0.8, pigs 1.2 and chicken 50.2. Agriculture contributes 31.23% in GDP and Livestock contributes 25.68% in Agriculture Gross Domestic Products (MOAD, 2015). Peste des petits ruminants (PPR) is an acute highly contagious and infectious viral disease of small ruminants like sheep and goats caused by a RNA virus of family Paramyxoviridae. PPR has been reported in various parts of Asia and Africa. PPR was first reported from Ivory Coast in West Africa in 1942 whereas this disease was first reported in Nepal in Central Terai in 1995. This disease has caused significant economic losses among small ruminant farmers of Nepal through high

morbidity and mortality pattern of the diseases. The major signs include high fever discharges from eyes, nose, mouth and severe diarrhea. The major sources of disease transmission are the discharges from eyes, nose, and mouth, and anus, close contact between infected and susceptible animals. In Nepal, PPR outbreak is associated with introduction of recently purchased animals from neighboring countries and abroad. National level PPR control program has been implemented by vaccination to prevent huge economic loss of the farmers. This present study was conducted to determine the status of PPR and sero-conversion of PPR vaccines in sheep and goats in midwestern region of Nepal.

MATERIALS AND METHODS

During the time of outbreak in midwestern region of Nepal from July 2015 to March 2016, the 247 serum samples were collected from suspected animals and tissue samples were collected from dead animals. The diagnosis was done based on history taking, clinical examination and postmortem examination. Serum samples of suspected animals were confirmed by using antibody ELISA test in Regional Veterinary Laboratory, Surkhet. Vaccination program against PPR throughout the country was conducted after outbreak and the serum samples were collected again after three weeks of vaccination. These serum samples were also tested using antibody ELISA test.

RESULT AND DISCUSSION

Districtwise distribution of PPR

PPR is a common epidemic among the small ruminant population of the country. During the study, total 247 outbreak suspected serum samples were tested for PPR, out of which 153 (61.9%) serum samples were confirmed to be PPR antibody positive. During the study period PPR have been confirmed from eight districts out of 15 districts in the Mid-western region of Nepal that is Jumla, Dailekh, Jajarkot, Dolpa, Mugu, Humla, Dang and Banke (Figure 1). No outbreaks were reported in remaining seven districts namely Bardiya, Surkhet, Ruknum, Rolpa, Salyan, Pyuthan and Kalikot of mid-western region of Nepal. In the region, total of 13 outbreaks occurred which caused death of 9230 (39%) out of 23670 infected goat and sheep. The highest number of PPR outbreak was reported in Dailekh (n=4) followed in Mugu (n=3) and one each in Jumla, Jajarkot, Humla, Dolpa, Dang and Banke. The highest number of outbreaks in Dailekh might be due to high movement of goats and sheep along the road corridor and in Mugu might be due to the common large pastureland. In Nepal, PPR has been reported from 68 districts out of 75 districts in past 20 years. Only in 2015, total 71 outbreaks of PPR was reported from 26 different districts in the country.

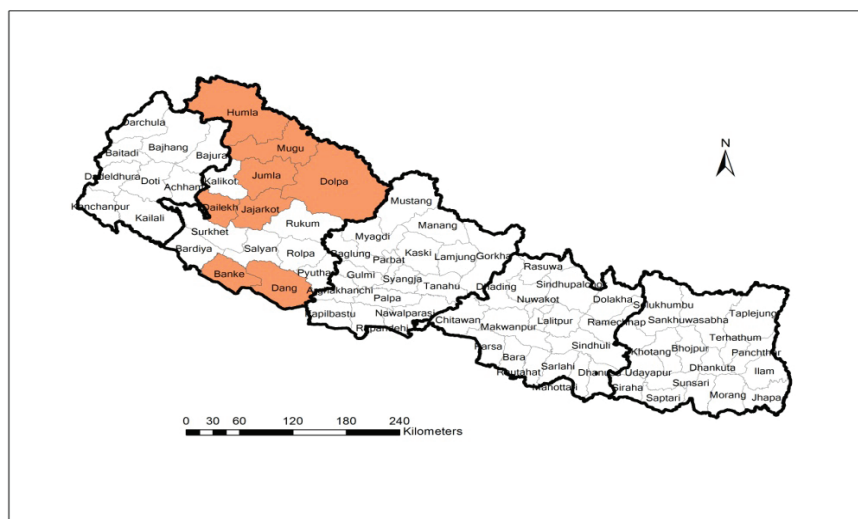


Fig. 1: PPR outbreak districts in mid-western region of Nepal

Total 1302 serum samples were collected after 3 weeks of vaccination from 11 districts of this region and tested by Antibody ELISA (Table 1).

Table 1: Sero-monitoring in different district

S.N.	District	Number of Vaccination	Sample Tested	Positive	Negative	Positive Percentage
1	Banke	500000	152	104	48	68.4
2	Bardiya	60000	120	81	39	67.5
3	Dang	60000	92	81	11	88.0
4	Surkhet	50000	232	157	75	67.7
5	Dailekh	35000	136	78	58	57.4
6	Jajarkot	35000	72	64	8	88.9
7	Rukum	25000	44	26	18	59.1
8	Rolpa	30000	60	36	24	60.0
9	Salyan	25000	158	112	46	70.9
10	Pyuthan	35000	126	99	27	78.6
11	Jumla	25000	110	82	28	74.5
	Total	430000	1302	920	382	70.7

Ecozone wise distribution of PPR

The Ecozone wise distribution of PPR in Midwestern region is shown in figure 2. Out of 13 outbreaks recorded in midwestern region, six outbreaks were reported from mountain region, five were reported in hills and two were in Terai regions. The highest outbreaks in mountain may be due to excessive movements of nomadic herd of sheep and goat, common pasture, low vaccine coverage in mountain whilst in hill region due to geographic difficulty and low sero-conversion of vaccine.

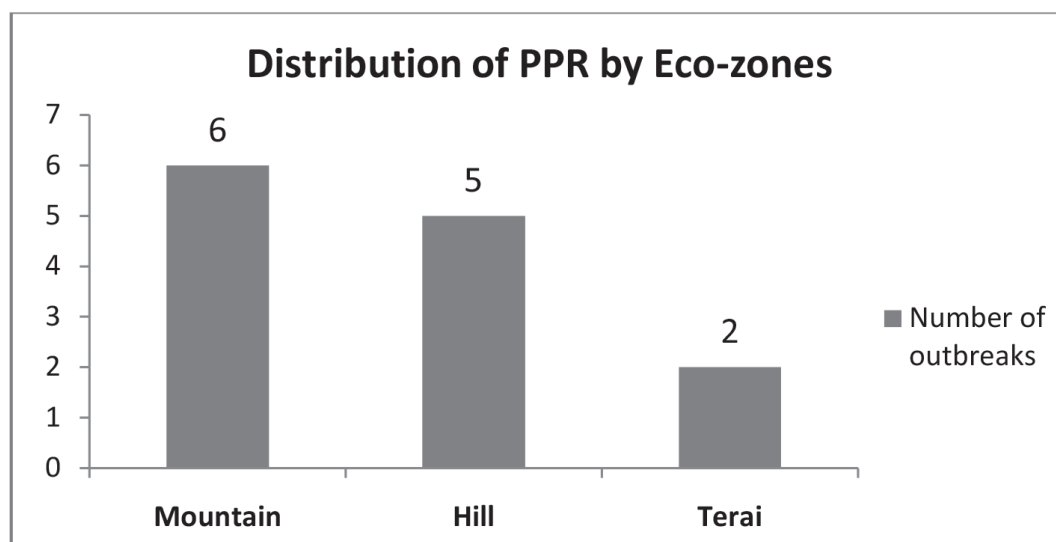


Fig. 2: The Ecozone wise distribution of PPR

Vaccination coverage and Seroconversion

The total population of goats in the country is 10.3 million and sheep is 0.8 million (MoAD 2015). Within the country, the mid-western region is a potential hub for goat and sheep farming. This region constitutes 25% (2.6 million) out of national goat population (10.3 million) and 45% (0.36 million) of total sheep population (0.8 million) of the country (RDLS, 2015). In order to control this disease, National PPR Control Program through vaccination has been implemented. The national vaccination coverage against PPR during 2015 was recorded as 22 % (VEC, 2015) whereas the vaccination coverage in this region is 14.3% (RVL, 2015). The national seroconversion of vaccine was found very low which 57.2 % (CVL, 2015) is whereas the average seroconversion in this region was found as 70.7 %.

The trend of sero-conversion of PPR vaccine in different districts in this region is shown in figure 3. The reasons behind low sero-conversion could be due to condition

of animals, vaccination skills of technicians, vaccine storage, transportation, sampling error and sincerity of farmers and technicians.

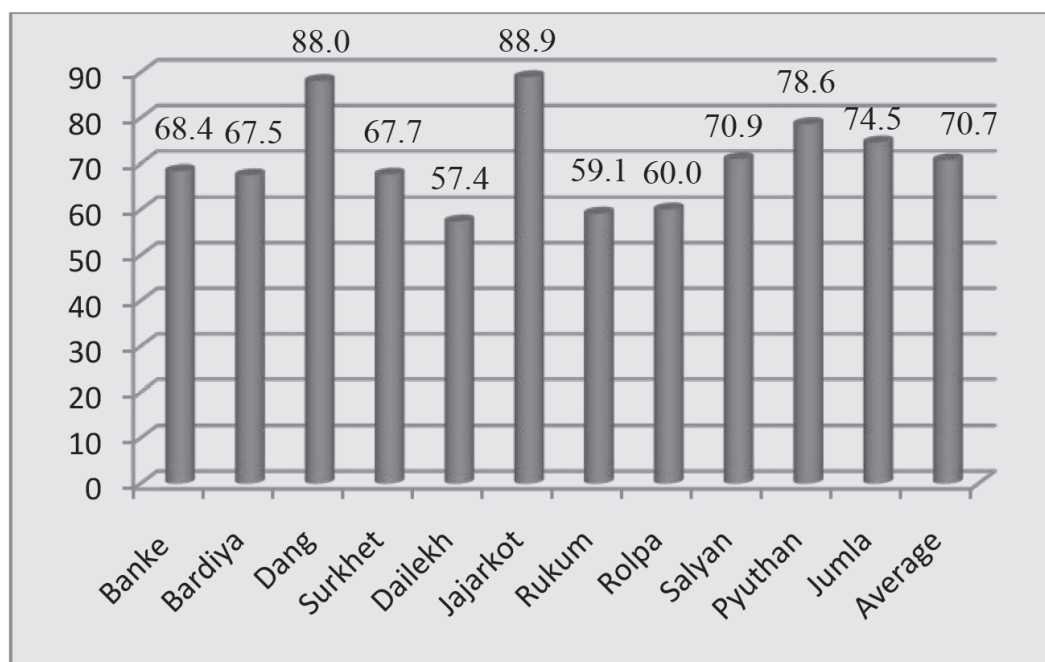


Fig. 3: The trend of sero-conversion of PPR vaccine in different districts of Midwestern region

CONCLUSION

PPR is a common problem among sheep and goat population of the country. There were total of 13 outbreaks from eight districts confirmed by using antibody ELISA test. In these districts, 39% (9230) of deaths were confirmed due to PPR out of 23670 infected sheep and goats which causes huge economic losses to the farmers. High number of outbreaks in mountain followed by hills may be due to excessive movements of nomadic herd of sheep and goats as per seasonal variation, common pasture, low vaccine coverage in mountain and hill region due to geographic difficulty and low sero-conversion of vaccine. Strategic vaccination campaigns against PPR, proper biosecurity, movement control, early diagnosis are the tools to control the PPR. The use of good quality vaccines in healthy animals with proper identification and without sampling error should be done for the best sero-conversion.

REFERENCES

CVL 2015, Annual Technical Report 2014/015.

MOAD 2015, Selected indicators of Nepalese Agriculture and Population, Nepal.

RDLS 2015, Annual Progress Report, Mid-western region, Birendranagar, Nepal

RVL, 2015 Annual Technical Report 2014/015.

VEC 2015, Annual Epidemiological Bulletin January –December, volume 11, number 3.

Participatory Silage Making and Hay and Silage Mixed Feeding Technology for Winter Feeding to Goats in Jumla, Nepal

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ABSTRACT

*This action research was carried out to demonstrate the method of silage making and its feeding, mixed with chopped hay to goats during feed scarcity periods as coping strategy for winter days in Jumla. Nine farmers took part in on-farm silage production and feeding trial. A total of 29 kids and 16 does as treatment group and 12 kids and 19 does of the similar age group and status as control were selected to compare the body weight gain. The normal practice of feeding was 3-4 hours of grazing daily, occasionally combined with tree fodder (*Quercus* sp.) in communal pastureland and offering hay for snowfall days in pens. In addition to this, treatment groups were supplemented with mixed silage and chopped hay at the rate of 2% of body weight on dry matter basis, half each from the silage and hay. The mixture was given daily early in the morning for 45 days from 25th of December to 10th of February, when the average minimum temperature dropped from -2.4 to -5.4^o C. Goats consumed about 95% of the offered amount of silage whereas chopped hay was rejected by about 90%. The result showed that kids gained 0.49 kg with average daily gain of 10.88 g and does gained 2.44 kg with an average daily gain of 54.22 g for the treatment group. The difference between the initial and final body weight was positive for treatment group whereas it was negative for the control. Male kids grew faster by 8.6%, female kids by 6.7% and does by 12.6% for silage supplemented group compared to control. It indicated that proper fermented maize fodder silage can be obtained from pits prepared before winter and loss in body weight can be prevented by the supplementation of silage during cold days in high mountains when there is scarce of green forages.*

Keywords: action research, coping strategy, maize fodder, body weight

INTRODUCTION

Mountain region of Nepal has about 13% of the total goat population. Sinhal and Chyangra are the major breeds and mostly reared under migratory system. High mountain region is highly acknowledged for the communal rangeland pasture resources to meet the forage demands of seasonal migratory animals. But the feed supply is adequate only in the months of June-September; rest of the year animals are forced to face feed scarcity (Pandey and Tiwari, 2002). A substantial shortage of winter fodder as being an important constraint within the transhumant system (Farman and Tetlay, 1991; Dost, 1995). The crisis of animal feed during winter season not only causes nutritional stress but also reduces the capacity to resist the cold stress. In this season, farmers either sell their flocks in lower price or bring their flocks to mid hill districts in search of feed where they should pay more money as levy against grazing. So, the silage technology may be taken as one of the best options. Silage, which is anaerobically fermented green fodder, is valued throughout the world as a source of animal feed during lean months (Ragothaman, *et al*, 2010). The practice of making and feeding silage is limited only in government owned farms in Nepal, mostly focused to large ruminants. The reluctance in the adoption among the farmers may be due to the lack of on farm application of the technology. Furthermore, in high mountain region the temperature factor for proper fermentation of silage and goats' preferences for its consumption was also not assessed at community level. So, aiming for green roughage supplement through silage to local goats during winter period by utilizing maize fodder, this action-oriented study was carried out.

MATERIALS AND METHODS

An Organized farmers' group (OFG), Jolimungrah Pasu Sewa Samuha, in Dhapa VDC-7 in Jumla district was selected for on farm silage making and feeding practices. As per the decision made from the group meeting, 9 members voluntarily carried out the study on their farms. Irrespective of the variety, farmers sowed the maize seed estimating at double of the rate as for grain production between 3rd and 4th week of June. They took care themselves for the fodder in the field. After two to two and half months of sowing, maize crop was harvested, in 3rd week of September, before coming out the cobs. It was wilted on sunlight for about 3 hours and chopped properly with the help of chaff cutter machine at about the size of 2-3 inches long. Silo pits were dug as per the size required to the owner ranged from 1-3 cubic meters. A polythene sheet was laid down on the pits. Chopped fodder maize was filled with continuous trampling by participants to remove the oxygen as soon as possible from the pits. The pits were sealed with the same polythene sheet at first then with the soil and stones tightly. Sixty-two farmers from KalikaKhetu and Kanakasundari Village

Development Committees were visited the site and participated actively in the process of chopping and ensiling the maize fodder. After 3 months (3rd week of December) silo pits were opened one by one from a corner and evaluated using the sense of smell, taste and color. Hay prepared traditionally from local grasses (non-legume type) was collected from hay store owned by farmers. Hay was chopped with chaff cutter machine and mixed with silage properly before offering to goats. A total of 29 kids (3-12 months) and 16 non pregnant does of local breed Sinhal were selected to feed the calculated amount of silage and chopped hay. Likewise, 12 kids of the similar age group and 19 does were taken as control group i.e. without silage and hay. Body weight was recorded at each fifteen days' interval, using spring weighing balance. The refusal of feeds if any was weighed once at fortnight. The health status was also observed during the trial period. Daily 3-4 hours grazing occasionally combined with tree fodder (*Quercus* sp.) in communal pastureland near by the village and offering hay for snowfall days in the pens was the normal practice of feeding. In addition to this, treatment group was supplemented with mixed silage and chopped hay at the rate of 2% of body weight on dry matter basis, half each from the silage and hay. The mixture was given in common feeder to the goats of individual farmers daily early in the morning from December 25 to February 10 (45 days). The period of one week was considered as an adaptation period. Weather data were collected from climatic field office, Jumla. The quantitative data were analyzed using simple statistical tools and qualitative data as description.

RESULTS

The weather situation

The average maximum and minimum temperatures in Jumla from January 2013 to February 2014 were collected and found that the months from November to February were the coldest months of the year when the average minimum temperature fell to -5.4°C in December (Figure-1). During the feeding trial period snowfall was recorded three times in the months of January and February. The rainfall was not recorded for the trial period. In the month of October when fodder was ensiled average maximum and minimum temperature was 25.8°C and 8.0°C which gradually decreased to 16.8°C and -5.4°C respectively by December when it was opened.

Quality of silage

Silage with pleasant aroma, nearly odorless, light sour taste and slightly golden with light green color was found within the pits. There was no mould growth appeared in all pits at opening.

Preferences of feeds

The mixture of chopped hay and silage was the new feed to goats. For the first day of offering the mixed, goats of two farmers did not like to eat it. When farmers added a little amount of common salt on the mixed, goats used to consume almost all the amount of silage offered. After few days, no salt was needed to accustom the feed. It was found that almost 95% of the offered amount of silage was consumed by goats whereas only 10% of chopped hay was consumed. The intake of chopped hay was nominal and the practically about 1% of the body weight of animals was consumed only from the silage on dry matter (DM) basis (assuming 35% DM content).

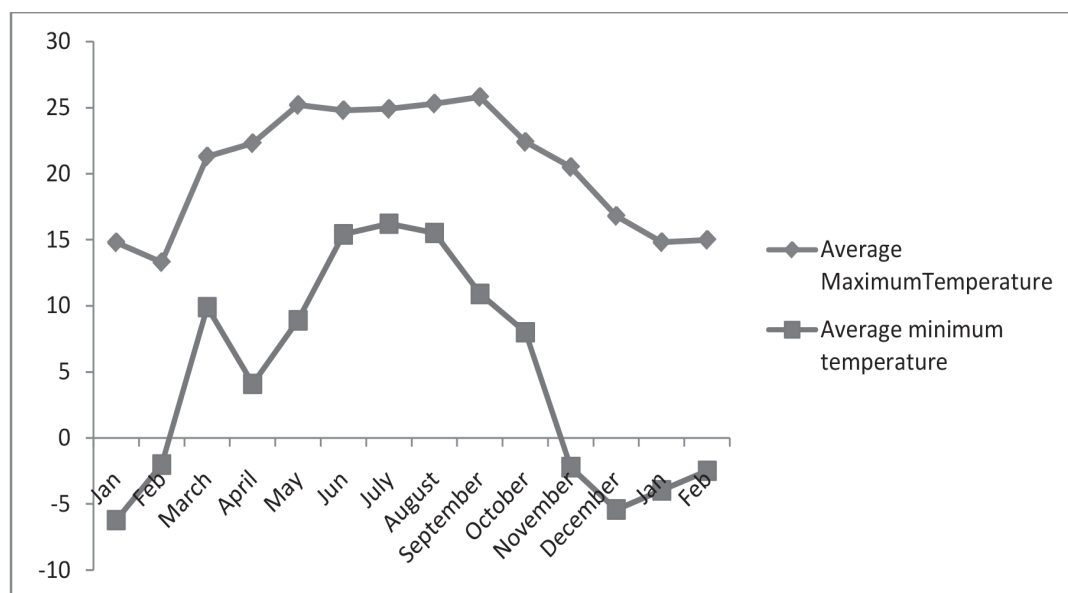


Fig. 1: Average maximum and minimum temperature (°C) in Jumla during January 2013 to February 2014 (Source: Climatic field office, Jumla)

Body weight gain in goats

The initial and final average body weight is presented in Table 1. It reveals that the average initial body weight of the kids was lower in treatment group than in the control group, whereas the average body weight of doe was lower in control group than that of treatment. The same trend was observed for the final body weight.

The total weight gain in kids was 0.49 kg/kid, with average daily gain of 10.88 grams and in does it was 2.44 kg with an average daily gain of 54.22 gm for the treatment group during the trial period, whereas kids and does lost their body weight by 0.03 and 0.84 kg/ animal respectively for the group without silage supplement.

Table 1: Initial and final body weight of goats (Kg) supplemented silage and hay mixed feed as treatment and without the mixture as control in Jumla, 2014

Animals	Control		Treatment	
	Initial BW	Final BW	Initial BW	Final BW
Kids	11.83(12)	11.75	9.44(29)	9.93
Does	25.31(19)	24.47	26.15(16)	28.59

Note: Figures in parenthesis indicate the number of animals

While considering the sex of kids, the difference (weight gain or loss) between the initial and final body weight was positive for both the males and females in treatment group whereas it was positive only for male kids in control group (Fig. 2). Male kids grew faster by 8.6% and female kids by 6.7% and does by 12.6% for silage supplemented group than the animals without the supplementation (Table 2).

Goat health status

Health condition of the goats was also observed during the period of feeding trial. No clinical signs of contagious diseases were noticed during the 45 days of feeding trial. Two kids with lower body scores were infested with external parasites (lice).

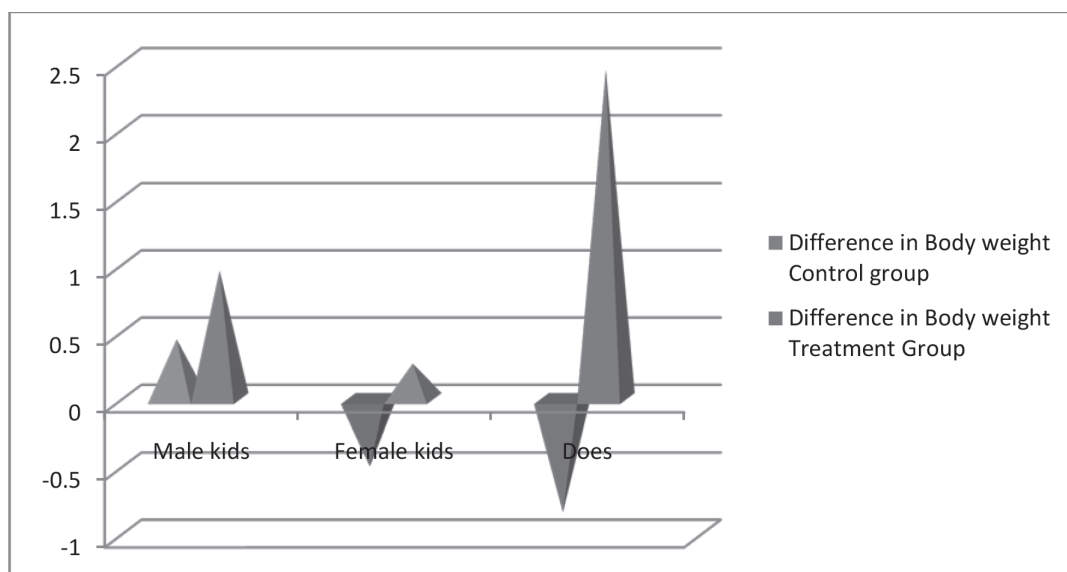
**Fig. 2:** Positive and negative body weight gain in goats supplemented silage and hay mixed feed as treatment and without the mixture as control in Jumla, 2014.

Table 2: Body weight gain of goats and differences (%) between supplemented silage and hay mixed feed as treatment and without the mixture as control in Jumla, 2014

Body weight gain in percentage			
Animals	Control group (C)	Treatment Group (T)	Difference between C&T
Male kids	4.67	13.27	8.59
Female kids	-4.10	2.61	6.70
Does	-3.32	9.33	12.65

DISCUSSION

The temperature record over the period of one year in Jumla shows that the environmental temperature fell down abruptly from 8 to -2 °C from October to November, December to February were the coldest months. The quality of silage in silo pit was found good may be due to well fermentation and formation of lactic acid due to the proper ambient temperature and anaerobic condition within the sealed pit . Regarding the aroma/odor, it was mentioned that nearly odorless is the characteristic of well fermented silage (Charley B and Schmidt, 2014). More preferences in consuming the silage may be due to its green nature as compared to the chopped dry hay. The normal practice among the farmers was offering hay without chopping. The findings matches, while compared to dry roughages (wheat and rice straw) between whole and chopped forms but in contrast to the findings goats' preferred chopped hay of clover and other high quality forages over the un chopped material (Abdel and Alla ,1999). The findings of Abi *et al.*, (2008) matches the present finding, which revealed that in transhumant system male kids, female kids and does gained lower body weight in winter period than the summer period and they also found that male kids gained more live weight than female in winter period. In the same study, does lose the body weight during the period.

In this experiment too low gain in body weight of the goats may be due to the low availability of forage in pastureland combined with cold stress and the result obtained positive weights was practically the supplement of silage only. The lowest average daily weight gain/goat was observed on transhumance system was also supported by Khanal (2012) and Osman *et al.*, (2009). Malisetty *et al.*, (2013), revealed that Nellore ram lambs gained average daily body weight of 32.33 g with sole silage-based feed ration. The lost in body weight during winter season in migratory ewes was also found in a study of Dhaubadel (2000).

CONCLUSION

Goats in high mountains areas are either confined around the farmers' settlement or brought down to the mid hills pasture during colder days of the year. While around the villages, it is crucial to meet the feed demand of animals. The practice of making silage from the maize fodder from pit silo was successful at the community level. Similarly, body weight loss due to deficit of feed combined with cold stress in goats could be prevented by the supplement of a little amount of maize silage (approximately 1% of body weight on DM basis) during winter season.

ACKNOWLEDGEMENT

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REFERENCES

- Abdel-Moneim A.Y , M.S Abd-Alla (1999).Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt DOI: [http://dx.doi.org/10.1016/S0168-1591\(99\)00045-3](http://dx.doi.org/10.1016/S0168-1591(99)00045-3)
- Abi Saab, S. E. Hajj1, B. Abi Salloum and E. Rahme (2008). Seasonal and altitudinal variations on adaptation, growth and testicular activity of Baladi goats with vertical transhumance in eastern Mediterranean. *Lebanese Science Journal*, Vol. 9: No. 1, 2008.
- Charley B and Renato J. Schmidt (2014). Use your senses to evaluate silage.Retrieved from <https://www.progressiveforage.com/forage-types/silage>
- Dhaubhadel TS (2000). Stress caused by a shortage of winter feed as a constraint upon the migratory system of sheep management in the western hills of Nepal. *Proceeding of the 3rd National Animal Science Convention (NASA), Lalitpur, Khumaltar, Nepal*.Aug 27-28, 2000, pp 97-102.
- Dost, M. (1995). Fodder production in Northern Areas. Report number Pak/86/027. *National Agriculture Research Council, Park road, Islamabad, Pakistan*. pp. 1-53.
- Farman, A. and K. A. Tetlay, 1991. Dynamics of livestock development in Northern Areas of Pakistan: *AKRSP, Gilgit*. pp. 20 - 21.

- Khanal B. (2013). Effect of feeding system on growth Performance of high hill goat *The Proceedings of National Workshop on Research and Development Strategies for Goat Enterprises in Nepal*. (Eds. Gurung T.B., Joshi B.R., Singh U.M., Paudel K.P., Shrestha B.S., Rijal K.P. and Khanal D.R.), NARC, Kathmandu, April 2013. PP 233-239.
- Malisetty V, Yerradoddi RR, Devanaboina N, Mallam M, Cherala HK, Admal RR and Manthani GP (2013). Effect of feeding maize silage supplemented with concentrate and legume hay on growth in Nellore ram lambs, *Vet World* 6(4):209-213, doi:10.5455/vetworld.2013.209-213.
- Osman, J.S., Waldron, D.F., Griffin, D.B. and Savell.J.W. (1999). Effect of breed-type and feeding regimen on goat carcass traits. *J. Anim. Sci*, 77:3215-3218.
- Panday, S.B. and Tiwari, M.R. (2002). Fodder resources and pastoral systems in Nepal. In: *Proceedings of the 5th meeting of the Temperate Asia Pasture and Fodder Network (TAPAFON)*, RNR Research Centre Bajo, Wangdue, Bhutan, 30th Apr– 4th May, Pp 71-78.
- Ragothaman, Venkataramanan. Chirukandoth, Sreekumar. Rishipal, Anil kumar. Selvaraj, P., Nainar, M. Vidhya. and Iyue, Mathagowder. (2010) Effect of jaggery on the quality and intake levels of maize silage. *Trop. Ani. Health and Prod.* 42(5):1027-1029.
- Yokota H., T.Okajima and M.Olishima (1991). Effect of environmental temperature and addition of molasses on the quality of Napier grass (*Pennisetum Purpureum Schum.*) silage. *AJAS* 1991 vol, 4(No.4): 377-382.

Status of Antibiotic Residues in Poultry Meat of Nepal

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ABSTRACT

Antibiotic residues in livestock products are the burning issue in animal production as well as public health sector. Imprudent use of antibiotics in poultry production increases the risk of antibiotic resistance thus increasing risk in both animals and humans. To determine the trend of antibiotic residues presence in poultry meat, a study was conducted from June 2016 to May 2017 in Kathmandu, Kaski, and Chitwan districts of Nepal. A total of 92 breast samples of broilers (Kathmandu-30, Kaski-40 and Chitwan-22) were collected randomly from different selling outlets of the study areas and tested for residues of commonly used antibiotic viz. Enrofloxacin (EX), Ciprofloxacin (CIP), Streptomycin (STR) and Chloramphenicol (CHL) using the Enzyme Linked Immunosorbent Assay (ELISA) technique. Out of 92 samples, 57 (62%) samples were found positive for antibiotics residue of which 38% samples were positive for STR residue, 15.2% for CIP and 8.7% for EX. However, none of the samples showed positivity for CHL residue. The level of STR was determined to be higher in all districts in comparison to other antibiotics. The meat samples from Kaski were found to contain higher level of STR (56.1%) while highest percentage of CIP (16.1%) was detected in Kathmandu. Also, presence of EX was found highest in Kathmandu compared to other districts. The study concluded that antibiotics residues have been abundantly found in poultry meat which can have serious effect in human health. Hence, to mitigate this, routine antibiotics residues monitoring and surveillance programmes in food animal products should be conducted to ensure the consumer safety.

Keywords: Ciprofloxacin, Chloramphenicol, Enrofloxacin, Poultry meat, Streptomycin.

INTRODUCTION

Antibiotics are used in animal agriculture especially in poultry industry, not only for therapeutic and prophylactic purposes, but also as a growth promoter to enhance the health and productivity of flocks through feed or drinking water (Donoghue, 2003 and Ahmed, 2016). Currently, approximately 80% of all food-producing animals receive antimicrobial medication at some point as treatment or prevention of disease or growth promoter (Pavlov *et al.*, 2008). The use of antibiotic drugs in food-producing animals may result in the presence of residues in meat and offal. The consumption of these trace levels of antimicrobial residues in foods from animal origin may have consequences on the indigenous human intestinal microflora which constitutes essential component of human physiology. This flora acts as a barrier against colonization of the gastrointestinal tract by pathogenic bacteria and plays an important role in food digestion (Shankar *et al.*, 2010). The massive use of antibiotics and failure to follow the withdrawal period of drugs leads to the consumption of antibiotics at low levels which may increase the risk of occurrence of microbial drug resistance and causes hypersensitivity reaction and disruption of normal intestinal flora in human (Beyene *et al.*, 2016, Nisha *et al.*, 2008).

Many of the drugs used as growth promoters affects the meat quality resulting in poor eating quality. These substances must be administered only in recommended concentration and their respective withdrawal times must be followed to decrease the potential risk to the consumers' health and to ensure the reduction of their residues in edible tissues to an acceptable level. (Kozarova *et al.*, 2004). Several groups of antibiotics including quinolones and fluoroquinolones are frequently used in veterinary medicine for treatment and prevention of diseases, thereby reducing famers' losses (Omotoso and Omojola, 2015). Specifically, Enrofloxacin (EX), Streptomycin (STR), Ciprofloxacin (CIP) and Chloramphenicol (CHL) have been widely used to treat and cure infections both in human and veterinary medicine. In Nepal, since the poultry sector is commercialized comparatively, huge amount of antibiotics is used in this sector. Due to the short lifespan of broiler, it is likely that the withdrawal period is not followed as required. The antibiotic residues in the bodies of chicken may pose adverse effect to human health and may cause undesirable changes in bowel microflora and induce immunological reaction in susceptible persons (Mahmoudi *et al.*, 2014). There is a perception among consumers that the food supply contains high concentrations of drug or hormone residues causing significant health concerns or problems (Donoghue, 2003). However, very few studies have been conducted in Nepal to estimate the level of antibiotic residues in poultry meat in Nepal. The objective of this was to detect the presence of antibiotic residues in chicken meat samples from three major cities of Nepal.

MATERIALS AND METHODS

The meat samples were collected from meat shops of Chitwan, Kaski and Kathmandu from June 2016 to May 2017. A total of ninety-two samples of breast meat from broilers (Kathmandu-30, Kaski-40 and Chitwan-22) were collected randomly from different selling outlets (only one sample from each outlet) in and around selected cities. The outlets were chosen randomly and purposively. About 50g of each sample was cut aseptically from the breast meat and transferred in self-sealing colorless polythene bags. The bags were labeled and transported in a cool box containing chiller packs to Animal Health Research Division (AHRD), Nepal Agriculture Research Council, Khumaltar, Kathmandu. All the meat samples were stored at -40 °C until the time of analysis.

During analysis, the chicken meat samples were thawed overnight in a refrigerator. Each meat sample were cut into small pieces and blended at 2000 rpm in a tissue blender for two minutes. Ten grams of blended meat tissue and 10 ml of deionized water were taken in 50 ml polypropylene centrifuge tube. Extraction method was adopted for sample analysis as per manufacturer's kit (Bioscientific Corporation, USA).

Sample preparation for EX

The meat sample was homogenized with a mortar and pestle. Then one gram of homogenized sample was kept in four ml of 35% Methanol or sample extraction buffer I as provided in the kit. The solution was vortexed at maximum speed and then centrifuged for 10 minutes at 4,000 *g at room temperature (20-25°C). Half ml of the supernatant was transferred to a tube and 25 µl of meat extraction buffer II plus 1.5 ml of 35% Methanol or sample extraction buffer was added and vortexed. After mixing the solution, 50 µl of the supernatant was taken for the assay. The sensitivity (detection limit) and specificity (cross reactivity) of EX was one parts per billion (ppb) and 100% respectively.

Sample preparation for CIP

Fat from the sample was cleared and homogenized with mortar and pestle. About one g of homogenized sample was mixed with four ml of 70% methanol. The solution was vortexed for 10 minutes at maximum speed. It was then centrifuged for five minutes at 4000*g at room temperature (20-25°C). Then 0.5 ml of the supernatant was transferred to a tube and 0.5 ml of 1X sample extraction buffer was added on it. After mixing the solution, 50 µl of the supernatant was taken for the assay. The sensitivity and specificity of CIP was 3.5 ppb and 100% respectively.

Sample preparation for STR

Fat from the sample was cleared and homogenized with mortar and pestle. About 0.5 g of homogenized sample was mixed with 4.5 ml of 1X sample extraction buffer and the solution was vortexed for three minutes at maximum speed. Then 0.2 ml of the homogenized sample was taken and centrifuged for five minutes at 4000*g at room temperature (20-25°C). Then 0.5 ml of the supernatant was transferred to a new tube and incubated at 75°C for five minutes. The solution was vortexed for one minute and then centrifuged for five minutes at 4000*g at room temperature. The 0.2 ml of the supernatant was transferred to a new tube and mixed with 5 µl of 1X sample extraction buffer. The solution was vortexed. 50 µl of mixture was used for the assay. The sensitivity and specificity of STR was 5 ppb and 100% respectively.

Sample preparation for CHL

Reasonable amount of sample was homogenized with a suitable mixer and about 3 g of the homogenized sample was mixed with 6 mL of ethyl acetate. The mixture was then vortexed for 3 min at maximum speed and centrifuged for 5 mins at 4,000 x g at room temperature (20 – 25°C). 4 mL of the ethyl acetate supernatant was transferred into a new vial and dried the sample in a 60-70°C water bath under reduced pressure. The dried residue was dissolved in 2 mL of n-hexane in which 1 ml of 1X sample extraction buffer was added. Then the mixture was vortexed at maximum speed for 2 mins and centrifuged for 10 mins at 4,000 x g at room temperature. The upper hexane layer was discarded. 100 µl of the lower aqueous layer was used for the assay. The sensitivity (Detection Limit) and specificity (Cross Reactivity) of CHL was 0.0075 ppb and 100% respectively.

Enzyme Linked Immunosorbent Assay (ELISA) test procedure

The manufacturer's instruction was followed for ELISA (Bioscientific Corporation, USA) testing and the concentration of all the tested antibiotics were calculated. There were separate ELISA test Kits for EX, CIP and STR and CHL antibiotics.

RESULT

The result of this study indicated that residues of antibiotics EX, STR, CIP and CHL were detected in samples of broiler breast muscles by ELISA. Out of 92 chicken meat samples, 57 (62%) samples were found positive for antibiotics residue of which 35 samples (38%) were positive to STR residue, 14 (15.21%) samples were positive to CIP, 8 (8.7%) samples were found positive for EX while none of the samples were positive for CHL (Figure 1).

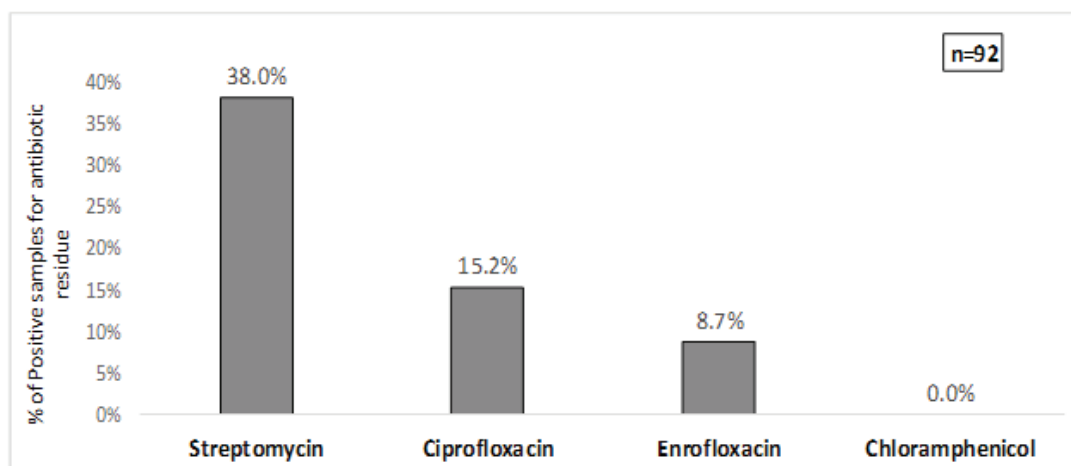


Fig. 1: Positive meat samples for antibiotic residue.

The presence of STR was determined in 57.5% of the meat samples from Kaski whereas only 30.0% of samples from Kathmandu and 13.6% of samples from Chitwan showed the presence of STR residue. In case of CIP, 16.7% of meat samples collected from Kathmandu was found to be positive whereas only 15.0% of samples from Kaski and 13.6% of samples from Chitwan showed the presence of CIP. Likewise, 13.3% of meat samples from Kathmandu was found with residues of CX.

The CHL was not detected in samples from any districts (Figure 2).

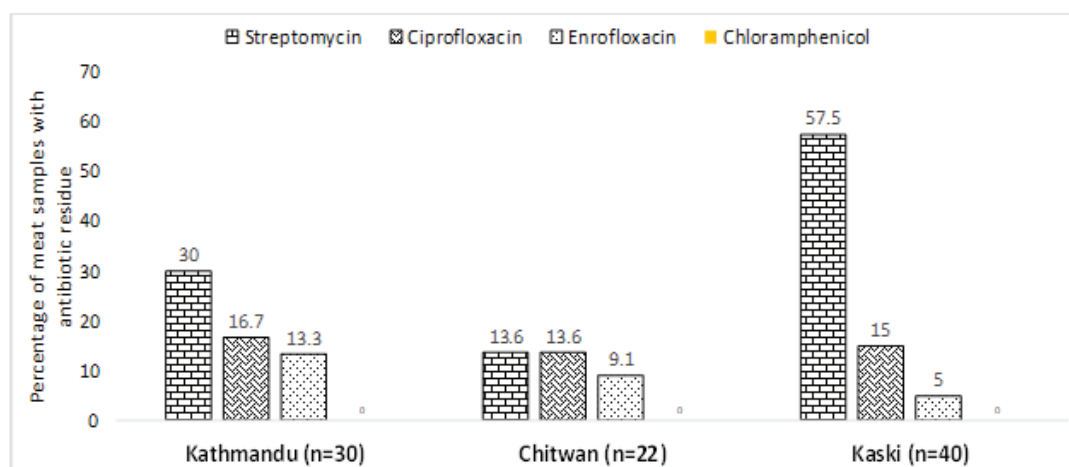


Fig. 2: Positive meat samples for antibiotic residues on the basis of location.

DISCUSSION

In the present study, out of 92 samples assayed by ELISA, 8 (8.7%), 35 (38.0%), 14 (15.2%) and none had detectable levels for EX, STR, CIP and CHL residues respectively. Similarly, higher level of CIP (50%) residues were found in a poultry meat in a similar study conducted in Nigeria (Omotoso and Omojola, 2015). The similarity in the result might be due to frequently administered of Fluoroquinolones in poultry rearing in Nepal and Nigeria. The CIP is also a marker residue for EX that is approved for use as a veterinary drug and when it is metabolized, its pharmacologically active metabolite, CIP, is produced (Tyczkowska, *et al.*, 1989). The detection of CIP during market screening often reflects the use of both EX and CIP (Navratilova *et al.*, 2011; Botsoglou and Fletouris, 2001).

The present study revealed that the concentration of STR was found higher than other antibiotics in all districts. Samples (57.5%) from Kaski were found to contain STR residues followed by Kathmandu with 30.0% of positive samples. The high occurrence of STR may be due to the use of antibiotic in poultry for the preventive measure.

CONCLUSION

The results of the present study suggest that fluoroquinolones have been abundantly used in poultry. To mitigate this, routine antibiotics residues monitoring and surveillance programmes in food animal products should be conducted to ensure the consumer safety. Awareness regarding the rational use of antibiotics and its serious effects on the human health should be initiated to educate the farmers. The use of rapid screening test along with quantitative methods to establish minimum residual levels of different types of antibiotics in meat along with respecting withdrawal period for antibiotics in food animals need to be established. The misuse of antibiotics in the poultry farm should be discouraged and be used only with veterinarian's prescription.

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REFERENCES

- Ahmed, A. M., & Gareib, M. M. (2016). Detection of Some Antibiotics Residues in Chicken Meat and Chicken Luncheon. *Egyptian Journal of Chemistry and Environmental Health*, 2(2): 315-323.
- Beyene, T. (2016). Veterinary Drug Residues in Food-animal Products: Its Risk Factors and Potential Effects on Public Health. *Journal of Veterinary Science and Technology*, 7(1): 285-291. doi: 10.4172/2157-7579.1000285
- Botsoglou, N. A., & Fletouris, D. J. (2001). *Drug residues in foods : pharmacology, food safety, and analysis*. New York: Marcel Dekker.
- Donoghue, D. J. (2003). Antibiotic residues in poultry tissues and eggs: human health concerns? [Review]. *Poultry Science*, 82(4): 618-621. doi: 10.1093/ps/82.4.618
- Kožárová, I., Máté, D., Hussein, K., Raschmanová, K., Marcincak, S., & Jevinová, P. (2004). High-performance liquid chromatographic determination of sulfadimidine residues in eggs. *Acta Veterinaria*, 54(5-6). doi: 10.2298/avb0406427k
- Mahmoudi, R., Gajarbeygi, P., Norian, R., & Farhoodi, K. (2014). Chloramphenicol, sulfonamide and tetracycline residues in cultured rainbow trout meat (*Oncorhynchus mykiss*). *Bulgarian Journal of Veterinary Medicine*, 17(2): 147-152.
- Navrátilová, P., Borkovcová, I., Vyhnálková, J., & Vorlová, L. (2018). Fluoroquinolone residues in raw cow's milk. *Czech Journal of Food Sciences*, 29(6): 641-646. doi: 10.17221/22/2011-cjfs
- Nisha, A. R. (2008). Antibiotic residues - A global health hazard. *Veterinary World*, 1(12): 375-377. doi: 10.5455/vetworld.2008.375-377
- Omotoso, A. B., & Omojola, A. (2015). Fluoroquinolone residues in raw meat from open markets in Ibadan, Southwest, Nigeria. *International Journal of Health and Animal Science Food Safety*, 2(1): 32-40. doi: 10.13130/2283-3927/4739
- Pavlov, A., Lashev, L., Vachin, I., & Rusev, V. (2018). Residues of antimicrobial drugs in chicken meat and offals. *Trakia Journal of Sciences*, 6(1): 23-25.
- Shankar, Manjunatha, P., Shivamallu, C., Ranjith, & Shivakumar, D. (2010).

Rapid Methods for detection of Veterinary Drug residues in Meat. *Veterinary World*, 3(5): 241-246.

Tyczkowska, K., Hedeem, K. M., Aucoin, D. P., & Aronson, A. L. (1989). High-performance liquid chromatographic method for the simultaneous determination of enrofloxacin and its primary metabolite ciprofloxacin in canine serum and prostatic tissue. *Journal of Chromatography B: Biomedical Sciences and Applications*, 493, 337-346. doi: [https://doi.org/10.1016/S0378-4347\(00\)82739-5](https://doi.org/10.1016/S0378-4347(00)82739-5)

Effect of Inclusion of Sickle Pod (*Cassia Tora* L.) Seed Meal in Diets on Performance of Japanese Quail (*Coturnix coturnix japonica*)

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ABSTRACT

A study was conducted at IAAS Livestock Farm, Rampur, Chitwan from June 9 to July 20, 2011 with an aim to find out the effect of inclusion of Sickle pod (Cassia tora L.) seed meal in the diets on the performance of Japanese quail to reduce the cost of production. The experiment was conducted in complete randomized design (CRD) consisting of five treatments, i.e. 0% (T1), 5% (T2), 10% (T3), 15% (T4) and 20% (T5) of Cassia tora L. seed meal in diets with three replications having seven birds in each treatment replication. The isocaloric, isonitrogenous diet each containing 2750 ME Kcal/kg and 27% CP was fed to the ten days old chicks ad libitum for thirty-two days. The chemical composition of C. tora seed was found to contain 87.6% DM, 20.51% CP, 13.8% CF, 4.3% EE and 12.6% total ash. Cumulative feed intake of birds in T2 was significantly higher than T5 ($P<0.05$), but did not differ from that of T3 and T4 ($P>0.05$). The cumulative body weights of birds in T1, T2, T3 and T4 were significantly higher as compared with T5 ($P<0.05$). Overall average feed conversion ratio was significantly narrower ($P>0.05$) in T2 (4.62) than T5 (5.28). Benefit cost ratio was the highest for T4 (1.58) and the lowest for T5 (1.40). C. tora seed meal could be included up to 15% in the diets without any detrimental effect on the performance of Japanese quail.

Keywords: *Cassia tora, Japanese quail, feed intake, body weight, benefit cost ratio*

INTRODUCTION

Availability of quality feed at a reasonable cost is a key to successful operation in poultry industry. To sustain in the competitive market, it would be wise to use unconventional feed to the diet formulation to reduce the production cost for poultry. Use of oilseed cakes and industrial by-products could have better practical importance but due to adoption of genetically modified crops there is need of other unconventional protein source be incorporated in feed (Chadd, 2004) and it needs

large experimentation and trial before recommendation to farmers level.

Cassia tora L. also known as taapre, sickle senna or ringworm plant is an annual weed or shrub, widely distributed in the tropical region of the world and in Nepal, it covers moist tropical and sub-tropical region whose seeds are 2 mm in diameter; light grey and cylindrical in shape (Baral and Kurmi, 2006). *Cassia tora* is specifically used in the medicinal purposes from the long time period (Baral and Kurmi, 2006). *Cassia tora* seed contains 21.12% CP, 64.5% NFE, 7.73% EE, 16.64% DCP, 59.4%TDN, 5.56% total ash, 0.87% calcium and 0.72% Phosphorous (Banerjee, 2004). In some preliminary research *Cassia* seed have been safely incorporated up to 10% in animal feed without affecting animal health and performance (Sood *et al.*, 1990).

Quail is a poultry species that is easy to rear, do not need large amount of capital and can be kept in empty areas around the house by family members with fast return within six weeks time. Moreover, they require less amount of feed (780 gm-1 kg) than broilers and are more resistant to diseases. They are also fast-growing birds; day-old chicks matured at 4 weeks period for table purpose and lay eggs in 36 days of age (Osti, 2002). In the present study an attempt has been made to assess the potential of including *Cassia tora* L. seed meal in diets on the performance of Japanese quail.

MATERIALS AND METHODS

The experiment was conducted at livestock farm of Institute of Agriculture and Animal Science (IAAS), Rampur, Nepal from June to July 2011 to study *Cassia tora* L. as a feed ingredient in Japanese quail ration. The experiment was started with 10 days old quail chicks and continued up to 42 days of age. The experiment was laid out in Completely Randomized Design (CRD) with five dietary treatment groups (T_1 , T_2 , T_3 , T_4 and T_5) and three replications. Each dietary treatment group consists of 21 chicks distributed in three replicated pens (R_1 , R_2 and R_3) with seven chicks in each. The layout of experiment is shown in table 1.

Table 1: Layout of experiment

Dietary treatments	Number of birds per replication			Total number of birds
	R_1	R_2	R_3	
T_1	7	7	7	21
T_2	7	7	7	21
T_3	7	7	7	21
T_4	7	7	7	21
T_5	7	7	7	21
Grand total				105

T_1 = 0% inclusion of *Cassia tora* seeds in diets, T_2 = 5% inclusion of *Cassia tora* seeds in diets, T_3 = 10% inclusion of *Cassia tora* seeds in diets, T_4 = 15% inclusion of *Cassia tora* seeds in diets, T_5 = 20% inclusion of *Cassia tora* seeds in diets

Experimental starter/grower quail diets were prepared which include *C. tora* L. seed meal excluding the basal diets. Nutrient levels of the diets were adjusted in accordance with NRC (1984) feeding standard using the computer-based feed formulation program (WINFEED 2.8, 2010). The composition of the diets in the experiment is shown in Table 2.

Table 2: Percentage of ingredients composition of Japanese quail starter/grower diets

Feed ingredients	T_1	T_2	T_3	T_4	T_5
Maize	41.05	35.6	29.61	23.74	17.43
Rice polish	5.38	6.03	6.56	7.04	7.75
De-oiled cake (DOC)	0.50	1.54	3.38	5.58	6.41
<i>Cassia tora</i> seed	0.00	5.00	10.00	15.00	20.00
Soya cake	41.81	39.03	36.03	33.00	30.06
Molasses	0.50	0.50	1.01	1.15	2.64
Meat and bone meal	5.00	5.00	5.00	5.00	5.00
Calcium dust	1.06	1.46	1.34	1.22	1.08
Vitamin + minerals	0.04	0.04	0.04	0.04	0.04
DL methionine	0.03	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00

T_1 = 0% inclusion of *Cassia tora* seeds in diets, T_2 = 5% inclusion of *Cassia tora* seeds in diets, T_3 = 10% inclusion of *Cassia tora* seeds in diets, T_4 = 15% inclusion of *Cassia tora* seeds in diets, T_5 = 20% inclusion of *Cassia tora* seeds in diets

The experimental quails were managed properly including housing environment, providing floor space, feeder and waterer space, litter management, sanitation and medication.

RESULTS AND DISCUSSION

Chemical composition of *Cassia tora* seed

The chemical composition of *C. tora* seeds and roasted *C. tora* seeds were analyzed in the laboratory and the result is presented in the Table 3. The results showed that the chemical composition of *C. tora* seed changes with roasting. The DM and CF percentage of the seeds increases with roasting whilst the CP content, EE and total

ash decreases on roasting.

Table 3: Analyzed chemical composition of *C.tora* seeds and roasted *C. tora* seeds used in the diets

	DM %	CP %	CF %	EE %	TA%
<i>C. tora</i> seeds	87.6	20.51	13.8	4.3	12.6
Roasted <i>C. tora</i> seeds	91.6	18.28	14.4	3.2	11.8

DM= Dry matter, CP= Crude protein, CF= Crude fiber, EE= Ether extract, TA= Total ash

The findings of chemical composition of *C. tora* seed through proximate analysis was well supported by the findings of Sigh (1964), Gohl (1975) and Taparia *et al.*, (1978). The CP content was found slightly higher than that found by Siruguri *et al.*, (2009) and Yen *et al.*, (1998) which was 17.8 and 17.7 percentage respectively. The percentage of CF and EE found was well supported by findings of Siruguri *et al.*, (2009) which was 13.6 % and 6.0 %.

Feed consumption, growth and feed conversion ratio

Inclusion of 5% *C. tora* significantly increased the feed intake than any other higher inclusion percentage. However, it was statistically similar to the control diet. Inclusion of *C. tora* had no influence on feed intake up to 15%, but inclusion of 20% significantly reduced the feed intake than control diet. Lower feed intake with increased amount of *C. tora* seed in the diets may be due to the presence of tannins and saponins in the seed which resembles the results by Katoch *et al.*, (1978). Inclusion of *C. tora* up to 15% gave similar body weight as control diet, however, increased inclusion of 20% significantly reduced the final body weight as compared to other treatments.

Table 4: Effect of *C. tora* seeds in diets on performances of Japanese quails at Rampur, Chitwan during 2011

Treatments	Feed intake	Final Body Weight	Feed Conversion ratio
T ₁ = 0% inclusion of <i>C. tora</i> seed	680.94±3.08 ^{ab}	147.14±5.67 ^a	4.63±0.19 ^b
T ₂ = 5% inclusion of <i>C. tora</i> seed	688.44±8.58 ^a	149.09±4.02 ^a	4.62±0.09 ^b
T ₃ = 10% inclusion of <i>C. tora</i> seed	674.68±4.31 ^{bc}	142.38±2.97 ^a	4.74±0.08 ^b
T ₄ = 15% inclusion of <i>C. tora</i> seed	672.58±3.38 ^{bc}	145.24±0.82 ^a	4.63±0.03 ^b
T ₅ = 20% inclusion of <i>C. tora</i> seed	666.95±3.78 ^c	126.43±3.98 ^b	5.28±0.14 ^a
F-value	8.009**	16.805**	16.893**

CV %	0.75	2.70	2.49
LSD _{0.05}	9.18	6.980	0.2153
SEM	2.91	2.22	0.014

Means in column with different superscripts differ significantly ($p < 0.05$).

LSD= Least significant difference, CV= Coefficient of variation, SEM= Standard error of mean, NS, not significantly different; * significant at 5% level of significance; ** significant at 1% level of significance.

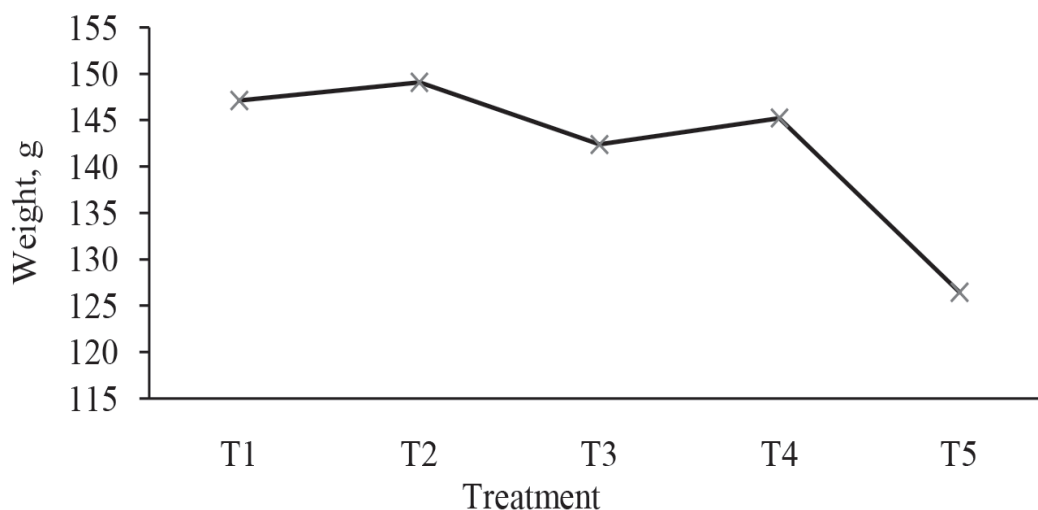


Fig. 1: Trend of final body weight of Japanese quail under different level of *C. tora* seed meal included in their diets

Figure 1 shows that, weight was significantly difference with inclusion of different level of *C. tora* seed meal in Japanese quail diets. It was found slight increased in 5% inclusion of *C. tora* seed meal but sharply decreased with 20% inclusion of *C. tora* seed. The present results are well supported by the findings of Singh *et. al.*, (2001) who recorded increased body weight gain in all *C.tora* fed up to 15%. The nonsignificant difference in body weight gain in control diet and 10% inclusion of *C. tora* seed meal on the diets are in the agreements with findings of Murty and Iyer (1957).

Feed conversion ratio was significantly higher in 20% *C. tora* inclusion in quail diet as compared to all other treatments. The remaining treatments were statistically similar, showing no differences in feed conversion ratio up to 15% inclusion as compared to control diet. The feed consumption was higher on 20 percent inclusion of *C. tora* seed meal in diets due to higher level of feed consumption than weight gain. The

present result was well supported by the findings of Singh *et al.*, (2001) who reported the lower feed conversion ratio in *C. tora* fed groups with lowest in 5% inclusion in broiler diets.

Economics of quail production

Except feed cost, other cost was constant and feed cost was only factor that differ the total production cost of Japanese quail. Economic analysis of Japanese quail under different level of *C. tora* seed meal included in the diets is presented in Table 6. The production cost per kg starter/ grower diets was maximum (Rs. 29.61) for T₁ and inclusion of *C. tora* seed in the diets had gradually decreased the production cost with minimum (Rs. 24.43) for T₅ as *C. tora* is an unconventional feedstuff and the price per kg was lower than other feed ingredients used.

Table 5: Economic analysis of Japanese quail under different level of *C. tora* seed meal included in the diets at IAAS, Rampur, 2011

Particulars	Treatments				
	T ₁	T ₂	T ₃	T ₄	T ₅
Cost of starter/ grower feed/ kg, Rs	29.61	28.22	26.96	25.69	24.43
Cost of production/bird, Rs	58.01	57.28	56.04	55.13	54.14
Income/bird, Rs	82.27	89.45	85.42	87.14	75.85
Net income/bird, Rs	24.26	32.17	29.38	32.01	21.71
Benefit/cost (B/C)	1.41	1.56	1.52	1.58	1.40

T₁= 0% inclusion of *C. tora* seed, T₂= 5% inclusion of *C. tora* seed, T₃= 10% inclusion of *C. tora* seed, T₄= 15% inclusion of *C. tora* seed and T₅= 20% inclusion of *C. tora* seed

Cost of production per bird was high in T₁ (Rs. 58.01) and low in T₅ (Rs. 54.14). The net income per bird was maximum (Rs. 32.17) on T₂ and minimum (Rs 21.71) on T₅. However, benefit/cost (B/C) ratio was high for T₄ (1.58) than T₂ (1.56) and low benefit/cost ratio was recorded on T₅ (1.40).

The results show that the inclusion of *C. tora* seed meal in the diets of Japanese quail decreases the cost of production of starter/grower diets, thus were more profitable than basal diets. However, higher percentage of inclusion decreases the benefit/cost ratio.

CONCLUSION

Feed intake, final bodyweight and feed conversion ratio was highest in 5% inclusion of *C. tora* seed meal in the diets. However, the total body weight, total feed intake and feed conversion ratio are non significant among control diet and diet containing 15% *C. tora* seed meal, and benefit cost ratio was highest for 15% *C. tora* included meal, *C. tora* seed could be included up to 15 % of the total feed in Japanese Quail diet.

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REFERENCES

- Banerjee, G. C. 2004. A textbook of animal husbandry. (8th ed.). Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, India. 636 p.
- Baral S. R. and P. P. Kurmi. 2006. A compendium of medicinal plants of Nepal. IUCN, Kathmandu. 372 p.
- Chadd, A. S., W. P. Davies and J. M. Koivisto. 2008. Practical production of protein for food animals. Protein sources for the animal feed industry. FAO, Italy, Rome. 72 p.
- Katoch, B. S., R. C. Katoch, N. C. Mahajan and K. B. D. Bhowmik. 1978. Utilization of Indian horse-chesnut (*Aesculus indica*) kernals and wild isbgol (*Plantago lanceolata*) plant-meal as poultry feed ingredients. Indian Veterinary Journal **55**: 781-87.
- Murty, V. N. and S. G. Iyer. 1957. *Cassia tora* Linn. Seed in poultry ration. Poultry Science **36** (1): 210-211.
- National Research Council. 1984. Nutrient requirements of poultry (8th ed.). National Academy Press. Washington D.C., USA. 230 p.
- Osti, N. P. 2002. Supplementation of methionine and lysine in locally available feed ingredient-based diets on the performance of broiler chicken and quail. Unpublished Thesis, M. Sc. (Animal Science), Tribhuvan University, Nepal. 72 p.

- Sigh, N. 1964. Leaf protein extract from some plants of North India. Journal of Food Science and Technology **3**: 37-39.
- Singh, J., R. Kumar and R. K. Yadav. 2001. Impact of feeding chakwar (*Cassia tora*) seed on growth and meat production in broilers. Indian Journal of Poultry Science **36**(3): 308-310.
- Siruguru, V., C. Ganguly and R. V. Bhat. 2009. Utilization of mouldy sorghum and *Cassia tora* through fermentation for feed purpose. African Journal of Biotechnology **8**(22): 6649-6654.
- Sood, A. K., M. Sharma and B. S. Katoch. 1990. Improvement in the nutritive value of Indian horse-chesnut kernel and cassia seeds by chemical treatments for feeding of chicks. Indian Journal of Animal Sciences **60**(11): 1364-1369.
- Taparia, A. L., T. W. Talmale and V. V. Sharma. 1978. Utilization of *Cassia tora* seeds in growth rations of buffalo calves. Indian Journal of Animal Science **48**: 804-810.
- Yen G., H. Chen and P Duh. 1998. Extraction and identification of an antioxidative component from Jue Ming Zi (*Cassia tora* L.). Journal of Agricultural Food Chemistry **46**: 820-824.

Proximate Analysis of Ostrich Meat to Assess the Quality in Chilled and Frozen Condition

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ABSTRACT

Ostrich meat is very popular due to its healthy composition thereby increasing its demand globally. Therefore, one study has been made on quality of ostrich meats of different ages at chilled and frozen conditions by targeting product development. Meat samples from two differently aged, 10- and 14-months ostriches were collected and divided into three parts for each type. One part was analyzed immediately, one part was put in chilling at 4°C for 72 hours and next part was put in deep fridge at -20°C for 30 days. Proximate analyses were carried out for moisture (73.98 to 75.22%), protein (21.08 to 22.46%), total fat (2.43 to 3.27%) and total minerals (0.83 to 1.82%). There was subsequent decrement in moisture at cold storage practices, at 72 hours chilling by 1% and one month freezing by 2.3% and there was no significant (?) difference in water content due to the age of bird. The amount of protein was found increasing at chilling, freezing and ageing? total fat was significantly different due to the age as well as meat storage practices and total ash was in decreasing trend in case of chilling and freezing conditions but found no difference due to the age.

Keywords: ostrich meat, proximate analysis, deep fridge, storage, protein, moisture, total fat, mineral.

INTRODUCTION

Meat consumption in developing countries has been continuously increasing. There are economic, dietary and sensory aspects that make meat processing one of the most valuable mechanisms for adequately supplying animal protein to human populations (Heinz and Hautzinger, 2007). These circumstances coerce into the diversification of meat into variety of meat products. The total annual meat production in Nepal is 288 thousand MT in which buffalo contributes the largest supply (64.1%) followed by goat (17.46%), sheep (1.33%), pig (7.38%), and poultry (9.61%) (CBS, 2014/15). From the data of five years, it can be said that production and consumption of poultry meat is in increasing trend in Nepal.

Introduction Over the last decade there is still observed a growing interest in ostrich farming and husbandry worldwide (Cooper et al., 2008). In Nepal also ostrich farming is in increasing trend just like as livestock and livestock products especially as a source of foods leathers, feathers and eggs. Its meat has been started to retail in meat mart since 2008 in Nepal. Ostrich meat is very popular due to the low level of cholesterol, calories, fat free, high level of iron and proteins, rich of omega 3, 6, and 9 with low saturated fat contents of 0.5% with rich of taste and easy to prepare. That's why ostrich meat is increasing rapidly and gaining popularity globally among the health-conscious red meat lovers.

Protein levels are high in ostrich meat, of about 28% in average, and the most frequent amino acid is creatinine (Nitzan *et al.*, 2002). Tenderness is the most appreciated ostrich meat characteristic. This is due to its low levels of saturated fat and its collagen to protein ratio, which are part of the connective tissue, and are responsible for meat texture, making it easy to digest and to chew. In addition to the low collagen level (0.44% in average), the arrangement of the muscle fibers, which are transversally oriented, may also explain its tenderness (Balog *et al.*, 2006).

The characteristic red color of ostrich meat can be explained by its high pigment content (22 mg Fe/g). Pigment content varies among different ostrich muscles, with *Flexor cruris lateralis*, *Iliofibularis*, and *Iliotibialis cranialis* presenting more intense color. Meat color is also influenced by water holding capacity, as higher water content results in higher absorption of radiation and lower reflection, making the meat darker (Balog and Almeida, 2007). This study was carried out to assess the quality of ostrich meats produced in Nepal which were of different ages and stored in chilled and frozen conditions

MATERIALS AND METHODS

Meat sample collection and processing

Meat samples from two differently aged, 10- and 14-months' ostriches were collected from outlet of Gangoliya Rupandehi by maintaining cold chain. Ice box and ice packs were used for cold chain maintenance and the temperature was in the range of 2-3°C. Collected samples were divided into three parts for each type. One part was analyzed immediately. Among the remaining two parts, samples were divided equally, packed in polyethylene pouches (thickness 40 microns) and sealed. Thereafter, one part was put in chilling at 4°C for 72 hours and next part was put in deep fridge at -20°C for 30 days prior to analyses. Proximate analysis Moisture, protein, fat, total ash, and crude fat as ether extract of ostrich meats of two different treatments were analyzed as per the method mentioned in Meat Science by Lawry (1998). The carbohydrate contents and crude fiber contents in ostrich meat were assumed to be zero.

RESULTS AND DISCUSSION

Proximate constituents such as moisture, protein, fat, total ash, and total fat as ether extract of ostrich meats of two different treatments were analyzed results were interpreted on Table 1. The carbohydrate contents and crude fiber contents in ostrich meat were assumed to be zero. Observations were taken at fresh meat, chilled and frozen meats after 72 hours.

Table 1: Proximate analysis of ostrich meat of different treatments

Treatments	Moisture %	Protein %	Total fat %	Total ash %	
T1 (Fresh 14 months' ostrich meat)		73.98 ^{ae}	22.46 ^a	2.43 ^a	1.82 ^a
T2 (Chilled 14 months' ostrich meat)		72.86 ^b	22.98 ^{ab}	2.51 ^a	1.56 ^a
T3 (Frozen 14 months' ostrich meat)		71.67 ^c	23.35 ^b	3.03 ^b	1.24 ^b
T4 (Fresh 10 months' ostrich meat)		75.22 ^d	21.08 ^c	2.85 ^c	1.32 ^b
T5 (Chilled 10 months' ostrich meat)		74.38 ^e	21.77 ^{cd}	3.02 ^b	1.02 ^c
T6 (Frozen 10 months' ostrich meat)		73.54 ^a	22.18 ^d	3.27 ^d	0.83 ^d
F. Pr.		p<0.05	p<0.05	p<0.05	p<0.05
LSD		0.615	0.845	0.221	0.276
SEM		0.205	0.188	0.067	0.085
CV%		1.5	0.9	4.4	7.8

Values are the means of triplicate analyses. The different superscripts on each mean value in same column indicates significantly different.

Moisture content of older and younger ostrich fleshes were found 73.98% and 75.22% respectively at fresh condition. There was subsequent decrement in moisture at cold storage practices. At 72 hours of chilling moisture of older ostrich flesh was reduced almost one percent whereas at frozen condition for one month, moisture was reduced by 2.3%. Moisture contents of ostrich were significantly different at both conditions such as age and storage practices ($p<0.05$) (Table 1). In case of younger ostrich flesh, the moisture loss was found in similar pattern as older ostrich flesh. The noticeable loss in moisture might be due to the drip loss at chilling condition. Unlikely chilling condition, the loss in moisture at frozen conditions might be the sublimation of exudates at the time of thawing.

The results in this study were very comparable to the findings of Naseva *et al.*, (2010). In his study, ostrich meat contains 74.52 % water. He also mentioned that the values were very similar to chicken flesh 75.58 % and beef flesh 74.21 %. The

protein amounts 22.62 % in the ostrich meat, 17.57 % in the chicken and 21.22 % in the beef; the fats quantity is 0.34 % in the ostrich meat, 5.2 % in the chicken and 1.9 % in the beef, and the minerals take 1.2 % of the ostrich meat, 0.9 % of the chicken and 1.1 % of the beef.

Regarding protein content, ostrich flesh was found a bit higher protein compare to other meat animal fleshs. The range of protein was 21.08 in younger bird of 10 months to 22.46% in older bird of 14 months. The amount of protein was found a bit increasing at chilling and more at freezing conditions. However, within the same origin of flesh, there were no significant differences in protein contents at both cold storage practices ($p < 0.05$) (Table 1). The condition might be due to the loss of water as drip loss at chilling condition and by sublimation at freezing condition and thereby increased solid contents.

In one of the studies by Nitzan *et al.*, 2002, protein level in ostrich meat was assessed about 28% in average, and the most frequent amino acid is creatine. Protein contents in this study were found lower than the abovementioned study but found similar to the results of Naseva *et al.*, (2010). The major factor might be the environmental condition where ostrich was reared or and the minor one might be the analytical errors.

Total Fat, extracted as ether extract, contents in ostrich fleshs were found significantly different within the age as well as storage practices ($P < 0.05$) and ranged 2.43 to 3.27% (table 4.1). Total fat includes fat from residual adipose tissues and intramuscular fats. Fat contributes in palatability of meat and considered eating quality. In this study, difference was insignificant in fat contents between normal and chilled flesh from 14 months' ostrich, but it was statistically different within the meats from younger ostrich.

Fat contents estimated in this study were found higher compare to the study made by Naseva *et al.*, (2010), which was less than 1%. The condition might be due to the error in sampling such as from different anatomical cut up parts or higher content of adipose tissue in the flesh. However, Cooper *et al.*, 2009 also mentioned that fresh ostrich meat contained 0.56 to 3.57% total fat. Similarly, Paleari *et al.*, (1998) reported that the percentage of fat is the lower for ostrich meat (1.6%) compared with turkey and bovine, with values of 3.8 and 4.5%, respectively and the results were very comparable to the current study. The fat-to-protein ratio is the key characteristic that favors ostrich meat as a healthy red meat. This ratio was low in ostrich meat (0.07) as compared with turkey and bovine, 0.18 and 0.22, respectively (Paleari *et al.*, 1998).

Total ash is the combination of all minerals available in the sample. Meat contains

1-2.5% total minerals (Subba, 2010). In this study the range of total ash was 0.83 to 1.82%. The study conducted by Naseva *et al.*, (2010) stated that there were about 1.2 % total minerals in ostrich meat which were similar to chicken flesh (0.9 %) as well as to beef (1.1 %). However, value of total minerals was a bit higher in the present study. Results from the current study were statistically different to each other except fresh and chilled meats from 14 months old ostrich ($p < 0.05$) (Table 1). Total minerals were in decreasing trend in case of chilling and freezing conditions because of the loss of minerals in exudates during chilling or thawing process. Among the ash contents, minerals such as phosphorus, manganese, and iron are higher, and sodium is lower in ostrich meat than either beef or chicken.

CONCLUSION

This study was conducted first time in Nepal to study on Proximate Analysis of ostrich meat to assess the quality in chilled and frozen condition. This study revealed the carbohydrate contents and crude fiber contents in ostrich meat were assumed to be zero. Observations were taken at fresh meat, chilled and frozen meats after 72 hours

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REFERENCES

- Anonymous (1992) Meat and meat products for Human Nutrition in Developing Countries. FAO Food & Nutrition Paper 53, FAO Rome.
- Balog, A. and Almeida P. (2007) Ostrich (*Struthiocamellus*) carcass yield and meat quality parameters. *Rev. Bras. Cienc. Avic.* **9**(4).
- Cooper, R. G, Jimenez S, Barbera T, Sendra R, Alvarez P (2006). Quality characteristics of ostrich (*Struthiocamelus*) burgers. *Meat Sci.* **73**: 295-303.
- Cooper, R. G. (1999) Ostrich meat, an important product of the ostrich industry: A Southern African perspective. *World's Poult. Sci. J.* **55**: 389-402.
- Hoffman, L. C., Fisher P (2001) Comparison of meat quality characteristics between young and old ostriches. *Meat Sci.* **59**: 335-337.
- Honikel, K.O. (1987) The water binding of meat. *Fleischwirtschaft* 67(9), 1098-

1102.

Horbańczuk, Sales J., Celeda T., Konecka A., Zieba G., Kawka P (1998) Cholesterol content and fatty acid composition of ostrich meat as influenced by subspecies, *Meat Sci.* **50**: 385-388.

Sales J. (1998). Fatty acid composition and cholesterol content of different ostrich muscles. *Meat Sci.* **49**: 489-492.

Sales J., Hayes J. P (1996). Proximate, amino acid and mineral composition of ostrich meat. *Food Chem.* **56**: 167-170.

Semen Extender Ratio and Season's Effects on Hatchability of Turkeys in Nepal

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ABSTRACT

This study was carried out to identify the effects of season and semen extender ratio on hatchability of artificially inseminated turkeys of Chitwan, Nepal. Trials were conducted using four treatments of different dilutions of semen with skim milk powder in the ratio of 1:1 (T1), 1:2 (T2), 2:3 (T3) and 3:4 (T4). The 32 turkeys were artificially inseminated in 4 days interval for 3 months. The dose of insemination was 0.03 ml and insemination were done around 6:00 p.m. Trials were done in winter and spring seasons of 2017 to find out the seasonal variation on hatchability of turkey. The data were analyzed using GenStat3.6.2, computer software. The hatchability was noted on total eggs as well as fertile eggs. In total eggs, it was found that hens with T1 (67.80%) gave higher hatchability than T2 (63.82%), T3 (63.04%) and T4 (63.98%) whereas in fertile eggs, T3 (83.26%) and T4 (83.01%) gave higher hatchability than T1 (82.96%) and T2 (82.90%). Hatchability rate was almost similar in both spring and winter seasons in total eggs with non-significant differences. On the other hand, in fertile eggs, the hatchability rate was found higher in spring season. Similarly, seasonal effect on hatchability rate of various semen extender ratios in total eggs and fertile eggs was also carried out. In total eggs, T1 and T3 produced higher hatchability rate in spring than in winter; T2 had similar hatchability rate in both seasons and T4 had higher hatchability in winter than in spring. However, there were no significant differences on hatchability rate of semen extender ratio due to seasonal variation. In fertile eggs, all the 4 dilutions had more hatchability rate in winter season than in spring with significance ($P < 0.05$). The hatchability was highest in T1 (85.19%) in winter season. The present study provides evidence that in total eggs of turkey inseminated with 1:1 semen dilutions provides more hatchability rate than other dilutions, whereas fertile eggs have more hatchability with dilutions 1:2 and 2:3 than others and hatchability rate based on total eggs is higher in spring than winter.

Keywords: Artificial Insemination, Semen Extender Ration, Hatchability, Fertility

INTRODUCTION

Turkey farming is flourishing in Nepal day by day due to its healthy meat. There are about 1 lakh Turkeys in Nepal contributing 2 quintal of meat per day. Turkey could be one of the potential commodities in enhancing the livelihood and nutritional security in the rural households of developing countries. However, promotion and commercialization of this species is scarcely done at government and non-government level. It has been experienced by the commercial growers that female turkeys get injured due to tearing of skin down to muscles by heavy males during mating. In this regard, artificial insemination is followed in turkey farming which has been found to be successful.

Artificial insemination in turkey began in 1930 when Beltsville based researchers William Burrow and Joseph Quinn in 1937 reported groundbreaking methods for semen collection and Artificial Insemination (AI) for poultry. The artificial insemination in turkey has continued to flourish and this has aided turkey farms since large number of poultry can be gotten through artificial insemination. Its use in turkey breeding is widespread due to the development of heavy, broad breasted bird with a conformation which makes natural mating difficult. As a result, it has become the only method for economic poultry production and some 95% of this success results from an artificial insemination breeding program (Marire, 2011). Low fertility in turkeys, due to unsuccessful mating caused by large body size of the tom and reduced libido is a serious and costly problem in the production of hatching turkey eggs (Merck pub., 2008). Artificial insemination is a common practice in the poultry industry with the turkey industry in North America and Europe using it almost exclusively to produce hatching eggs. It is also important as with AI, a greater number of females (about 18) can be fertilized than with natural mating where 1 tom can be used to fertilize 5 females only. The use of AI in turkey is enhanced with the improvement of diluents and breeding in appropriate season. As very small amount of semen is ejaculated at a time i.e. 0.2 to 0.5 ml so, appropriate diluents make it possible to increase its volume thereby enabling the spread of semen over many more turkeys. Important too is the decrease in ratio of toms from 1 to 10 with natural mating to 1 to 30 with Artificial Insemination (Tara, 1998). In past, some researchers have successfully employed various diluents for turkey semen like ringers' lactate, sodium phosphate buffer, egg yolk, egg white, milk and tyrode solutions. Diluent or extender refers to the aqueous solution used to increase the volume of the ejaculate to a level appropriate for making multiple inseminations (Salisbury *et al.*, 1978; Foote, 1980). Undiluted semen dies or loses its fertilizing capacity in about 4-6 hours at room temperature. Its life may be slightly prolonged by slow cooling to 100C (Gadea, 2003). Dilution reduces the

number of spermatozoa per ml of semen. Munro (1938) found a decline in sperm numbers with increasing dilution, but the decline is only appreciated when the dose of insemination contained less than the minimum number of spermatozoa required for optimum fertility (Brown *et al.*, 1970; LLinskii *et al.*, 1975). Loss of fertility following excessive dilution of semen could result by inseminating chickens weekly with semen diluted 1:4. Sexton (1986) reported that fertility of stored turkey semen diluted 1:2 was lower than that of semen diluted 1:1 and 2:1.

There have been very limited studies carried out in Nepal focusing to assess hatchability and fertility of Turkey to date. Very small amount of semen is ejaculated at a time i.e. 0.2 to 0.5 ml. Thus, appropriate diluents could make it possible to increase its volume thereby enabling the use of semen over many more turkeys. The influence of weather (i.e. season of production) on hatchability has not been adequately studied in the chicken. This may also influence egg quality before incubation especially during summer when environmental temperatures are high (Tona *et al.*, 2007). Considering the above-mentioned facts, present experiment was carried out specially aiming to know the most appropriate season of AI and the semen-extender ratio to increase hatchability of turkeys in Chitwan, Nepal.

MATERIALS AND METHODS

This study was conducted on a commercial turkey farm at Chitwan, Nepal in 2017. Ten months old laying turkeys were taken for study purpose. The experiment was done in imbalanced design due to the differences in the availability of turkeys for insemination according to the differences in the season. Altogether 32 turkeys were separated in different cages without any Tom for 3 months. Tom was isolated from hens a day before insemination. All those turkeys were given proper similar care, feed as well as sufficient time for grazing in the field. The diluent was prepared by adding two spoonful of water in one spoonful of skim milk powder. Collection of semen from Tom was done with the help of tuberculin syringe after massaging and stimulating its sex organ and kept in a glass beaker. Then semen and diluent were mixed at different ratios. The mixture was then inseminated to all turkeys equally i.e. 0.03 ml using tuberculin syringe.

Different trails were conducted using various dilutions i.e. dilution ratio of 1:1 (T1), 1:2 (T2), 2:3 (T3) and 3:4 (T4). Insemination was performed in every 4 days. From the day after insemination, eggs were collected from inseminated turkeys thrice a day. Each egg was marked and kept in hatchery for hatching. Every day, for 3 months i.e. from winter to spring, the number of marked fertile eggs from hatchery was recorded. As different dilutions were done on different days, the hatchability rate of different

dilution rate was recorded accordingly. The data collected during the experimental period were analyzed using GenStat3.6.2, computer software. The statistical model used in analyzing the data of this experiment is presented here under:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

Where,

Y_{ijk} is the adjusted mean of the hatchability

μ is the overall mean

a_i is the effect of i^{th} season

b_j is the effect of j^{th} semen extender ratio

$(ab)_{ij}$ is the effect of j^{th} semen extender ratio in i^{th} season

e_{ijk} is the residual (error) term that is assumed to be normally and independently distributed

RESULT AND DISCUSSION

Hatchability Based on Total Eggs

Results of present study revealed that the overall mean hatchability based on total eggs was 64.1. Season; semen extender ratio and the interaction between season and semen extender ratio had no significant influence on hatchability of artificially inseminated turkeys based on total eggs as per this study. However, higher rate of hatchability was observed for spring season as compared to winter season. Similarly, greater value of hatchability was observed in case of the turkeys inseminated with the diluted semen at the rate of 1:1 (T1). The result indicated that there is high hatchability rate of artificially inseminated turkey in spring than in winter. However, there are no such significant differences. It was found that T1 has high hatchability rate than others though there was no significant differences observed. Likewise, T1 and T3 have higher hatchability rate in spring than in winter; T2 has similar hatchability rate in both seasons whereas T4 has higher hatchability in winter than in spring. However, there were no significant differences on hatchability rate of semen extender ratio due to seasonal variation (Table 1).

Table 1: Effect of season and semen extender ratio on Hatchability (%)

Factors	Hatchability based on total eggs	Hatchability based on fertile eggs
Overall mean	64.1	83.1
Seasons		
Winter	64.07	83.68
Spring	64.59	82.31
Significance	NS	**
Semen extender ratio		
1:1	67.80	82.96
1:2	63.82	83.26
2:3	63.42	83.01
3:4	63.98	82.90
Significance	NS	NS
Semen extender ratio X Season		
1:1 X Winter	67.65	85.19
1:1 X Spring	68.00	80.00
1:2 X Winter	63.97	83.34
1:2 X Spring	63.61	83.14
2:3 X Winter	62.17	83.76
2:3 X Spring	65.08	82.00
3:4 X Winter	64.60	83.00
3:4 X Spring	63.16	82.76
Significance	NS	*

Note: *Significant at 95% confidence limit ($p < 0.05$), **Significant at 99% confidence limit ($p < 0.01$), NS: Non-significant

Hatchability Based on Fertile Eggs

The overall mean hatchability based on fertile eggs was 83.1%. Hatchability based on fertile eggs was significantly affected by the season of insemination ($P<0.01$) and semen-extender ratio ($P<0.05$). But the semen extender ratio had no significant influence on hatchability. The result indicated that there is high hatchability rate of artificially inseminated turkey in winter than in spring season with significance of $P<0.01$. It was found that T2 has high hatchability rate than others, however there was no significant differences observed. All the 4 dilutions had more hatchability rate in winter season than spring with significance of $P<0.05$. The hatchability was highest with T1 in winter season (Table 1).

Similar to our result, hatchability rate of fertile eggs in winter season was higher than spring season (Tona *et al.*, 2007). In case of month wise hatchability whereas winter (57.676%) shows the highest followed by summer (54.135%) and monsoon or rainy season (49.134%) (Chowdhury, 2004).

CONCLUSION

Based on the findings of present study, it can be concluded that total eggs of turkey inseminated with 1:1 semen dilution provides more hatchability rate than other dilutions, whereas fertile eggs have more hatchability rate with dilutions 1:2 and 2:3 than other dilutions. Similarly, Hatchability rate was almost similar in both spring and winter seasons in total eggs with non-significant differences. On the other hand, in fertile eggs, the hatchability rate was found higher in spring season. Knowing the above findings, turkey farmers can pre-plan the best season, semen-extender ratio for insemination and use the knowledge of interaction of season and semen-extender ratio.

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REFERENCES

- Brown, K. I.; Nestor, K. E and Topscher, M. (1970). A comparison of various methods of estimating spermatozoa concentration of turkey semen. *Poultry Sci.*, **49**: 295-299.
- Burrows, W. H. and Quinn, J. P. (1937). The collection of spermatozoa from the domestic fowl and turkey. *Poult. Sci.*, **16**: 19-24.
- Chowdhary, M.M.I. (2004). Effect of season on hatchability of duck eggs. DOI:10.3923/ijps.419.421.
- Foote, R. H. (1980). In “*Reproduction in farm Animals* (E.S.E., Hafez, ed.)
- Gadea, J. (2003). Semen extender used in the artificial insemination of Swine. *Spanish Journal of Agricultural Research*, **1**(2): 17-27.
- LLinskii, E. V.; Ten, E. B.; Tolstov, V. A. and Strelkova, L. I. (1975). Artificial Insemination of caged hens. *A.B.A.* **43**(5) no. 2048.
- Marire B. N (2011). Farm Animals, Features, Husbandry, Health and Production.
- Merck...Pub., (2008). Artificial insemination: Introduction. Disclaimer/Feedback. www.merckmanuals.com/vet/poultry/ar...
- Mounro, S. S. (1938). The effect of dilution and density on the fertilizing capacity of fowl sperm suspensions. *Canad. J. Res. D.*, **16**:281-299.
- Salisbury, G. W.; Vandermark, N. L. and Lodge, J. R. (1978). *Physiology of reproduction and artificial insemination in cattle*, 2nd ed. W. H. Freeman and Co., p. 247-417.
- Sexton, T. I. (1986). Comparison of Commercial diluents for holding turkey semen 24 hours at 40C. *Poult Sci.*, **67**:131-134.
- Tara W. (1998). Spotting top-notch toms-males turkeys. How Researchers are re Volutionizing turkey production-*Agric. Res.*
- Tona, K., Onagbesan, O., Ketelaere B.De., Bruggeman, V. and Decuypere, E. (2007). A model for predicting hatchability as a function of flock age, reference hatchability, storage time and season. *European Poultry Science*. **71**(1): S,30-34.

Seroprevalence of Avian Influenza (subtype H9) in ducks at AI risk areas of Nepal

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ABSTRACT

A study was conducted in duck population of Nepal from August-2013 to March-2014 as to find out the seroprevalence of Low pathogenic Avian Influenza (LPAI) subtype H9. Altogether 300 blood samples were taken from avian influenza risk areas of Nepal by using purposive sampling method and were analyzed in virology laboratory, Animal Health Research Division, NARC, Khumaltar. The serum samples were processed for Haemagglutination Inhibition (HI). Of the 300 serum samples, 16 samples showed HI positive (at > 3rd well) at two-fold dilution revealing revealing 5% Seroprevalence of LPAI H9 and overall average antibody titre 6.27. The seroprevalences of six districts were found to be Chitwan (10%), Sunsari (6%), Kathmandu (2%), Banke (8%), Bhaktapur (2%) and Jhapa (4%) with their mean antibody titres in log form were found to be 6.8, 6.3, 5, 6, 6 and 6.5 respectively. These samples when categorised into three divisions of influenza risk areas viz. Buffer zone, Border market area and Outbreak area, the seroprevalence were found to be 8%, 6% and 2% and their mean antibody titre in log₂ form were found to be 6.6, 6.3 and 5.5 respectively. The statistical analyses revealed that there is significant difference ($P < 0.05$) in age wise prevalence on group above 6 months and below 6 months. However, no significant difference ($p > 0.05$) occurred in prevalence by district, sex and influenza risk area.

Keywords: LPAI, HI, Seroprevalence, titre, chi-square

INTRODUCTION

Avian influenza subtype H9 that has been identified only in low pathogenic form (LPAI) should not be neglected by what the name suggests. This is so because H9 subtype infection is usually asymptomatic, but it is highly associated with virus shedding as it transmits easily from migratory bird to backyard poultry (e.g.: duck), from it to caged poultry population and even poultry to human population. Nepal is highly vulnerable to LPAI infection by migratory birds, from China (Tibet as well as North China) and Siberia (Russia), millions of them flying South West and South across the entire spread of the Northern boundaries, covering thickly populated areas beneath (both human and poultry: fowl as well as duck) (FAO and OIE 2009). The H9 subtype such as H9N2 virus strain is likely to get reassortment with other subtypes of Avian influenza i.e. can mutate to Highly Pathogenic Avian Influenzas (HPAI). The antigenic shift may result from mutation which is the genetic reassortment and adaptation of influenza viruses from one species to another. Antigenic shift results in new influenza A subtype (WHO, 2006) This causes high mortality in poultry population causing huge economic losses as well as grave public health hazards.

MATERIALS AND METHOD

The present study was conducted from August-2013 to March-2014. The potential site for avian influenza risk areas such as near migratory bird habitat in buffer region, outbreak areas and important poultry market areas of Nepal were selected. The selected district includes Chitwan, Sunsari, Jhapa, Kathmandu, Bhaktapur and Banke district. Altogether 300 blood samples were collected by using purposive sampling method and carried in icebox maintaining 4°C temperature and serum was separated later. All the samples were analyzed in virology laboratory, Animal Health Research Division, NARC, Khumaltar.

The serum samples were processed for Hemagglutination Inhibition (HI) test under OIE Manual of diagnostic Tests and Vaccines for Terrestrial Animals, 2014 and the standard Antigen and Antisera was bought from Charles River (USA). The HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells was considered to show inhibition.

RESULT AND DISCUSSION

The serovalence of serum samples of duck with HI positive (at >3rd well) at two-fold dilution revealed 5% *Seroprevalence of LPAI H9 and overall average antibody titre as 6.27*. The seroprevalence on risk areas categories Buffer zone, Border market

area and Outbreak area, were found to be highest in buffer zone (8%) and lowest in outbreak area (6%), antibody titre higher from buffer zone area. On district wise prevalence, Chitwan, Sunsari and Banke has higher prevalence than compared to Kathmandu, Bhaktapur and Jhapa, antibody titre being highest in Chitwan and lowest in Bhaktapur district. Age wise prevalence was found to be higher in age above 6 months (7.78%, antibody titre=6.53) than age below 6 months (2.25%, Antibody titre=5.33) while Sex wise prevalence was found to higher in female (6.53%) than male (4.80%). The seroprevalence and antibody titre on different research category is as shown in table 1 and 2.

Table 1: Statistical analysis on Seroprevalence

S.N.	Category	Groups	Positive samples (%)	Total Samples	Probability (P)	χ^2 value
	District	Chitwan	5(10) ^a	50		
		Sunsari	3(6) ^a	50		
1		Kathmandu	1(2) ^a	50	N.S	5.282
		Banke	4(8) ^a	50		
		Jhapa	2(4) ^a	50		
		Bhaktapur	1(2) ^a	50		
2	Risk areas	Buffer zone	8(8) ^a	100	N.S	8.997
		Outbreak area	6(2) ^a	100		
		Market area	2(6) ^a	100		
3	Age	Below 6 months	3 (2.25)	130	*	4..82
		Above 6 month	13(7.78)	154		
4.	Sex	Male	10 ^a	198	N.S	.542
		Female	6 ^a	86		

^{a,b} Indicate significant differences ($P < 0.05$) between the means of the groups, NS = not significant ($P > 0.05$)

Table 2: Statistical analysis on antibody titre

S.N.	Category	Groups	Positive samples	Titre	Probability(P)	χ^2 value
	District	Chitwan	5	6.8	N.S	
		Sunsari	3	6.3		
1		Kathmandu	1	5		18.415
		Banke	4	6		
		Jhapa	2	6.5		
		Bhaktapur	1	6		
2	Risk areas	Buffer zone	8	6.6	N.S	8.997
		Outbreak area	6	5.5		
		Market area	2	6.3		
3	Age	Below 6months	3	5.33	N.S	8.056
		Above 6 month	13	6.53		
4.	Sex	Male	10	6.3	N.S	7.557
		Female	6	6.4		

^{a,b} Indicate significant differences ($P < 0.05$) between the means of the groups, NS = not significant ($P > 0.05$)

There was no significance difference ($P > 0.05$) between antibody titre of different districts, risk areas, age and sex categories. The seroprevalence in this study was low as compared to seroprevalence study conducted at Iran which was 72.98% for LPAI H9N2 (Hadipour, 2011). The possible reason for such a low prevalence in duck is that the density of duck population and the market access of live duck is low. So, the spread of this virus is still limited in it.

Higher sero-prevalence in Chitwan district might be due to migratory bird habitat and the probable interaction and contamination of secretions of migratory birds with free ranging ducks. The absence of clinical signs of influenza in ducks despite high antibody titers in our study in samples from buffer zone area could be due to persistent exposure and acquired resistance of these birds to influenza virus in the environment. It supports the finding of Hadipour (2011) who found high antibody titers among chickens with no clinical symptoms observed.

Sero-prevalence in Banke district and Kathmandu district in our study was found to be 8% and 2% respectively. However, on a surveillance study of Avian influenza on 2005- 2006 by Panta et al., 2007, none of the duck sample were found positive in Kathmandu (n = 11) and Banke district (n = 4). Interestingly, the seroprevalence of Avian influenza subtype H9 on two different age group describes that both young group and adult age group has antibody for H9 and the adult group having higher mean antibody titre in $\log_2$ form as 6.33. High antibody titre in adult age could be correlated to frequency of exposure. Higher exposure causes subsequent increase in antibody titre.

The sero-prevalence of H9 on adult age group is higher than young age group. This finding is similar to that of Karki *et al.*, (2014) who found that older ducks had higher proportion of Influenza A seropositive in ducks from Kathmandu district (Karki *et al.*, 2014). This might be linked to the foraging behaviour as the range of foraging in adult is wider as well as longer time for exposure. Also, higher mean antibody titre in $\log_2$ form as 6.33 was found in adult age group. High antibody titre in adult age could be correlated to frequency of exposure. Higher exposure might have caused subsequent increase in antibody titre. The seroprevalence of Outbreak area on our study was found to be lowest (2%) than samples taken from buffer region area and Market area. In study done by Hymann (2012), none of the sample was positive for H9 but found seroprevalence for H5 and H7 from samples taken from H5N1 outbreak area in Cote d'Ivoire.

CONCLUSION

No significant difference occurred between prevalence of samples taken from buffer zone, market area and outbreak area. However, the high antibody titer and prevalence rate on buffer zone explains that this area would be high-risk area for virus transmission and mutation of LPAI leading to HPAI outbreak. Chitwan district and Sunsari has high prevalence of LPAI as compared to other districts. The scavenging ducks with high antibody titre is likely to transmit H9 virus to the farmer or nearby poultry farm. Hence, requiring surveillance of AI on this district needs to be highly prioritized. The age group above 6 months has high probability of transmitting infection than compared to age below 6 months requiring need for sero monitoring of this age group. Finally, surveillance (epidemiological, laboratory), supervision, monitoring and quarantine activities should be furthermore strengthened. Workers involved in the surveillance and transporting poultry and duck flocks must be protected with proper clothing and standard procedure for disposal of suspected poultry and its products should be developed and implemented.

REFERENCES

- Aamir, U.B., U. Wernery, N. Ilyushina and R.G. Webster. 2007. Characterization of avian H9N2 influenza viruses from United Arab Emirates 2000 to 2003. *Virology* **361**: 45-55.
- Acharya, S., M.P Acharya and T. Khanal. 2012. Sero-Surveillance of Avian Influenza Subtype H9N2 on live bird market of Kathmandu Valley. *Souvenir* 2012. NVA. P37 Available at: <http://www.nva.org.np/files/publication/Souvenir.pdf> (Retrieved on Jan 4, 2013).
- FAO and OIE. 2009. Situation of HPAI in Nepal. AICP, Kathmandu, Nepal.
- Hadipour M. 2010. Seroprevalence survey of H₉N₂ avian influenza virus in backyard chickens around the Caspian Sea in Iran. *Vet journal*. 20p.
- Hadipour, M. 2010. Prevalence of antibodies to H9N2 Avian Influenza virus in Backyard Chickens around the Maharlou Lake in Iran. *Pakistan Veterinary Journal*. 2p.
- Hymann, E., A. Kouakou, A. Koassi, Y. Koffi. 2012. Surveillance for avian influenza in poultry and science, West Africa, 2006-2008.
- Karki, S., Lupiani, B., Budke, C.M., Manandhar, S. and Ivanek, R., 2014. Cross-Sectional Serosurvey of Avian Influenza Antibodies Presence in Domestic Ducks of Kathmandu, Nepal. *Zoonoses and public health*, 61(6), pp.442-448.
- Office International Des Epizootic. 2009. Country Affected Areas (H5N1) in 2009. Date/Place of Latest Outbreak Affected Populations. (19-10-2009). Available at: <Http://www.oie.org/AI.html>. 2009 (Retrieved on Jan 2, 2013).
- OIE. 2014. Avian Influenza. OIE terrestrial manual 2014. Available at: <http://oie.org/manual.html>. (Retrieved on Jan 8, 2014).
- Pant, G., W. Sellar and T. Paul. 2006. Surveillance of Influenza in Nepal. 2004-2005. *Avian Disease*. Vol **51**. Available from: <http://www.jstor.org/er/10.2307/4493224?uid=2129&uid=2&uid=70&uid=4&sid=21104242408467> (Retrieved from Jan 13, 2013).
- WHO 2006. World Health Organization. Influenza research at the human and animal interface. Report of a WHO working group. WHO: Geneva, Switzerland? Available from: http://www.who.int/csr/resources/publications/influenza/WHO_CDS_EPR_GIP_2006_3C. (Retrieved on Feb 2, 2013).

Management System Including Health Care and Marketing of Pigs Adopted by Farmers in Dhankuta and Terhathum Districts

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ABSTRACT

Study was carried out to explore the management system of pig including health care and marketing, adopted by farmers in Hattikharka of Dhankuta and Phakchamara of Terhathum districts. A semi-structured questionnaire was distributed to a total of 200 pig farmers of selected sites. Farmers were surveyed to acquire information on management systems of pig such as, housing, breeding, feeding, health care and marketing. Majority of pig farmers kept Pakhribas black pig, chawanche and their crosses. Population of local pigs was found three times more than improved breeds in both sites. Generally, 1-5 pigs were raised by a farmer. Bamboo, wood and mud were mostly used as housing material. Stall feeding system was adopted by most of the farmers. Locally available feeding materials such as, rice bran, rice polish, maize, sisno, karklo, swill feeding, leftover food and green grasses like rayo, latte sag, kande jhar, twigs of fodder, khubhindo, pumpkin etc were mostly used for pig. Rai, Sherpa, Tamang, Gurung, Limbu, Magar, BK and Shrestha communities were mainly rearing pigs. Diarrhea, gastroenteritis, piglet mortality, agalactia, repeat breeding, parasitic infestation, etc were reported as the common diseases in pigs. Drenching against worm was adopted by few farmers and drugs like albendazole and fenbendazole were common. Vaccination against swine fever was also reported by the farmers and is in increasing trend as a preventive measure. According to farmers, demand for pigs is more than supply in the market and all the pigs are marketed and consumed within the district.

Keywords: Management system, health care, marketing, pig, Dhankuta and Terhathum

INTRODUCTION

Pig (*Sus scrofa*) farming enterprise is one of the important economic activities in Nepal. Livelihood, food and nutritional security and employment opportunities of several communities are also associated with pig husbandry. The population of pigs is estimated to be about 1.2 million (1,203,230) heads in Nepal and pork meat production is 20,135 metric ton (CBS, 2016). Among which about 50% pig population

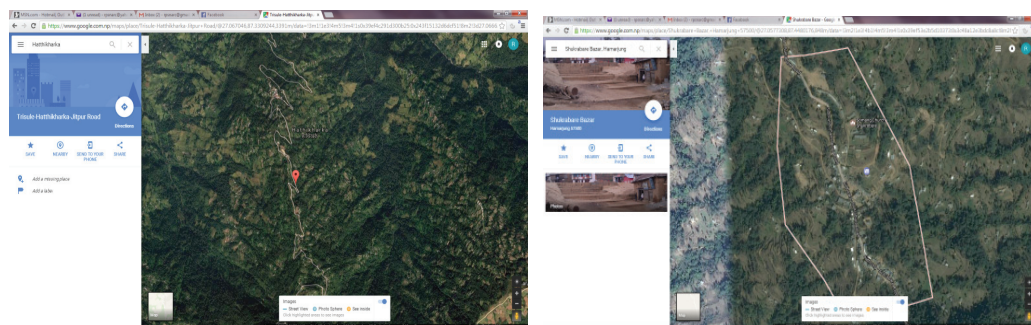
is represented by Nepalese breeds, such as *Pakhribas black*, *Chawanche*, *Hurrah* and *Bampudke*, while the introduced breeds are *Hampshire*, *Landrace*, *Tamworth*, *Yorkshire*, *Duroc* etc. Management system includes housing, breeding, feeding and health care and marketing as well. *Pakhribas black* pig is a synthetic breed developed by Nepali scientists for higher production in the prevailing agro-ecological conditions, using three different breeds (*Seddleback*, *Fayun* and *Tameworth*).

Pig farming has been accepted socially and culturally by certain ethnic groups only. However, its trend is changing gradually due to urbanization. The farm size is usually smaller, but the commercialization has been started. In comparison to other livestock, crop cycle of pig farming is shorter. Generally, two farrowings per sow per year can be obtained having 8–12 piglets in a single farrowing with maximum 15% piglet mortality during weaning period. Pig farming trend is increasing in Nepal due to reduced cultural biases against pigs and new government programs to support pig farming as a low cost means to create income for poverty alleviation and to improve food security. About 48% increase in the Nepal pig population in 14 years (MoAD, 2016) shows how fast this industry is growing. Pig farms are not homogeneously distributed in Nepal. Pig farming is most prevalent in the eastern and central regions of the country where the pig-raising ethnic community predominates and is also more common in peri-urban areas because of an increased demand of pig meat in urban areas. Hence, this study aims to represent the scenario of different existing systems associated with pigs and identify commonly occurring diseases in pigs in Dhankuta and Terhathum districts.

MATERIALS AND METHODS

In 2015-16, a survey was conducted to the pig farmers of different two places viz. Hattikharka (Figure 1A) and Phakchamara (Figure 1B) of Dhankuta and Terhathum districts, respectively. A semi-structured questionnaire was distributed to a total of 200 pig farmers of selected sites (100 from each). Required data were collected from District Livestock Service Office, Dhankuta and Terhathum. The questionnaire format included number of pigs, breeds, housing system, feeding system, health care, common diseases and marketing.

The data generated were stored in a Microsoft Excel spreadsheet (Microsoft Corporation) and descriptive analysis was performed.



A.

B.

Fig. 1: Map of study areas (A. Hattikharka of Dhankuta and B. Phakchamara of Terhathum) source: www.google.com/maps

RESULTS AND DISCUSSION

Population of Pig

Total population of pig in Dhankuta and Terhathum districts was presented in the following Table 1. Local breed was found two times more than other breeds (crosses, improved) of pig. Local breed included mainly pakhribas black pig, chawanche and Bandel.

Table 1: Population of pig in Dhankuta and Terhathum (in number)

Breed/District	Local	Others (crosses, improved)	Total
Dhankuta	26,803	11,387	38,190
Terhathum	21,697	13,158	34,855
Total	48,500	24,545	73,045

Source: Annual Report, DLSO, Dhankuta and Terhathum (FY 2072/73)

Breeds of pigs

It was found that 70% farmers raised indigenous/local breed followed by mixed (crosses of local and improved- 22%) and improved breed (8%) of pig as given in Figure 2. Population of indigenous pigs was found about 3 times more than mixed and improved breeds in both sites. Rearing more indigenous pigs might be due to tradition to raise black pigs and people like black color of pig. Indigenous breeds like pakhribas black and chawanche look like mainly black. Such breeds are also popular due to their positive attributes. Indigenous pigs are successfully reared in low investment as well as well adopted in Nepalese climate in comparison to exotic breeds.

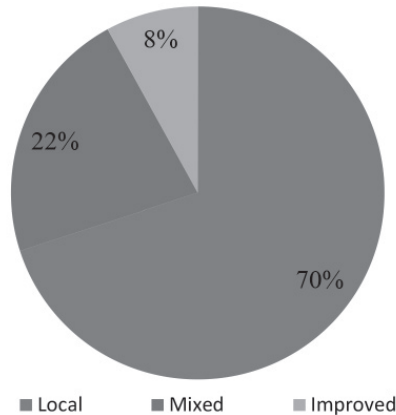


Fig. 2: Breeds of pigs raised by farmers

In this figure, people were encouraging towards rearing improved breeds also because of urbanization. Improved breeds included Hampshire, Yorkshire, Landrace, Duroc and Meishan.

Pigs raised per household

Most of the farmers were raised pig as an average of 2.5 pigs (range: 1-5 pigs) per household (Figure 3). It might be because farmers want to utilize their kitchen wastes, locally available grasses and other materials like kudo. This report is similar made by Joshi (2006) who stated that farmers kept 2-3 pigs per household. This also might be because few ethnic groups like Rai people rear pigs for their religious and ethnic value. But due to urbanization, people were encouraging towards commercial scale also.

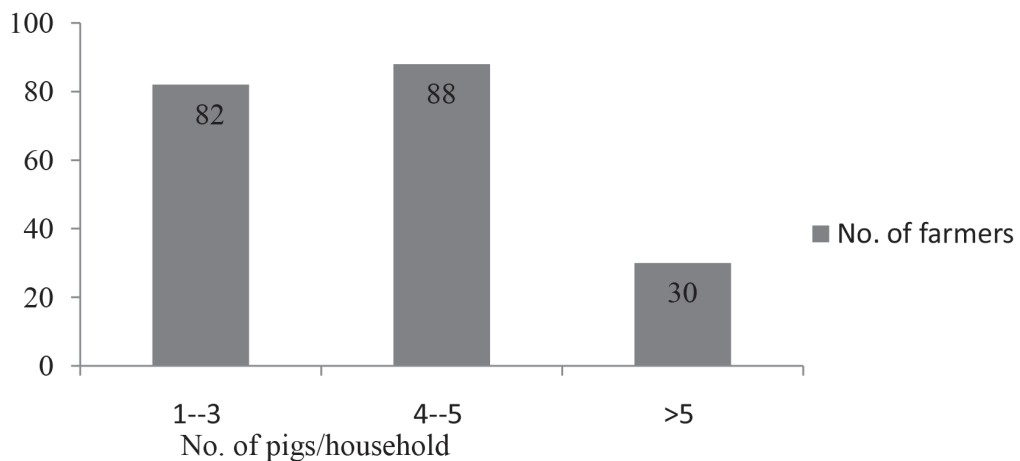


Fig. 3: Pigs raised by farmers

Housing system and materials

Majority of pig sty/house (90%) were made up of locally available materials like bamboo, wood and mud rather than concrete as shown in Figure 4. It is because of low economic status of people. People were showing interest to adopt semi-intensive and intensive housing system rather than free range. About 10% people were raising pigs in concrete houses which indicate people are encouraging towards rearing pigs commercially.



A.



B.



C.



D.

Figure 4. Pigs raised under different management conditions (A, B: houses made up of bamboo, wood and mud; C, D: houses made up of concrete)

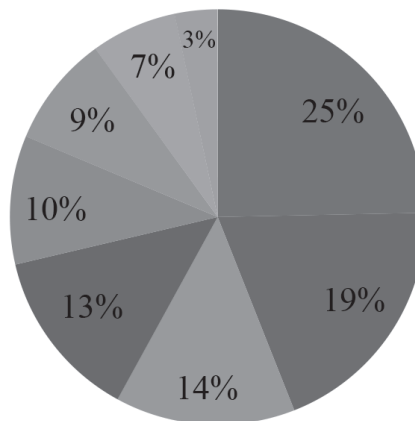
Feeding system

Stall feeding system was adopted by majority of pig farmers (98.5%) rather than others (1.5%). Because it is easy to feed and utilize most of the waste materials as feed and family members also can give time to enough care.

Farmers used locally available feeding materials that were rice bran, rice polish, maize, *sisno*, *karkalo*, swill feeding, leftover feed from home/hotel and cooked food and green grasses like *rayo*, *latte sag*, *kande jhar*, twigs of fodder, *khubhindo*, pumpkin and other forages etc. Pig farmers used mainly above ingredients as feed because they want to get profit from their own resources.

Pigs reared by different community

Generally Rai, Sherpa, Tamang, Gurung, Limbu, Magar, BK and Shrestha communities were reported for rearing the pigs. Above ethnic group of people had been raising pigs since long ago. It was found that involvement of Rai people in pig rearing was 25% that is followed by Sherpa (19%) and Tamang, Gurung etc (Figure 5). It is said that ethnic groups like Rai caste people rear pigs for their religious and ethnic value. Involvement of Bahun and Kshetri to rear pigs was found nil.



Common diseases of pig

According to respondents, the common diseases encountered in pigs were diarrhea, gastroenteritis, piglet mortality, agalactia, repeat breeding, parasitic infestation, abortion as given in Table 2. Among 200 respondents, 85% farmers faced problem of diarrhea. Similarly 81% farmers encountered gastroenteritis. These problems may be due to feeding of unbalanced, unhygienic feeding materials and internal parasite infestation. Piglet mortality might be due to coldness during parturition/newly born piglets, hypoglycaemia, iron deficiency, weakness of mother, low milk production.

Table 2: Common diseases of pigs

Common diseases	Yes	%
Diarrhoea	170	85
Gastroenteritis	162	81
Piglet mortality	153	76.5
Agalactia	130	65
Parasitic infestation	120	60
Abortion	68	34
Others	60	30

Drenching against worm was being adopted by few farmers and the drugs were albendazole and fenbendazole. Vaccination against swine fever was being used, increasing in trend, for prevention. Iron injection for piglet was also being practiced frequently as prevention from piglet anemia and mortality.

Marketing system

There was no fixed system for marketing; however, there was a regular live animal and pork haat bazaar system developed in Dhankuta municipality and a few highway roadside bazaars to which farmers brought their live animals for sale to local traders. The traders then sold the pigs to the major traders or they sold to the butchers who slaughtered the pigs and sold the meat at retail meat shops. The major traders took the live pigs to other districts like Sunsari also. Sometimes farmers slaughtered the pigs in their own home and sold the meat nearby prefix consumers. Generally all the pork meat was consumed in Dhankuta and Terhathum districts.

CONCLUSION

Dhankuta and Terhathum districts have been seen as potential area of interest for pig farmers because supply is less than demand. Raising pigs towards commercial scale was increasing in trend and majority of people like black pigs. Also, people of different communities are raising pigs. Farmers need knowledge on proper method of mixing of ingredients and feeding practices so that they can get more profit from locally available ingredients. Awareness towards diseases prevention like parasitic diseases, piglet mortality should be provided to the farmers time to time. Encouragement towards deworming, vaccination and sanitation should be given to pig farmers

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REFERENCES

- CBS (Central Bureau of Statistics). 2016. National Sample Census of Agriculture, Nepal.
- DLS (Department of Livestock Services). 2072-73. Annual Report of Department of Livestock Services (DLS), Dhankuta and Terhathum, Government of Nepal.
- Joshi, D. D. (2006). Pig farming system in Nepal: how can research and development enhance benefits to the poor farmers of Nepal? NZFHRC, Kathmandu, Nepal. pp. 48–56.
- MOAD: (2016) Statistical information on Nepalese Agriculture (2072/73). Government of Nepal, Ministry of Agriculture Development. http://www.moad.gov.np/downloadfile/yearbook2016_1363677455.pdf.

Serodiagnosis of Toxoplasmosis using Lateral Flow Chromatographic Immunoassay among Animals and Humans in Sunsari District of Nepal

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ABSTRACT

Toxoplasmosis is a cosmopolitan parasitic zoonosis, infecting human and other warm-blooded animals worldwide. This disease has economic importance in regard to animal reproduction, and it leads to abortions and neonatal complications in humans. This study was carried out to determine the seroprevalence of Toxoplasma gondii in sheep, cattle, cats and human in Inaruwa and surrounding areas of Sunsari district, Nepal. Altogether 336 blood samples, of which 50 from sheep, 92 from cattle, 44 from cats and 150 from human were collected and tested immediately using lateral flow chromatographic immunoassay (Toxo IgG/IgM Combo Rapid test®). Associated biometric information such as age, sex, pregnancy status, occupation, association with cat was recorded and analyzed to determine the association of risk factors with the disease. Data were analyzed using R 3.2.2 (The R foundation for Statistical Computing, 2015). Seroprevalence of toxoplasmosis was detected 12.00% (95% CI: 4.53- 24.31%) in sheep, 8.70% (95% CI: 3.83- 16.42%) in cattle, 36.36% (95%CI: 22.41- 52.23%) in cats and 12.67% (95% CI: 7.80- 19.07%) in human. In case of human, 31 to 45 years age group were found more susceptible to toxoplasmosis (21.74%, OR: 6.4) in comparison to 21 to 30 years (10.0%) and up to 20 years (4.17%) age groups. Toxoplasmosis was found highly significantly associated with abortion (58.33%, OR= 15.4, P=0.0001) in human in the tested individuals. Regarding occupation, 20.83% butchers were seropositive followed by farmers (15.52%), housewives (10.0%) and diagnostic lab technicians (8.0%). Female and higher age group showed high prevalence of toxoplasmosis in all studied species. Therefore, this assay is a useful method for the serological screening of toxoplasmosis in different animals and humans.

Keywords: Animals, Human, Nepal, Seroprevalence, Toxoplasmosis

INTRODUCTION

Toxoplasmosis is a global zoonosis occurs in almost all warm-blooded animals including human beings and birds, is caused by *Toxoplasma gondii*. Based on serological investigations, it is reported that upto one third of the world's human population has been infected to this parasite (Innes, 2010; Pereira *et al.*, 2010; Montoya and Liesenfeld, 2004). The parasite is known to cause congenital disease and abortion both in humans and livestock species (Dubey and Beattie, 1988; Remington *et al.*, 2001). In most countries, toxoplasmosis comes as the second cause of abortion in prevalence after chlamydial abortion (Dubey *et al.*, 2013). Therefore, the infection has an economic and clinical significance in many sheep and goat producing countries.

A broad spectrum of animals can be infected by ingestion of raw or undercooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts from the feces of infected cats (Dubey, 2004; Lake *et al.*, 2002). People usually acquire infection via ingestion of tissue cysts in undercooked meat, consuming food and water that has been contaminated with sporulated oocysts, or by accidentally ingesting oocysts from the environment, or vertically by transplacental transmission of tachyzoites. Once infected, humans may remain infected for the entire life. Clinically, patients may have headache, disorientation, drowsiness, hemiparesis, reflex changes, and convulsions and may become comatose (Dubey, 2010). Infection with *T. gondii* during pregnancy can result in fetal death, neonatal death or various congenital defects, such as hydrocephalus, central nervous system abnormalities and chorioretinitis (Zewdu *et al.*, 2013).

The majority of infections are asymptomatic and unapparent or latent, but in sheep and goat clinical toxoplasmosis most often reported. Infections during pregnancy can cause abortions, stillbirths, mummification or resorption of the fetus (Hill and Dubey, 2002). There are numerous serological procedures available for the detection of IgG and IgM antibodies; these include the Sabin–Feldman dye test (DT), indirect hemagglutination assay (IHA), indirect fluorescent antibody assay (IFA), modified agglutination test (MAT), latex agglutination test (LAT), enzyme-linked immunosorbent assay (ELISA) and complement fixation test (CFT) (Pal, 2007). For the diagnosis of *T. gondii* infection, detection of the organism itself is confirmative but very difficult.

Seroprevalence in different populations may vary according to different environments, social customs and habits. In Nepal, seropositivity rates were 57.9% (Rai *et al.*, 1994); 30.6% (Upadhayay *et al.*, 1989) and 50.6% (Jaiswal *et al.*, 2014) in human using different serological tests. Nepal is a country of vast diversification in geotopography.

The positive rate is reported to vary from place to place (Tizard *et al.*, 1977; Feldman, 1982; Suzuki *et al.*, 1987). Cats are the most popular pet in the world and are now found in almost every place where humans live (Driscoll Carlos, 2009; Sah *et al.*, 2016). No doubt Nepal has unknown number of domestic, stray and wild cats. Usually cats do not show the clinical signs even during shedding of oocysts. So, cats have a key and crucial role in the epidemiology of toxoplasmosis. Therefore, expanding the basic knowledge about *T. gondii* infection in both humans and animals in Nepal is a matter of importance. Therefore, lateral flow chromatographic immunoassay (LFCIA) (Toxo IgG/IgM Combo Rapid test[®]) was used in sheep, cattle, cats and humans. Prevalence of toxoplasmosis investigation is very important in Nepal for surveillance and monitoring for future planning control strategy. By considering these points, the present research work has been aimed with the following objectives: (1) To determine the seroprevalence of toxoplasmosis in sheep, cattle, cat and human in Sunsari District and (2) To identify risk factors of *T. gondii*.

MATERIALS AND METHODS

Study Area

Study areas lie in Sunsari district in Terai, plain region of eastern Nepal. This district is located 26.6276° N latitude and 87.1822° E longitude, having 1257 Km² area and consists of 763,487 human population with 610/Km² population density (Central Bureau of Statistics Nepal, 2014).

Study population and collection of samples

Altogether 336 samples (50 sheep, 92 cattle, 44 cats and 150 human) were examined. The type of the study was cross-sectional. Random sampling method was used for sheep, cattle and cat whereas human sampling was done among risk group of people. Human blood samples were collected in Birat Diagnostic and Research Center, Inaruwa, Sunsari and few samples from home visit from fingertips for housewives and for butcher from Sunsari district. Similarly, blood samples of sheep, cattle and cats were collected from farmers' house in Inaruwa and surrounding areas and were examined at the spot.

Basic relevant history and data like age, sex, status of body condition- pregnant, aborted, non-pregnant, availability of cats, occupation of human and cooking method of meat etc were also taken from patient attendants both from human and animals during blood collection.

Lateral flow chromatographic immunoassay (LFCIA)

For rapid test, Toxo IgG/IgM Combo Rapid Test®, CTK Biotech USA was used. This kit is a lateral flow chromatographic immunoassay and the test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *T. gondii* antigens conjugated with colloidal gold (*T. gondii* conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The M line is pre-coated with monoclonal anti human IgM for detection of IgM anti-*T. gondii* antibody, the G line is pre-coated with reagents for detection of IgG anti-*T. gondii* antibody, and the C line is pre-coated with a control line antibody. A drop of blood (35 µl) was kept into well of kit and same amount diluent supplied by kit was put into well and development of color line was observed within 10 minutes. Presence of a burgundy colored G /M line indicated a *T. gondii* IgG/IgM positive test result. Absence of any test lines (M and G) was considered a negative result.

Data Analysis

All the collected data were compiled in Microsoft Excel. Data were analyzed using R 3.2.2 (The R foundation for Statistical Computing, 2015). Seroprevalence percentage, OR values and P value at 95 % significance level were calculated to show the association of risk factors with disease and results were tabulated.

RESULTS

Toxoplasmosis in different species

Humans as well as animals like sheep, cattle and cat were found to be infected with toxoplasmosis. The odds of toxoplasmosis was 6.0 times higher in cat than cattle (Table 1).

Table 1: Seropositivity of toxoplasmosis in human, sheep, cattle and cat

Species	Positive/ tested sample	Prevalence %	95% confidence interval (CI)	Odds Ratio (OR)	P value at <0.05
Human	19/150	12.67	7.80- 19.07		
Sheep	6/50	12.00	4.53- 24.31	1.4	0.5298
Cattle	8/92	8.70	3.83- 16.42	Reference	
Cat	16/44	36.36	22.41- 52.23	6.0	0.0002

Toxoplasmosis in humans

Female had higher prevalence of toxoplasmosis than male. The odds of toxoplasmosis was 6.4 times higher in humans aged between 31-45 years in comparison to those below 20 years. About 58.33% aborted women were seropositive and the odds of toxoplasmosis was 15.4 times higher in aborted females than non-pregnant ($p=0.0001$, OR: 15.4) (Table 2).

The highest prevalence (20.83%) of toxoplasmosis was found in butcher while all students tested were seronegative (Table 3).

Table 2: Distribution of toxoplasmosis in humans

Variables	Positive/ tested person	Prevalence %	95% CI	Odds Ratio	P value at <0.05
Sex					
Female	15/115	13.04	7.49- 20.60	1.2	0.8016
Male	4/35	11.43	3.20- 26.74	Reference	
Age					
Upto 20 years	1/24	4.17	0.11- 21.12	Reference	
21-30 years	8/80	10.00	4.42- 18.76	2.5	0.3882
31-45 years	10/46	21.74	10.95- 36.36	6.4	0.0866
History of individual					
Abortion	7/12	58.33	27.67- 84.83	15.4	0.0001
Non pregnant	8/96	8.33	3.67- 15.76	Reference	

Table 3: Distribution of toxoplasmosis in human based on their occupation and their association with cat

Variables	Positive/tested person	Prevalence %	95% CI	Odds Ratio	P value at <0.05
Occupation					
Butcher	5/24	20.83	7.13- 42.15	3.0	0.2146
Housewife	3/30	10.00	2.11- 26.53	1.3	0.7976

Farmer	9/58	15.52	7.35- 27.42	2.1	0.3628
Diagnostic Lab technician	2/25	8.00	0.98- 26.03	Reference	
Student	0/13	0.00			
Contact with cat					
Yes	17/120	14.17	8.47- 21.71	2.3	0.2813
No	2/30	6.67	0.82-22.07	Reference	

Toxoplasmosis in sheep

The distribution of toxoplasmosis in sheep is presented in Table 4. The prevalence of toxoplasmosis was higher in female (13.33%), sheep older than 18 months (20%) and in pregnant sheep (16.67%).

Table 4: Distribution of toxoplasmosis in sheep

Variables	Positive/tested animal	Prevalence %	95% CI	Odds Ratio	P value at <0.05
Sex					
Female	4/30	13.33	3.76- 30.72	1.4	0.7232
Male	2/20	10.00	1.23- 31.70	Reference	
Age					
Below 6 months	0/8	0.00	0.00- 36.94		
6-18 months	2/22	9.09	1.12- 29.16	Reference	
Above 18 months	4/20	20.00	5.73- 43.66	2.5	0.3238
History of animal					
Pregnant	2/12	16.67	2.09- 48.41	1.8	0.5845
Non pregnant	2/18	11.11	1.38- 34.71	Reference	

Toxoplasmosis in cattle

The distribution of toxoplasmosis in cattle is presented in Table 5. Female, cattle over 2.5 years old and pregnant cattle showed higher prevalence than respective another group.

Table 5: Distribution of toxoplasmosis in cattle

Variables	Positive/ tested animal	Prevalence %	95% CI	Odds Ratio	P value at <0.05
Sex					
Female	7/77	9.09	3.73-17.84	1.4	0.7615
Male	1/15	6.67	0.17-31.91	Reference	
Age					
2.5 years	0/5	0.00	0.0-52.18		
> 2.5 - 5 years	3/42	7.14	1.5-19.48	Reference	
> 5 years	5/45	11.11	3.71-24.04	1.6	0.5252
History of animal					
Pregnant	1/10	10.0	0.25-44.5	1.1	0.6115
Non pregnant	6/62	9.68	3.63-19.88	Reference	

DISCUSSION

The study revealed variable rate of seroprevalences of toxoplasmosis among human, sheep, cattle and cats in the study area. Although toxoplasmosis is considered harmless for non-pregnant women, it is potentially harmful during pregnancy, especially at the first trimester (Giannoulis *et al.*, 2008). In this study 12.67% seropositive was found in human beings. Similar findings were reported by many other scientists in abroad. Slight lower prevalences were reported 10% in women in Egypt (Amany and Merwad, 2012); 10.66% in human in Bangladesh (Hossain, 2016) and 12.4% in women in Bangladesh (Samad *et al.*, 1997). High rates 15.89% in women with different gynaecological diseases (Samad *et al.*, 1993) and 38.5% in human (Ashrafunnessa *et al.*, 1998) were observed in Bangladesh using different serological tests. But this finding

seemed very low from previous reports in Nepal. In Nepal, high rates 54.8% (Rai *et al.*, 1994); 57.9% (Rai *et al.*, 1993) and 50.6% (Jaiswal *et al.*, 2014) were observed in human by using different serological tests. The positive rate is reported to vary from place to place (Suzuki *et al.*, 1987).

The present study demonstrated association between toxoplasmosis and different age groups of humans. The highest prevalence 21.74% found among age 31 to 45 year supported by findings of reports 44% among 36-40 years age group in Iran (Jafar *et al.*, 2014) and 27.9% in age group 31-45 year in Bangladesh (Margia, 2015).

Toxoplasmosis was significantly ($P < 0.05$) associated with abortion in human. So, this parasite is harmful for pregnant women because it causes abortion. It may be due to ingestion of contaminated vegetables with oocysts or consumption of undercooked meat containing cysts.

According to occupation of people, butchers were found higher seropositive 20.83% compared to other occupational groups viz. farmer (15.52%), housewife (10%) and diagnostic lab technician (8%). It may be due to lack of awareness among slaughterhouse workers and there is no hygienic condition maintained at shop and also no trend to wear gloves during meat cutting and selling. Interestingly, this study found majority of the seropositive people had come in contact with cat (14.17%) by any means whereas people who were not in contact with cat found low (6.67%). The finding showed that 36.36% cats were seropositive. This finding is slightly higher than a report 33.33% (Samad *et al.*, 1997) and lower than 42% (Lamaj *et al.*, 2016). This finding is 2.5 times higher than a report made by Sah *et al.*, (2016) but they did work to detect oocysts in cat faeces in Bangladesh.

It was found that anti-*T. gondii* antibody is prevalent in sheep (12.0%) and cattle (8.7%). These findings are almost similar to 14% in sheep (Nunes *et al.*, 2015) and 8.57% in cattle (Azizi *et al.*, 2014). Surprisingly in all the studied species of animals, highly appreciable findings were that female, older age group (above 18 months in sheep and above 5 years in cattle) and pregnant animals were found more susceptible to *T. gondii*. The higher seroprevalence in female as compared to male might be attributed to the management system in those females are retained in the farm for longer periods for breeding purpose than males. Few males are retained for mating while the majority are culled and sold for cash purpose. The hormonal difference in relation to stress of lactation and pregnancy leading to immunosuppression may also increase susceptibility to toxoplasmosis in females (Dubey and Lappin, 1998). Higher seroprevalence in older age group compared to young is consistent with earlier studies and is the result of higher likelihood of ingestion of oocysts with increasing age (Dubey, 2010; Carneiro *et al.*, 2009; Ramzan *et al.*, 2009; Halos *et al.*, 2010).

CONCLUSION

Based on LFCIA, the seroprevalences of toxoplasmosis were 12.67%, 12.00%, 8.70% and 36.36% in human, sheep, cattle and cats, respectively. With above results of the experiment, it is suggested that this assay is highly useful as a serodiagnostic tool in *T. gondii* infections. As this parasite has zoonotic importance, the knowledge should be disseminated to the people and animal raisers so that precautions can be taken in time.

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REFERENCES

- Amany, M. and Merwad, A.M. (2012). Epidemiology and molecular detection of zoonotic *Toxoplasma gondii* in cat feces and seroprevalence of anti-*Toxoplasma gondii* antibodies in pregnant women and sheep. *Life Science Journal*, **9**: 133-146.
- Ashrafunnessa, Khatun, S., Islam, M.N. and Huq, T (1998). Seroprevalence of toxoplasma antibodies among the antenatal population in Bangladesh. *Journal of Obstetrics and Gynaecology Research*, **24**(2): 115-9.
- Azizi, H., Shiran, B., Boroujeni, A.B. and Jafari, M. (2014). Molecular survey of *Toxoplasma gondii* in sheep, cattle and meat products in Chaharmahal va Bakhtiari Province, southwest of Iran. *Iranian Journal of Parasitology*, **9**(3): 429-434.
- Carneiro, A.C.A.V., Carneiro, M., Gouveia, A.M.G., Guimaraes, A.S., Marques, A.P.R., Vilas-Boas, L.S. and Vitor, R.W.A. (2009). Seroprevalence and risk factors of caprine toxoplasmosis in Minas Gerais, Brazil. *Veterinary Parasitology*, **160**: 225-229.
- Driscoll Carlos, A., Clutton Brock, J., Kitchener Andrew, C. and Brien, O.S.J. (2009). The Evolution of House Cats. *Scientific American* 2009. New York: Nature Pubg. Group. Retrieved 26 August 2009.
- Dubey, J.P. and Beattie, C.P. (1998). Toxoplasmosis of Animals and Man. Boca Raton, Florida: *CRC Press*; pp.220.
- Dubey, J.P., Hill, D.E., Remington, D.W., Kwox, O.C. and Sue, S. (2013). High

- prevalence and genotypes of *Toxoplasma gondii* isolated from organic pigs in northern USA. *Veterinary Parasitology*, **188** (1): 14-18.
- Dubey, J.P. and Lappin, M.R. (1998). Toxoplasmosis and neosporosis. In: Greene CE, editor. Infectious diseases of the dog and cat. 2nd edition. Philadelphia: *WB Saunders*; pp. 493–509.
- Dubey, J.P. (2004). Toxoplasmosis – a waterborne zoonosis. *Veterinary Parasitology*, **126**: 57–72.
- Dubey, J.P. (2010). Toxoplasmosis of Animals and Humans. 2nd edition. Boca Raton, Florida: *CRC Press*; pp.313.
- Feldman, H.A. (1982). Epidemiology of *Toxoplasma* infection. *Epidemiology Review*, **4**: 204-13.
- Giannoulis, C., Zournatzi, B., Giomisi, A., Diza, E. and Tzafettas, I. (2008). Toxoplasmosis during pregnancy: a case report and review of literature. *Hippokratia*, **12**(3):139–143.
- Halos, L., Thebault, A., Aubert, D., Thomas, M., Perret, C., Geers, R., Alliot, A., Escotte-Binet, S., Ajzenberg, D., Darde, M.L., Durand, B., Boireau, P. and Villena, I. (2010). An innovative survey underlining the significant level of contamination by *Toxoplasma gondii* of ovine meat consumed in France. *International Journal for Parasitology*, **40**: 193–200.
- Hill, D. and Dubey, J.P. (2002). *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clinical Microbiology and Infectious Diseases*, **8**: 634–640.
- Hossain, B. (2016). Serodiagnosis of toxoplasmosis in human and animals using lateral flow chromatographic assay. *MS Thesis*, Department of Parasitology, BAU, Bangladesh.
- Innes, E.A. (2010). A Brief History and Overview of *Toxoplasma gondii*. *Zoonoses and Public Health*, **57**: 1–7.
- Jafar, Y.Y., Nabi, J., Sameri, M.J. and Noorshahi, N. (2014). Seroprevalence of Anti-*Toxoplasma gondii* Antibodies Among pregnant woman in South Khuzestan, Iran. *Jundishapur Journal of Microbiology*, **7**(5): e9998.
- Jaiswal, S., Pokhrel, T., Sharma, S., Ranabhat, S.R., Yadav, D.K. and Koirala, B. (2014). Seropositivity Rates of Toxoplasmosis and Syphilis in Pregnant Women Visiting Western Regional Hospital, Nepal. *International Journal of*

Health Sciences & Research, **230**(4): 9230-42.

- Lake, W.C., Khachaeramb, W., Koizumic, S. and Maruyama, S. (2002). Seroprevalence of *T. gondii* infection in domestic goats in Satun Province, Thailand. *Veterinary Parasitology*, **127**: 17–22.
- Lamaj, S., Dharmo, G. and Dova, I. (2016). Epidemiology of *Toxoplasma gondii* infection of cats in southwest of Albania. *Journal of International Academic Research for Multidisciplinary*, **4**(2): 76-81.
- Margia, U.H. (2015). Seroprevalence of toxoplasmosis in women of Mymensingh and Rangpur districts. *MS Thesis*, Department of Parasitology, BAU, Bangladesh.
- Montoya, J. and Liesenfeld, O. (2004). Toxoplasmosis. *Lancet*, **363**: 1965-1976.
- Nunes, A.C.B.T., Silva, E.M.V., Oliveira, J.A., Yamasaki, E.M., Kim, P.C.P., Almeida, J.C., Nunes, K.B. and Mota, R.A. (2015). Application of different techniques to detect *Toxoplasma gondii* in slaughtered sheep for human consumption. *Brazil Journal of Veterinary Parasitology, Jaboticabal*, **24**(4): 416-421.
- Pal, M. (2007). Zoonoses. 2nd edition. *Satyam Publishers*, Jaipur, India.
- Pereira, K.S., Franco, R.M. and Leal, D.A. (2010). Transmission of toxoplasmosis (*Toxoplasma gondii*) by foods. *Advances in Food and Nutrition Research*, **60**: 1–19.
- Rai, S.K., Shibata, H., Sumi, K., Kubota, K., Hirai, K., Matsuoka, A., Kubo, T.T., Basnet, S.R., Shrestha, H.G. and Mahajan, R.C. (1994). Seroepidemiological study of toxoplasmosis in two different geographical areas in Nepal. *Southeast Asian Journal Tropical Medical Public Health*, **25**(3): 479-84.
- Rai, S.K., Yano, K. and Kubo, T.T. (1993). Immuno-serological study of human toxoplasmosis in Nepal. Proceeding Third Congress Asia Pacific Societies of Pathologists and Eleventh National Convention, *Bangladesh Society of Pathologists*, pp. 98.
- Ramzan, M., Akhtar, M., Muhammad, F., Hussain, I., Hiszczynska Sawicka, E., Haq, A.U., Mahmood, M.S. and Hafeez, M.A. (2009). Seroprevalence of *Toxoplasma gondii* in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. *Tropical Animal Health and Production*, **41**: 1225–1229.
- Remington, J.S., McLeod, R., Thulliez, P. and Desmonts, G. (2001). Toxoplasmosis. *Infectious diseases of the fetus and newborn infant*, 5th ed. W. B. Saunders,

Philadelphia, pp. 205-346.

- Sah, R.P., Talukder, M.H. and Hossain, M.B. (2016). Toxoplasmosis in cats and its zoonotic potential at Bangladesh Agricultural University campus. *3rd International Exhibition on Dairy, Aqua and Pet (IEDAP)* held at BICC Dhaka, Bangladesh, pp. 300-303.
- Samad, M.A., Dey, B.C., Chowdhury, N.S. and Akhtar, S. (1997). Sero-epidemiological studies on *Toxoplasma gondii* infection in man and animals in Bangladesh. *Southeast Asian Journal of Tropical Medicine and Public Health*, **28**(2): 339-43.
- Samad, M.A., Rahman, K.B. and Halder, A.K. (1993). Seroprevalence of *Toxoplasma gondii* in domestic ruminants in Bangladesh. *Veterinary Parasitology*, **47**(1-2): 157-9.
- Suzuki, H., Yamamoto, Y. and Matsumoto, K. (1987). A serological study of *Toxoplasma* infection in Nagasaki by ELISA. *Japanese Journal of Parasitology*, **36**: 118-24.
- Tizard, I.R., Chauhan, S.S. and Lai, C.H. (1977). The prevalence and epidemiology of toxoplasmosis in Ontario. *Journal of Hygiene Cambodia*, **78**:275-82.
- Upadhyay, M.P., Rai, S.K., Bhandari, R.K. and Bista, P. (1989). Serological study of *Toxoplasma gondii* infection in Kathmandu. Nepal. *Journal of Institute Medicine*, **2**: 211-5.
- Zewdu, E.G., Hailu, A., Tessema, T.S., Desta, K.T., Medhin, G., Vitale, M., Di Marco, V., Cox, E. and Dorny, P. (2013). Seroepidemiology of *Toxoplasma gondii* infection in women of child-bearing age in central Ethiopia. *BMC Infectious Diseases*, **13**: 101.

Survey on Good Hygiene Practices in Retail Meat Shops in Butwal Municipality, Nepal

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ABSTRACT

Present study was conducted to assess good hygiene practices in retail meat shops for safe and wholesome meat production as well to understand different roles performed by delegated institutions in ensuring quality meat production. A descriptive survey design was used to answer questions concerning the current status of meat hygiene and sanitation practices in the retail meat shops in Butwal municipality. Meat handlers from the meat shops were interviewed through a structured questionnaire. A total of 190 retail meat shops were analyzed randomly to assess their meat hygiene knowledge. It was found that 93.68% of the meat handlers had no regular health check up, 38.42% of the butchers didn't use protective clothes, 95.26% of meat shops had no evisceration facility, 77.37% of meat shops disposed their waste materials in open space nearby, 96.84% lack lirage facilities and 99.47% did not follow ante-mortem and post-mortem inspection. There was a knowledge gap about Slaughterhouse and Meat Inspection Act 1999 within 96.32% of the meat handlers. The study recommends implementation of Slaughterhouse and Meat Inspection Act 1999 which has been approved in 1999 by the GoN to promote hygiene practices and animal welfare thereby improving the quality standard of meat shops and finally the quality of meat.

Keywords: Food borne diseases, Hygiene practices, Meat and meat products, Retail meat shop, Slaughterhouse and Meat Inspection Act (SIMA) 1999, Zoonosis.

INTRODUCTION

Food borne diseases are common in developing countries because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipments, and lack of education for food-handlers (WHO, 2004). Bacteriological quality of meat and meat products is strongly influenced by the prevailing hygienic conditions during their production and handling. Food borne illnesses are prevalent in all parts of the

world and the toll in terms of human life and suffering is enormous. According to WHO, contaminated food contributes to 1.5 billion cases of diarrhea in children each year, resulting in more than three million premature deaths (WHO, 1999). Those deaths and illnesses are shared by both developed and developing nations.

In the absence of modern slaughterhouses in Nepal, animal slaughtering was common in streets, riversides and open pasturelands (Joshi, 2003). In 1999, the government legislated “Slaughterhouse and Meat Inspection Act 1999” which has been approved in by the then GoN to make meat inspection scientific and to ensure the production of safe and hygienic meat to safeguard the health of the consumer. But it’s unfortunate that till today, the act has failed to be implemented (The Himalayan Times, 17 November 2009). Some of the literatures claim that Slaughterhouse and Meat Inspection Act has not been enforced to date (TLDP, 1999; Joshi *et al.*, 2003; Pant, 2007; Parajuli, 2007; Joshi, 2009).

The major retail outlets of meat in Nepal are the butcher’s shops. Butchers slaughter goats and poultry in their premises with poor hygienic conditions (Upadhyaya, 2012). The meat available at retail outlets comes through a long chain of slaughtering and transportation, where each step may pose a risk of microbial contamination. The sanitary conditions of abattoirs and its surrounding environment are major factors contributing to bacterial contamination of meat.

Butwal municipality inaugurated to build the slaughterhouse to ensure the supply of safe, wholesome meat and meat products to consumers but is not yet operating as it is not completed. There are very few studies on standards of meat shops and their hygienic conditions in Nepal and there is a need to explore existing understanding and knowledge regarding importance of good hygienic practice of retail meat shops. So, this study was conducted to reveal hygienic status of meat shops and to add up in the existing knowledge and further, help in implementation of effective good hygiene practice in meat shops.

MATERIALS AND METHODS

Sample selection

A total of 190 retail meat shops were selected randomly from 376 meat shops in Butwal municipality for this study.

Data collection

Data was collected from primary and secondary source.

Primary data collection

The primary data was collected through, key informant interview, questionnaire survey, formal and informal discussion, direct observations.

Key informant interview

To develop further idea of the study site, informal discussion and interview with key informant was done, workers and other knowledgeable persons were taken as the key informants. The interview was focused on the hygiene practice in shop during the process, impacts of unhygienic meat in consumers and its possible health risks measures.

Questionnaire survey

Among the 376 retail meat shops, 190 butcher shops were randomly selected (which accounts for about 50.53% of the total size). The method of survey was adopted from Czaja & Blair, 1996 where the interviewer introduced him/herself to the respondent and explained the purpose of the questionnaire and assured the respondent that the information would be handled confidentially before commencing with the questions. The interviewer also ensured that the respondent understood the objectives and importance of the study.

The questionnaire survey was focused on the personal and general hygiene practices, type of shop, storage of meat, hygienic practices during handling, sources of water used, slaughtering and marketing practices, cleaning and disinfection of equipment and premises as well as knowledge about Slaughterhouse and Meat Inspection Act (SIMA).

Statistical analysis

Analysis was done by carrying out different statistical procedure for quantitative analysis of data as per required. The data collected was tabulated and analyzed statistically by using Statistical Package for Social Science (SPSS) and Microsoft-Excel.

RESULTS

Results and discussion of the Questionnaire survey in Butwal municipality are presented below. A total of 190 retail meat shops out of 376 were analyzed on random basis.

Status of registered meat shops

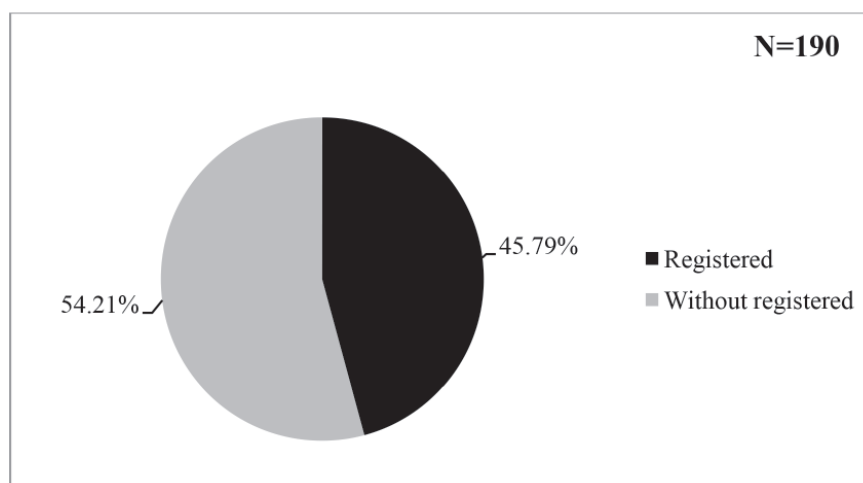


Fig. 1: Status of registered meat shops

From the study, 45.79% meat shops were found to be registered in the municipality. Regulation of meat shops, slaughterhouse is primarily the function of municipal bodies in urban areas. It was found 93.16% responded that there was no effective monitoring system of meat shops (Figure 1).

Educational status of meat handlers and training of meat handlers on meat handling practice and meat hygiene

Table 1 summarizes 70% of the butchers did not have formal education and 17.55% of the meat handlers indicated that they had received training in meat handling practice and meat hygiene while the remaining 82.45% of the meat handlers did not take training regarding meat handling practice and meat hygiene.

Table 1: Information regarding educational status of meat handlers and training of meat handlers on meat handling practice and meat hygiene

Characteristics		Percentage (%)
Educational status (n=190)	Formal education	30%
	No formal education	70%
Meat handlers who receive training (n= 190)	yes	17.55%
	No	82.45%
Frequency of training, if yes	Once a year	17.02%
	Others	0.53%

Practice regarding the hygienic status of the meat shop workers

On the survey on hygienic practice, 38.42% of meat shops personnel never used protective clothes whereas 61.58% of meat handlers were using personal protecting equipment (PPE) in the form of apron and all of them handled meat with bare hands without covering their hair with hair nets (Table 2).

Table 2: Practice regarding hygienic status of the meat shop workers

Characteristics		Percentage (%)
Wearing Protective cloths	Yes	61.58%
	No	38.42%
Cutting instrument	Stainless steel	1.05%
	Iron	98.95%
Jewellery materials	Not worn	15.79%
	Worn	84.21%
Handling money	Self (with bare hands)	96.84%
	Cashier	3.16%
Eating habit during handling meat	Yes	60.53%
	No	39.47%

Table 3: Practice regarding slaughtering facility and meat storage in meat shops.

Characteristics		Percentage (%)
Species available	Single	92.63%
	More than one	7.37%
Lairage facility	Yes	3.16%
	No	96.84%
Evisceration facility	Yes	4.74%
	No	95.26%
Hot and cold water available	Yes	94.74%
	No	5.26%
Leftover meat	Store in refrigerator	78.95%
	Not store	21.05%
Covering off meat	Yes	71.58%
	No	28.42%
cleaning covering cloth, if yes	Once daily	70.53%
	Twice daily	1.05%

Table 4: Practice regarding hygienic status of meat shop environment

Characteristics		Percentage (%)
Control of fly and rodents	Yes	50%
	No	50%
Disposal of waste	Open space nearby	77.37%
	Municipal sewer	22.11%
	Soak pit	0.53%

Table 5: Practices regarding cleaning and disinfection process

Characteristics		Percentage (%)
Ceilings periodically cleaned	Yes	2.63%
	No	97.37%
Surfaces of wall, partitions and floor	Impervious	3.16%
	Non impervious	98.84%
Means of disinfection	Only with water	79.47%
	water and detergent	20.53%

Main sources of water supply

From the survey, it was found that 83.68% shops were connected to the municipality water supply which is the main supplier of the water control system while 16.32% used water from tube wells (Figure 2).

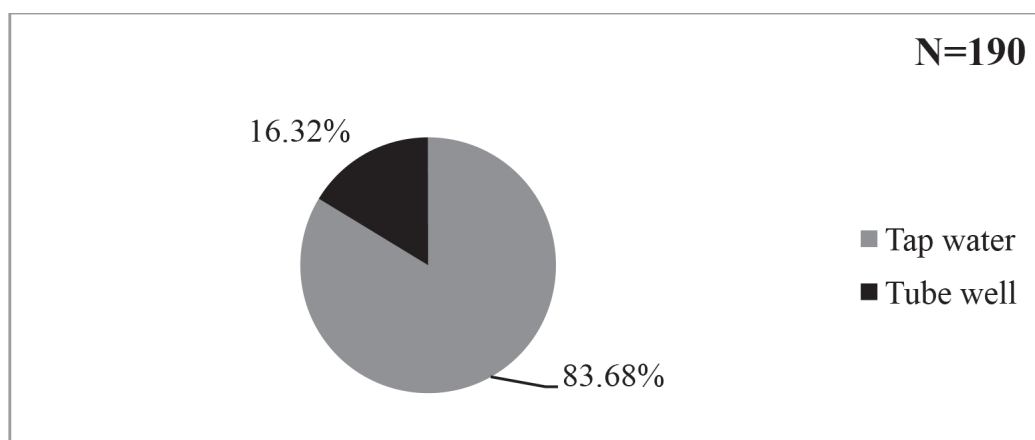


Fig. 2: Main sources of water supply

Practice regarding slaughtering facility and meat storage in meat shops

According to the survey, 92.63% of meat shops sold meat of single species, 3.16% of meat shops had a lairage facility and 4.74% of meat shops had evisceration facility. It was found 94.74 % of meat shop had hot or cold water as required, 78.95% of meat shops had a refrigerator for the storage of all leftover meat but the rest 21.05% had no such provision and 71.58% of meat shops were found covering the meat with red cloths and among them 70.53% cleaned the red cloth once daily (Table 3).

Practices regarding reporting of illness and routine medical examination

Figure 3 summarizes the practice of meat handlers regarding disease reporting. 16.32% of the meat handlers had the habit of reporting illness. There was no legal procedure which enforces the meat handlers for reporting illness. Only 6.32% of the meat handlers indicated that they went for routine medical examinations.

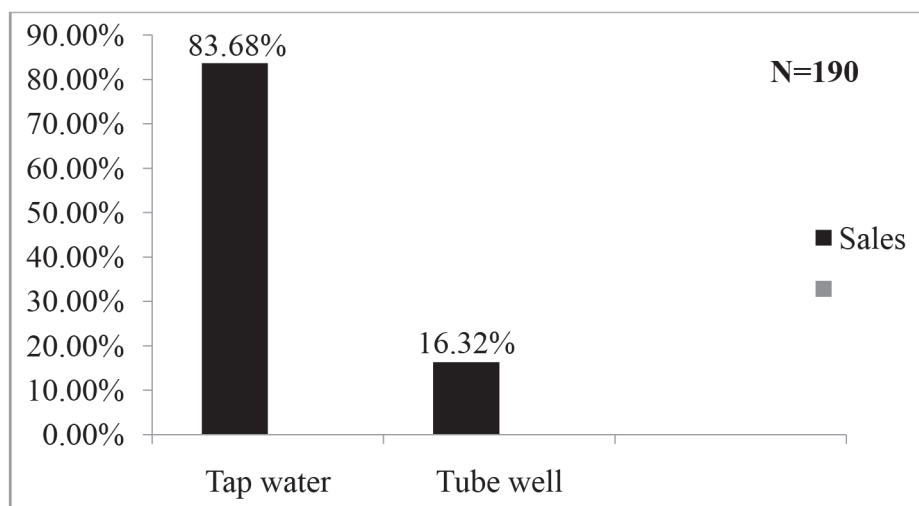


Fig. 3: Reporting illness

Practices regarding washing hands

Upon asking the respondents, 98.42% of meat handlers never washed their hands after handling suspected meat and those who washed their hands used only water and soap.

Practice regarding the hygienic status of the meat shop environment

It was found 50% of meat shops were not protected against dogs, rodents and insects. Further observation showed that there was no proper waste disposal system as a result, the pile up paunch contents and other solid wastes, faeces, horns, scraps of

tissue and other solid wastes were found near the meat shops which may serve as the residence for rodents, cats, and dogs. Only 22.11% of the meat shops responded that there was municipal system for the disposal of waste materials (Table 4).

Practices regarding cleaning and disinfection process

In this study, 97.37% of meat handlers indicated that they never cleaned or disinfected the wall surfaces, ceilings, ventilation while 2.63% indicated that the wall surfaces were being cleaned and disinfected periodically. Upon questioning the meat handlers regarding the procedures of cleaning and disinfection, 20.53% of them indicated that water and detergent were used to clean and disinfect the surfaces while 79.47% of meat handlers indicated that they use only water to disinfect the surfaces (Table 5).

Meat handler's knowledge about SIMA (1999)

Ante-mortem (AM) inspection was altogether absent. The respondents (the butcher shop owners and workers) were asked if they knew the provision in SIMA (1999). It was found that only 3.68% were well known about SIMA (Figure 5).

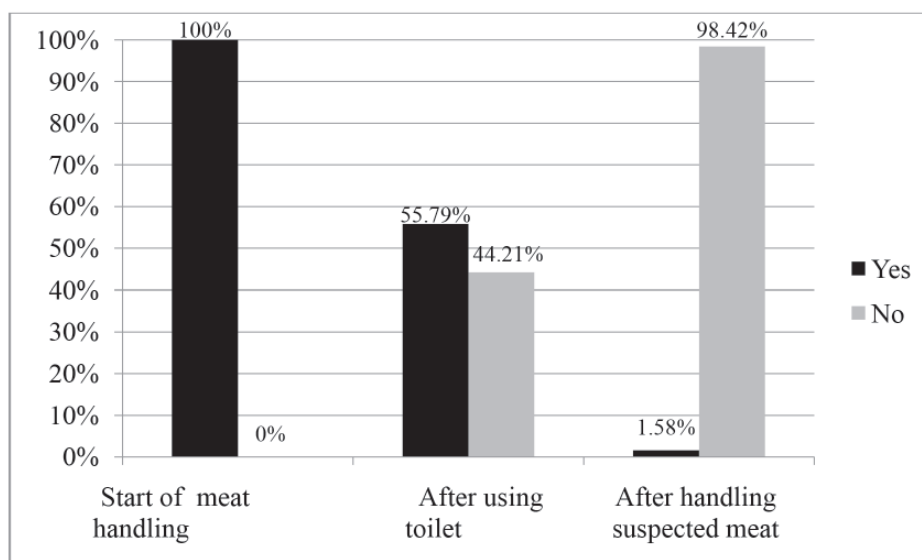


Fig. 4: Practices regarding washing hands

DISCUSSION

The study was carried out to assess the safety knowledge and practices in handling of meat. Personal and meat shop hygiene, waste disposal system, training and hygienic regulation of the meat shops were included in the study. In the current study, 17.02% of the meat handlers received training once a year from the municipality. This finding

has raised the key issue of illiteracy. If more educated people start these industries, then there is likeliness of adoption of safety measures and hygienic practices. Training and education of food handlers regarding the basic concepts and requirements of personal hygiene plays an integral part in ensuring a safe product to the consumer (Adams & Moss, 1997). To ensure this, there should be some form of induction training with regular updating and refresher courses for the food handlers. Meat handlers should furthermore understand the risks associated with contamination of food by microbiological hazards.

The purpose of wearing overalls is to protect both the food products and the meat handler from cross contamination. However, this study showed that 38.42% of the meat handlers did not wear aprons and they all handled food with their bare hands. Because meat handlers are probable sources of contamination for microorganisms, it is important that all possible measures should be taken to reduce or eliminate such contaminations. Hands are rarely free from micro-organisms (especially the bacteria *Staphylococcus aureus* that are present on the skin, nose and hair), it is of utmost importance that soap (preferably in a dispenser) and hot running water are used for this purpose, thus aiming to reduce the microbiological load on hands (Desmarchelier, Higgs, Mills, Sullivan, & Vanderlinde, 1999). To ensure that the meat handlers wash their hands with hot water and soap, Van Zyl (1998) suggested that soap and hot water, at 45°C, should always be available at the washing-basins. Poor personal hygiene practices such as negligence to wash hands after visiting the bathroom may result in up to 10^7 pathogens under the fingernails of the food handler. Organisms originating from infected food handlers include *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and faecal *streptococci* (Lawrie, 1998). However, in this study, 55.79% of meat handlers were found to wash their hands atleast after using toilet (Figure 4).

The aim of meat inspection is to provide safe and wholesome meat for human consumption (Herendaet *al.*, 2000). AM and meat inspection is concerned with three main areas: public health, animal health and animal welfare but 99.47% of meat shops in this study were found not to follow any practice of examination of live animals before and after slaughter. And, only 0.53% of meat shops were found to practice inspection at least after slaughter. In general, the level of understanding of meat hygiene/ meat inspection is very low (Figure 5).

The AM examination of animals represents 50% of meat inspection and it improves meat quality by making post-mortem (PM) examination more efficient (Kotwal & Agrawal, 2007). This is due to the fact that the judgment of a food animal whether it is fit for slaughter or not is initially made on the basis of AM findings. The

provision of a veterinary inspection of the live animals prior to slaughter is a basic legal requirement of most meat inspection system (Gracey *et al.*, 1999) and lack of practice of AM inspection therefore implies that SMIA (1999) is not enforced. Once a food animal qualifies the requisites of AM inspection i.e. the animal is clinically healthy, it is passed for slaughter; and the subsequent examination of the internal organs of the butchered animals is carried out to rule out occurrence of any disease or the condition. PM refers to any procedure or test conducted by a competent person on all relevant parts of slaughtered animals for the purpose of judgment of safety, suitability and disposition (Zade & Khan, 2007) and this judgment declares that the carcass is either fit for human consumption or it is conditionally held or condemn is made.

In the study area, it was found that proper garbage collection and disposal were lacking, and 77.37% vendors disposed garbage in an open place. Hygiene problems are not limited to meat shops but also associated with incorrect processing and marketing practices. According to the results of this study, 96.84% of the meat shop workers handled money while serving meat. Since, money is full of microbes, it can contaminate the food. Handling of foods with bare hands may also result in cross contamination; hence introduce microbes on safe food.

This study showed that 60.53% of meat handlers practiced smoking, eating or drinking while handling meat. Furthermore, 84.21% of the meat handlers were found wearing jewellery during meat handling (Table 5). Jewellery is a potential source of micro-organisms, because the skin under the jewellery provides a favourable habitat for contaminating microorganisms to proliferate (Trickett, 1997). Meat handlers are probable sources of contamination for microorganisms, it is important that all possible measures should be taken to reduce or eliminate such contamination.

CONCLUSIONS

Retail meat shops were found with a very poor hygiene level, lack of basic hygiene components such as waste disposal system, proper plant design and provision of facilities: like toilets, hand washing basins, which shows the high potential of cross contamination of meat. Under these conditions, the meat handlers as well as the consumers are equally at high risk of zoonotic diseases. Government and the concern authorities have to take initiative to the implementation of existing Slaughterhouse and Meat Inspection Act 1999 and training courses must be conducted for butchers and meat sellers to promote hygiene practices and animal welfare thereby improving the quality standard of meat shops and finally the quality of meat.

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REFERENCES

- Adams, M.R. and Moss, M.O. (1997). *Food microbiology*. Cambridge: The Royal Society of Chemistry.
- Czaja, R. and Blair, J. (1996). *Designing surveys: A guide to decision and procedures*, Pine Forge Press, Thousand Oaks, California, USA.
- Desmarchelier, P.M., Higgs, G.M., Mills, L., Sullivan, A.M. and Vanderlinde, P.B. (1999). Incidence of coagulase positive *Staphylococcus* on beef carcasses in three Australian abattoirs, *International Journal of Food Microbiology*, **47**: 221-229.
- Graceyet, J., Collins, D.S. and Huey, R. (1999). *Meat Hygiene*, 10th edition, London, Harcourt Brace and Company Limited.
- Henson, S. (2003). The Economics of food safety in Developing Countries, *ESA working Paper*, www.fao.org/es/esa. Assessed on 15/06/2013.
- Herendact, D., Chamberd, P.G., Ettriqui, Seneviratna, P. and DaSolva, T.J.P. (2000). *Manual on meat inspection for developing countries*, FAO, Rome, Italy, pp.193-195. <http://epaper.thehimalayantimes.com/HT/HT/2009/11/17/index.shtmltoforce>. Assessed on 15/06/2013.
- Joshi, D.D. (2009). Current Situation and Management of Animal Slaughterhouse and Meat Marketing (Nepali Version), *Souvenir on Annual General Meeting and World Veterinary Day*, 24-25 April. Nepal Veterinary Association, Kathmandu, Nepal, pp.16-21.
- Joshi, D.D., Maharjan, M., Johansen, M.V., Willingham, A.L., Sharma, M. (2003). Improving Meat Inspection and Control in Resource-poor Communities: the Nepalese Example, *ActaTropica*, **87**: 119-127.
- Kotwal, S.K. and Agrawal, R. (2007). Food Animals. In V. Singh, ed. *Universal Meat Hygiene in Public Health Care*, Lucknow, International Book Distributing

Co.; pp.105 – 159.

- Lawrie, R.A. (1998). *Lawrie's meat science*, 6th edition, Cambridge, Woodhead Publishing Limited.
- Pant, K.P. (2007). Impacts of the Regulatory Measures on the Farming Structure, Particularly on Small and Marginal Farms, *Paper presented in a Workshop on Policies for Bio-Secure Agriculture*, Dhulikhel, Nepal, pp.1-54.
- Parajuli, B.P. (2007). Present Meat Marketing Practices in Nepal, *Proceedings of the Meat Quality Food Safety, Safe Drinking Water Supply, Housing and Sewerage Plan Training Workshop*, **23**: 24 – 29.
- TLDP, (1999). *Marketing of Meat and Meat Products*, Third Livestock Development Project, JICA Publication.
- Trickett, J. (1997). *Food hygiene for food handlers*, London, Macmillan Press.
- Upadhyaya, M., Poosaran, N. and Fries, R. (2012). Prevalence and Predictors of *Salmonella spp.* in Retail Meat Shops in Kathmandu, *Journal of Agricultural Science & technology*, **2**: 1094.
- Van Zyl, A.P. (1998). *Red meat manual for veterinary public health*, Pretoria, Directorate Veterinary Public Health.
- WHO (1999). Food Safety Programme “Food Safety – An Essential Public Health Issue for the New Millenium”, 1999, (WHO/SDE/PHE/FOS/99.4), [hereafter “Food Safety An Essential Public Health Issue for the New Millenium”], at p. 7.
- WHO (2004). Regional Office for Africa “*Developing and Maintaining Food Safety Control Systems for Africa Current Status and Prospects for Change*”, Second FAO/WHO Global Forum of Food Safety Regulators, Bangkok, Thailand, pp. 12-14.
- Zade, N.N. and Khan, W.A. (2007). Meat Inspection: General Principles and Objectives of Meat Hygiene, *Universal Meat Hygiene in Public Health Care*, Lucknow, International Book Distributing Co. Ch. 3 (A)

Using Mapping, Global Positioning System (GPS) and Smart Phone Technology in Rabies Control Program in Kotagiri, Nilgiris District, India

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ABSTRACT

In this study, we utilized a catch-vaccinate-release approach for dogs in a canine rabies vaccination program in Kotagiri municipality, India. Following vaccination, surveys on dog population and their vaccination status was undertaken. A bespoke smartphone 'Mission Rabies' application was developed to facilitate data entry and team management. This global positioning system (GPS) enabled application captured the location of all vaccinated dogs and dogs sighted on post-vaccination surveys. In areas where coverage was below 70%, catching teams were re-deployed to vaccinate additional dogs followed by repeat survey. Out of 248 dogs captured, only 210 dogs were vaccinated because of the previous vaccination history within a year, and refusal of owner due to their misconceptions. In survey, 147 dogs were sighted of which 77 were seen marked with paints indicating a vaccination coverage of 52.4%. The total estimated dog population was found to be 475. Our study demonstrated that mobile technology enabled efficient team management and real-time data entry and analysis. The vaccination approach outlined in this study can serve as a guideline for rapid vaccination of large numbers of dogs with a high coverage rate in free roaming dog populations in India in the future.

Keywords: Rabies, Vaccination, Mark, Capture, Dog, Population

INTRODUCTION

Rabies is one of the deadliest zoonotic diseases responsible for more than 59,000 human deaths annually across the world (Fooks *et al.*, 2014, Hampson *et al.*, 2015). It is also the most lethal infectious disease with a case fatality rate of 100% even with advanced medical intervention (Fooks *et al.*, 2014). Rabies is a viral neurological disease affecting all mammals, including human beings. Canine rabies has been reported as one of the neglected diseases in the developing world (Lankester F *et*

al., 2014). The annual global cost of rabies has been estimated to be 8.6 billion US dollars and accounts for over 3.7 million disability-adjusted life years (Hampson K *et al.*, 2015).

In many developing countries, dogs are allowed to roam freely and are the principal reservoir for the disease, with almost all human cases of rabies contracted from bites of an infected dog (WHO, 2013). However, this disease is preventable. Mass vaccination of the dog population has been shown to be effective in eliminating this disease from many countries (Hampson *et al.*, 2015, Vigilato *et al.*, 2013 and Wells, 1954). This has led to the broad belief that the global elimination of canine transmitted rabies is possible through mass dog vaccination (Hampson *et al.*, 2009, Lembo *et al.*, 2010 and Morters *et al.*, 2014). Despite the feasibility of eliminating both canine and human rabies through widespread canine vaccination programmes, there is still limited investment for large scale dog vaccinations in endemic areas of many African and Asian countries (Hampson *et al.*, 2015).

India alone accounts for over 35% of the global rabies burden with over 20,000 annual deaths. Despite the need to develop and undertake mass canine vaccination programmes in India, there are few published reports of successful implementation of large-scale vaccination programmes (Kakkar *et al.*, 2012, Shahid *et al.*, 2015). There is a dearth of research relating to the Indian free-roaming dog population and practical implementation of effective mass canine vaccination on a scale that could be broadened to a state-wide or even national level (Belsare *et al.*, 2013, Davlin *et al.*, 2013 and Abbas *et al.*, 2014). A major challenge to the eradication of rabies in India is ensuring epidemiologically required vaccination coverage to break the Rabies transmission cycle in the dog population. There is a broad consensus that over 70% vaccination coverage in an area is sufficient to break the transmission cycle and reduce the incidence of rabies in dog and human populations (WHO, 2013).

Central point vaccination campaigns have been effective at accessing a large enough proportion of the dog population and make an impact on canine and human rabies incidence in parts of Africa (Jibat *et al.*, 2015). However, these approaches have been ineffective in reaching a high proportion in Indian dog population where the majority of dogs are free roaming (Belsare *et al.*, 2013). To progress towards the eradication of canine transmitted rabies in India, it is essential that effective field protocols are developed which facilitate mass canine vaccination with sufficiently high vaccination coverage to break the Rabies transmission cycle. Firstly, there is a clear need to develop field strategies which allow vaccination of large numbers of free roaming dogs in urban areas. Secondly, there is a need for improved methodologies which can rapidly assess whether the vaccination coverage achieved is high enough to result in widespread protective immunity within the dog population.

This study describes the development and implementation of a mass vaccination programme in Kotagiri, India using mapping techniques and smart phone technology

MATERIALS AND METHODS

Study area

Kotagiri (11.43°N, 76.88°E) is a panchayat town in the Nilgiris district in the Indian state of Tamil Nadu (Fig. 1).

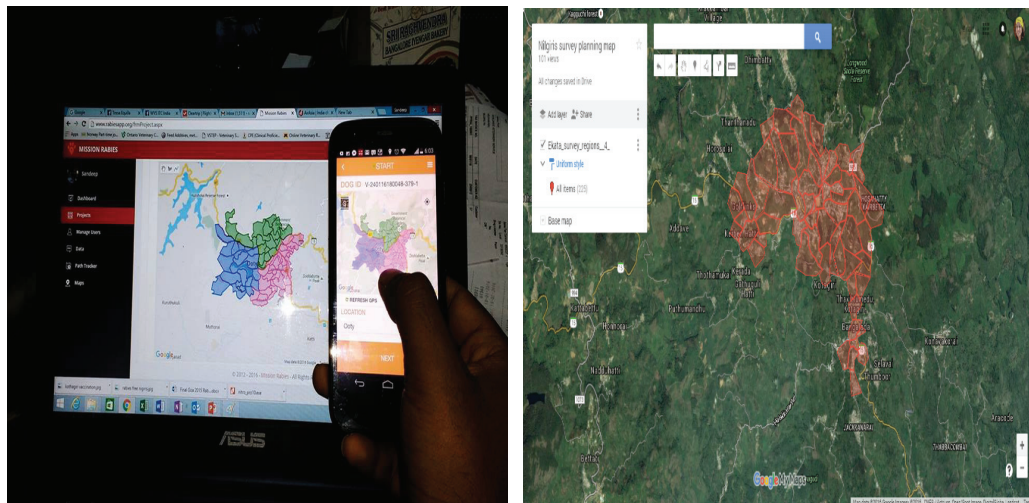


Fig. 1: Google map showing study area (left) and mobile application connected to server (right)

Study design

This study has two components: Dog vaccination and Survey. On the first day, the dogs were vaccinated and marked and followed up in the 2nd day. The survey was conducted to record the marked and unmarked dogs.

Study population

Dogs included in the study were those vaccinated, marked and released (VMR) by roaming vaccination teams and those sterilized as a part of the catch-neuter-vaccinate-return (CNVR) programme which ran in parallel to the rotating vaccination work. The study period was from 5th December 2014 to 16th April 2015.

Mission rabies application

A bespoke 'Mission Rabies' App was developed which enabled information about each dog vaccinated to be recorded on a smart phone on a real-time basis. This information was then synchronized via internet to a web-based server (Fig. 2). The

dataset for each dog vaccinated included GPS location, manually entered ward number, action taken (vaccinated, marked and released/vaccinated and released but not marked/previously vaccinated within one year, marked/taken to clinic), sex (male/female), ownership status (presented by owner/free roaming), approximate age (<3 months, >3 months), neuter status defined by presence/absence of an ear notch routinely performed at the time of surgery (neutered/not-neutered), body condition score (BCS: emaciated (1), underweight (2), healthy weight (3), obese (4), presence of alopecia (four point score of alopecia affecting a percentage of total body surface area; normal (no hair loss), mild (<20 % hair loss), moderate (20–80 % hair loss), severe (>80 % hair loss)), other disease (transmissible venereal tumour, wounds, lameness, other). Road boundaries were displayed on the app to enable teams to navigate through the ward and stay within boundaries. Three Samsung Galaxy Core 2 phones were used, one with each vaccination team and one with the surveyor.

Vaccinate-assess-move protocol

Once restrained dogs were vaccinated intramuscularly or subcutaneously (Nobivac® Rabies, MSD Animal Health), they were marked and released. Non-toxic paint along the top and back of the head was used for marking to allow for identification on post-vaccination surveys and prevent repeated vaccination.

Post-vaccination survey protocol

Following completion of vaccinations, a surveyor travelled around the ward by motorbike navigating using the smart phone map to cover every street within the municipality boundaries. Surveys were conducted in the morning between first light and 11 am and in the late afternoon between 3 pm and dusk. Only free roaming dogs were recorded, therefore dogs tied or confined to private property were not recorded. Each dog sighted was recorded in a 'Survey form' on the 'Mission Rabies' app. The data was then synchronized to the central server via internet as described above. A 'path tracker' function was incorporated into the app to record the path travelled during the survey.

Data analysis

Data summaries and maps can be viewed in real time on the 'Mission Rabies' app backend. Calculations of vaccination coverage were undertaken in Excel 2013 (Microsoft Inc., Redmond, WA). For more detailed analysis, both vaccination and survey datasets were downloaded from the server as CSV files. Road boundaries were imported into ArcGIS Desktop 10.3 and dog sighting (survey) and vaccination locations were labelled with the road according to GPS location. Data was then exported into Excel 2013 for cleaning and analysis. Maps were prepared for presentation in

QGIS Desktop 2.6.1 (QGIS development team, Open Source Geospatial Foundation Project).

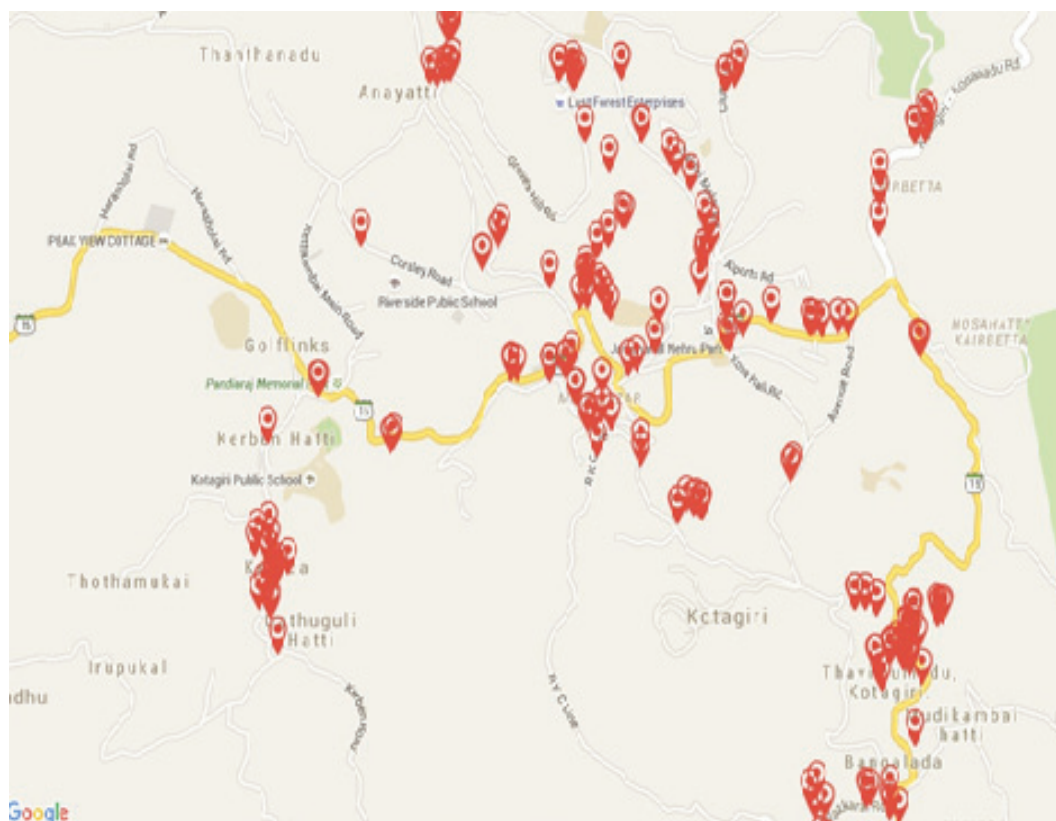


Fig. 2: Real time data and location data of vaccinated dog

RESULTS

Out of 248 dogs captured or brought by owner, 210 dogs were vaccinated as some dogs were already vaccinated within 1 year and some owners refused to vaccinate. In survey, 147 dogs were sighted of which 77 were observed marked with paints. This indicated a vaccination coverage of 52.4% (Fig. 4). The total dog population observed during the vaccination programme and survey was 475. Majority of dogs were found to be free roaming (58.8%) within vaccinate, mark and release (VMR) of which 51.4 % were male. The neuter status of free roaming and owned dog are shown in Table 1. 23.80% of the free roaming VMR population were neutered. The male-biased gender ratio is 1.74:1. Adults comprised the majority of population (87.8%) while puppy represented 12.9%. Adult and puppy vaccination with location data, vaccination coverage with location data and sterilization percentage of male and female stray dog are shown in Fig. 3, Fig. 4 and Fig. 5 respectively.

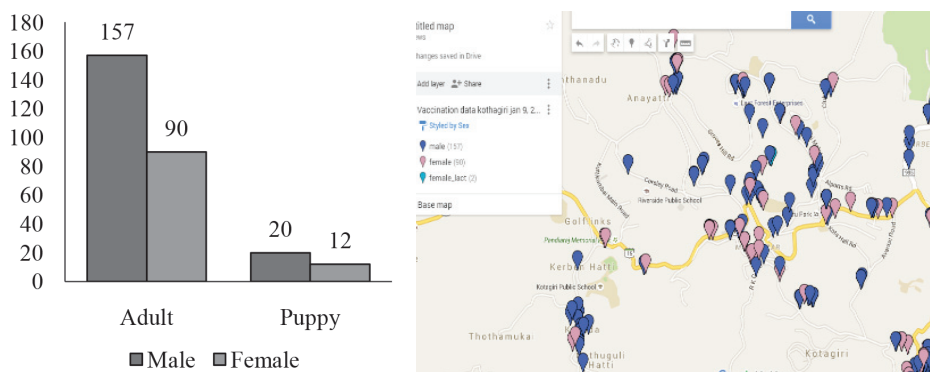


Fig. 3: Adult and puppy vaccination with location data

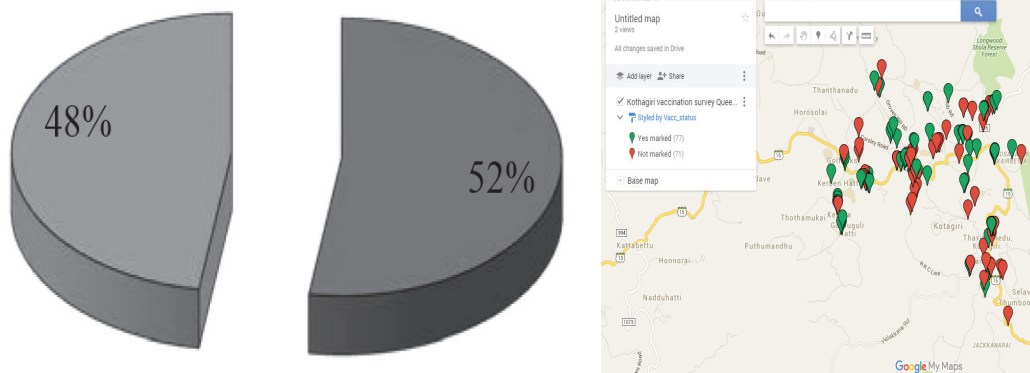


Fig. 4: Vaccination coverage with location data

Out of 147 dogs sighted, 77 were vaccinated.

Table 1: Neuter status within VMR population for owned and free roaming dog with location data

Neuter status	Free Roaming	Presented by owner	Total
Entire	69	120	189
Neutered	46	13	59
Unknown	1	-	1
Total	115	133	249

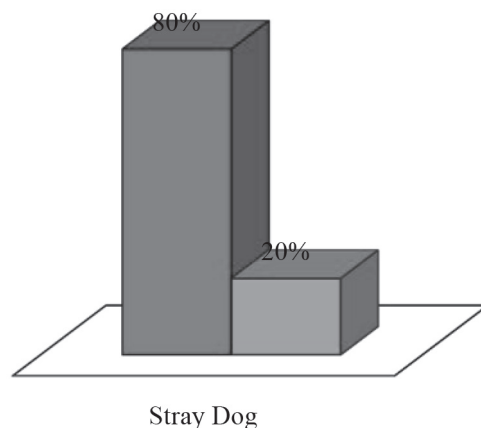


Fig. 5: Sterilization percentage of male and female stray dog

DISCUSSION

India has the highest number of human deaths from rabies globally (Hampson, *et al.*, 2015, Knobel *et al.*, 2005). The canine rabies vaccination coverage of more than 70 % dogs has been well documented in numerous countries to significantly reduce the incidence of rabies in both human and canine populations (Jibat *et al.*, 2015, Cleaveland *et al.*, 2003 and Kayali *et al.*, 2003). Despite the pressing need for effective canine rabies vaccination programmes in India owing to highest rabies burden, there have been few publications describing canine rabies vaccination strategies in the country (Kakkar *et al.*, 2012, Shahid *et al.*, 2013 and Davlin *et al.*, 2012). There is a paucity of publications which have demonstrated that a large number of dogs can be vaccinated in a short period of time maintaining a high vaccination coverage.

The vaccination coverage of 52.4% achieved in this study is higher than the similar study conducted in India who found 34% (Belsare AV, 2013) and 35.5% (Reece and Chaula, 2006) respectively. The geographical area of Kotagiri and dog handling was a challenge in our study. Likewise, misconceptions among owners hindered to achieve higher vaccination coverage (Reece and Chaula, 2006). In a 12-month period in northwest Tanzania, Hampson *et al.*, 2009, estimated that a target vaccination coverage of 60% is sufficient to avoid coverage falling below the critical threshold of 40% between annual campaigns.

Vaccination coverage of 70% in 16 wards and in 2 wards 68.7% and 68.8%, the similar study held in Ranchi using Smart Phone Technology (Gibson *et al.*, 2015) which is more than our finding of 52.83%. Smartphone application provided specific functionality of data entry and boundaries displayed on maps, enabled efficient and simple region wise direction of catching and in overall budget for mass vaccination

campaigns, the cost of using smart phones per dog vaccinated was minimal. Although mobile phone technology has been used in other epidemiological studies (Aanensen, Huntley, Feil and Spratt, 2009), this Ranchi program was the first study to report the development and implementation of a bespoke app tailored towards the collection of data relevant in canine rabies field work. This technology helped the project manager to instantly view maps of where the vaccination teams has been working or download the data in spreadsheet format for estimation of vaccination coverage enabling the prompt direction of teams back to areas with low coverage. This instant access to digitalized data saved significant management time in conversion of paper records into an electronic spreadsheet which made the application of the system more appealing and sustainable at the project management level. The resulting dataset also facilitates study of dog demographics and spatial analysis.

In this study, majority of dog were found to be free roaming (58.8%) and were male (51.4%). This finding is lower than observed by Gibson *et al*, 2015 (free roaming 65.6%), Hibi, 2011 (male 56.5%). The male biased gender ratio is 1.74:1 observed in this study is in line with the finding of (Pal, 2001) who found it to be 1.37:1. Perception of males as better guard dogs than females has played the role and to avoid the nuisance of owning a bitch in estrus or having to deal with unwanted puppies might be the reason for the observed bias (Daniels and Bekoff, 1989, Daniels, 1983). Unlike in Africa, where a large number of dogs are owned and can be vaccinated through static point vaccine approaches (Cleaveland S *et al.*, 2003), the vast majority of dogs in Kotagiri were not identifiably owned. In this study teams used butterfly nets to catch and restrain the large number of dogs which were not amenable to handling. The use of butterfly nets has been previously described in vaccination campaigns in Bali where the majority of dogs were not amenable to handling (Morters *et al.*, 2014) and has been found to be more effective and humane than other capture methods.

CONCLUSION

The simple method of marking vaccinated dogs followed by dog-sight surveys to estimate vaccination coverage in the abundant free roaming dog populations is a cheap and effective system to estimate vaccination coverage in real-time. Mass vaccination campaigns provide an ideal opportunity for gathering information about a large cross section of the population with minimal additional effort which can then be used to better direct resources and refine effective methods. Crucially, we developed a 'Mission Rabies' smartphone application (App) which allowed for rapid entry of field data and facilitated the real time assessment of vaccination coverage. This mobile technology ensured that areas of vaccination coverage below 70 % were immediately detected, thereby enabling areas with suboptimal vaccination coverage to be revisited by vaccination teams. If the same approach is rolled out

more extensively across rabies endemic areas including Nepal, this vaccination strategy has the potential to significantly reduce the incidence of rabies in both dog and human populations. Killing dogs does not stop the disease. Additionally, for developing countries including India and Nepal, open source software like ArcGIS for mapping and epitoools for collection of data relevant in canine rabies field can be applied.

REFERENCES

- Aanensen DM, Huntley DM, Feil EJ, al-Own F, Spratt BG. EpiCollect: linking smartphones to web applications for epidemiology, ecology and community data collection. *PLoS One*. 2009; 4:e6968. doi:10.1371/journal.pone.0006968.
- Abbas SS, Kakkar M, Rogawski ET. Costs Analysis of a Population Level Rabies Control Programme in Tamil Nadu, India. *PLoS Negl Trop Dis*. 2014; 8. doi:10.1371/journal.pntd.0002721.
- ANDERSON, R.M., JACKSON, H. C. M., MAY, R. M. & SMITH, A. M. (1981) Population dynamics of fox rabies in Europe. *Nature* **289**: 765-771
- ANON (1988) Report of WHO consultation on dog ecology studies related to rabies control, WHO/Rab Res/88.25. Geneva, WHO.
- ANON (2004) Assessing the burden of rabies in India. WHO sponsored national multicentric rabies survey 2003. Bangalore, Association for Prevention and Control of Rabies in India
- Anon: WHO expert consultation on rabies: first technical report. In Technical Report Series 931 Geneva, WHO; 2004.
- Belsare AV, Gompper ME. Assessing demographic and epidemiologic parameters of rural dog populations in India during mass vaccination campaigns. *Prev Vet Med*. 2013; 111:139–46. doi:10.1016/j.prevetmed.2013.04.003. Elsevier B.V.
- Beran, G. W. (1991) Urban rabies. In the Natural History of Rabies. 2nd edn. Ed G. M. Baer. Boca Raton, CRC Press. pp 427-443
- Cleaveland S, Kaare M, Tiringa P, Mlengeya T, Barrat J. A dog rabies vaccination campaign in rural Africa: impact on the incidence of dog rabies and human dog-bite injuries. *Vaccine*. 2003; 21:1965–73. doi:10.1016/S0264-410X(02)00778-8.
- Coleman PG, Fevre EM, Cleaveland S. Estimating the public health impact of rabies. *Emerging infectious diseases*. 2004; **10**(1):140–2. PMID: 15078611

- Coleman PG. & DYE, C. (1996) Immunisation coverage required to prevent outbreaks of dog rabies. *Vaccine* **14**: 185-186
- Conan A, Kent A, Koman K, Konink S, Knobel D. Evaluation of methods for short-term marking of domestic dogs for rabies control. *Prev Vet Med.* 2015; **121**(1-2):179-82.
- Davlin SL, VonVille HM. Canine rabies vaccination and domestic dog population characteristics in the developing world: A systematic review. *Vaccine.* 2012; **30**:3492-502. doi:10.1016/j.vaccine.2012.03.069.
- Fooks A.R., et al., Current status of rabies and prospects for elimination. *Lancet*, 2014. 384(9951): p. 1389-99. doi: 10.1016/S0140-6736(13)62707-5 PMID: 24828901.
- Gangaboraiah, 2007. Assessing the burden of human rabies in India: results of a national multi-center epidemiological survey. *Int. J. Infect. Dis.* 11, 29-35.
- Gibson et al., *BMC Infectious Diseases* (2015) 15 : 589 DOI 10.1186/s12879-015-1320-2.
- Gsell AS, Knobel DL, Cleaveland S, Kazwala RR, Vounatsou P, Zinsstag J. Domestic dog demographic structure and dynamics relevant to rabies control planning in urban areas in Africa : the case of Iringa, Tanzania. *BMC Vet Res.* 2012; **8**:236.
- Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, et al., Estimating the Global Burden of Endemic Canine Rabies. *PLoS Negl Trop Dis.* 2015; 9.doi:10.1371/journal.pntd.0003709.
- Hampson K, Dushoff J, Cleaveland S, Haydon DT, Kaare M, Packer C, et al., Transmission dynamics and prospects for the elimination of canine rabies. *PLoS Biol.* 2009; 7:e53. doi:10.1371/journal.pbio.1000053.
- Hampson K, Dushoff J, Cleaveland S, Haydon DT, Kaare M, Packer C, et al., Transmission Dynamics and Prospects for the Elimination of Canine Rabies. *Plos Biology.* 2009; 7(3):e1000053. doi: 10.1371/ journal. pbio.1000053 PMID: 19278295.
- Hiby LR, Reece JF, Wright R, Jaisinghani R, Singh B, Hiby EF. A mark-resight survey method to estimate the roaming dog population in three cities in Rajasthan, India. *BMC Vet Res.* 2011; 7:46. doi:10.1186/1746-6148-7-46. BioMed Central Ltd.

- Jibat T, Hogeveen H, Mourits MCM. Review on Dog Rabies Vaccination Coverage in Africa: A Question of Dog Accessibility or Cost Recovery? *PLoS Negl Trop Dis.* 2015; 9:e0003447. doi:10.1371/journal.pntd.0003447.
- Kaare M, Lembo T, Hampson K, Ernest E, Estes A, Mentzel C, et al., Rabies control in rural Africa: evaluating strategies for effective domestic dog vaccination. *Vaccine.* 2009; 27:152–60. doi:10.1016/j.vaccine.2008.09.054.
- Kakkar M, Venkataramanan V, Krishnan S, Chauhan RS, Abbas SS. Moving from rabies research to rabies control: lessons from India. *PLoS Negl Trop Dis.* 2012;6:e1748. doi:10.1371/journal.pntd.0001748.
- Kayali U, Mindekem R, Yémadji N, Vounatsou P, Kaninga Y, Ndoutamia AG, et al., Coverage of pilot parenteral vaccination campaign against canine rabies in N'Djaména, Chad. *Bull World Health Organ.* 2003; 81:739–44. Available: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2572337&tool=pmcentrez&rendertype=abstract>.
- Knobel DL, Cleaveland S, Coleman PG, Fèvre EM, Meltzer MI, Miranda MEG, et al., Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ.* 2005; 83:360–8.
- Korns RF, Zeissig A. Dog, fox and cattle rabies in New York State: Evaluation of vaccination in dogs. *Am J Public Health Nations Health.* 1948; 38(1):50–65. PMID: 18917504.
- Lankester F, Hampson K, Lembo T, Palmer G, Taylor L, Cleaveland S. Implementing Pasteur's vision for rabies elimination. *Science (80-)* 2014; 345:1562–4. doi: 10.1126/science.1256306 PMID: 25258065.
- Largi, O.P., Arrosi, J.C., Nakajataa. J. & Villa-Nova, A. (1988) Control of urban rabies. In *Rabies*. Eds J. B. Campbell, K. M. Charlton. Boston, Kluwer Academic Publishers. pp 407-422.
- Lembo T, Hampson K, Kaare MT, Ernest E, Knobel D, Kazwala RR, *et al.*, The feasibility of canine rabies elimination in Africa: dispelling doubts with data. *PLoS Negl Trop Dis.* 2010; 4:e626. doi:10.1371/journal.pntd.0000626.
- Morters MK, McKinley TJ, Horton DL, Cleaveland S, Schoeman JP, Restif O, *et al.*, Achieving Population-Level Immunity to Rabies in Free-Roaming Dogs in Africa and Asia. *PLoS Negl Trop Dis.* 2014; 8:e3160. doi:10.1371/journal.pntd.0003160.
- Pal SK. Population ecology of free-ranging urban dogs in West Bengal, India. *Acta*

- Theriol (Warsz). 2001; **46**:69–78. doi:10.1007/BF03192418.
- Reece JF, Chawla SK. Control of rabies in Jaipur, India, by the sterilisation and vaccination of neighbor-hood dogs. Vet Rec. 2006; **159**:379–83. doi:10.1136/vr.159.12.379.
- Shahid S, Kakkar M. Rabies control in India: a need to close the gap between research and policy. Bull World Heal Organ. 2015; **93**(2):131–2.
- Sudarshan, M.K., Madhusudana, S.N., Mahendra, B.J., Rao, N.S.N., Ashwath Nurayana, D.H., Abdul Raman, S., Meslin, F.X., Lobo, D., Ravikumar, K.,
- Suraweera W, Morris SK, Kumar R, Warrell DA, Warrell MJ, Jha P, *et al.*, Deaths from Symptomatically Identifiable Furious Rabies in India: A Nationally Representative Mortality Survey. PLoS Negl Trop Dis. 2012; **6**(10):e1847. Doi 10.1371/journal.pntd.0001847 PMID: 23056661.
- Totton SC, Wandeler AI, Zinsstag J, Bauch CT, Ribble CS, Rosatte RC, *et al.*, Stray dog population demographics in Jodhpur, India following a population control/ rabies vaccination program. Prev Vet Med. 2010; **97**:517. doi:10.1016/j.prevetmed. 2010.07.009. Elsevier B.V.
- Vigilato MAN, Cosivi O, Knöbl T, Clavijo A, Silva HMT. Rabies update for Latin America and the Caribbean. Emerg Infect Dis. 2013; **19**:678–9. doi:10.3201/eid1904.121482.
- Vigilato MAN, Cosivi O, Knöbl T, Clavijo A, Silva HMT. Rabies update for Latin America and the Caribbean. Emerg Infect Dis. 2013; **19**:678–9. doi:10.3201/eid1904.121482.
- Wells C. The control of rabies in Malaya through compulsory mass vaccination of dogs. Bull World Health Organ. 1954; **10**:731–42.
- WHO. Expert Consultation on Rabies. Second report. 2013.

Welfare Status of Indigenous Cattle in Terai and Mid-Hill of Nepal

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ABSTRACT

The aim of present study was to assess the status of welfare of indigenous cattle, based on certain animal-linked parameters: body condition (BCS), body hygiene (cleanliness), lameness, skin injuries, general condition, and flight distance. The study was ruled in eight herds (10 cows /herd) in Terai and Mid-hill region of Nepal from March to June 2014. Altogether 80 cattle kept in semi-intensive system were assessed using several indicators determined through specific methods. Out of 80 cattle, 2 (2.5%) had BCS between 1.5-2, considered as very thin cows; 49 (61.25%) had BCS between 2.25-2.75, considered as thin cows; 29 (36.25%) had BCS 3-3.75 considered as normal cows and no cows found with BCS more than 3.75 score; 69 (86.25%) had dung particles at their lower legs, 60 (75%) had dung particles at their flank region and 11 (13.75%) had dung particles at their udder region; 4 (5%) were moderately lame, 27 (33.75%) had hairless spot on their different body parts(hock, knee joints, neck), 15 (18.75%) had swelling joints (knee joints); 10 (12.5%) showed fear at the observer's approach. The general condition was good of all the assessed cows. The obtained results indicate that more than half of the assessed cows are thin and dirty which has a negative impact on their health and welfare. The main factors that affect the indigenous cattle welfare in Nepal are insufficiency of feeding, poor housing system, poor sanitation and hygiene maintenance, lack of treatment of injured/diseased cattle and somewhat poor human animal relationship.

Keywords: BCS, Dirty, Fear, Housing, Indigenous cattle, Lameness, Welfare status

INTRODUCTION

In Nepal, there are 7.2 million cattle (Statistical information of Nepalese Agriculture, 2012), among them 88% are indigenous and remaining are exotic. Nepali indigenous cattle are small sized and humped (*Bos indicus*). They are disease resistant and hardy in nature. They can survive in scarce condition without supplement of extra concentrate ration. They prefer grazing in the forests or rangelands where they can get green forage and fodders and medicinal plants as well. Although their milk production is low, they are very useful in many aspects for us. Hindu people worship

them as a form of goddess Laxmi. Their products like milk, milk fat, urine and dung have high medicinal value for the treatment of many diseases like, diabetes, asthma, cancer and many diseases of respiratory system. Their milk is used as panchamrit/ pure liquid given after religious celebration (Pathak *et al.*, 2003). The local price of urine, milk, and fat of these cattle is Rs. 300/litre, Rs.100/litre and Rs.1000/kg respectively which is too higher than the price of improved dairy cattle products (Guru, S., 2014). It has been found that their urine contains curcumin, the anti-cancer element so homeopathic pharmaceuticals are using it in their medicines, and it is not found in humpless improved dairy cattle's urine (Dixit, R., 2015). Their milk is rich source of vitamins and minerals with less cholesterol and used in the treatments of constipation. Their milk fat is useful in the treatment of asthma, sinus, common cold, snoring sound etc and for massage. Their dung and urine act as anti-bacterial so used to purify the houses and religious places in religious activities (Guru, S., 2014). But nowadays their number is decreasing day by day due to commercialization of dairy sector. Due to their low productivity, farmers are neglecting them and leaving them to the roadsides. Some hermitage and marginalized communities only keep the indigenous cattle and their poor economic condition is reflected in poor wellbeing of the cattle. They are not given nutritious feed, no specific treatments on diseased and injured condition, no deworming, no good housing and feed deficit due to deforestation and decreasing rangeland areas which are being invaded by alien species of plants and being covered by human waste materials. So, all these cause the decreased welfare condition of the indigenous cattle in Nepal.

“Welfare principally concerns both the physical and psychological wellbeing of an animal, which is largely determined by the standard of stockman ship, the system of Husbandry and the suitability of the animal for the environment” (Farm Animal Welfare Council, 2009). Nowadays the dairy cattle welfare represents a permanent concern in many countries due to the impact on animal health and productivity and ultimately upon public health (Broom, D. M., 1991).Traditionally, farm animals' welfare assessment has focused on the measurement of resources provided to the animal such as housing and its design criteria (Capdeville *et al.*, 2001). Later on, it was modified on animal-related parameters, such as body condition score (BCS), body cleanliness, lameness, skin lesions, injuries and swellings, animal human relationship and so on which provides exact welfare condition of farm animals, (Winckler *et.al.*, 2002). So, the aim of this research was to assess the welfare condition of indigenous cattle of Central Terai and Mid-hills of Nepal, to know the major welfare parameters which are in very low condition or decreasing the welfare and range dynamics of that regions affecting the feed availability to them, so that future strategy to increase their welfare condition can be made to conserve them to increase to their production.

MATERIALS AND METHODS

The study was conducted on Bageswori cattle conservation centre, Devght, Chitwan, in Terai and Mrighsthal cattle conservation centre, Pashupatinath kshetra, Kathmandu and Ramdi cattle conservation centre, Ramdi, Shyanjha in mid-hills of Nepal, from February to June 2014. The welfare of these cattle was assessed on the basis of several animal-linked parameters such as; body condition score (BCS), body Cleanliness, lameness, injuries, flight distance etc. Among 80 indigenous cattle, each cow was examined and evaluated by two expert examiners. For the assessment of some parameters like (lameness and flight distance) the cows were untied and moved outside the shed.

The body condition score (BCS) and cleanliness scoring was assigned according to the system elaborated by Vasseur *et al.*, (2013) with scores from 1 to 5. Thus, a fat cow is one with $BCS \geq 4$; a normal cow is one with $BCS 3-3.75$; a thin cow is one with $BCS 2.25-2.75$ and an emaciated/very thin cow is one with $BCS \leq 2$. For cleanliness manure contamination in three body regions: udder, flank and leg were observed. The skin lesions (mainly knee joints left, right; hock joints left, right and neck region) and lameness were assessed through the method proposed by Gibbons *et al.*, (2012). The general condition was established using the method proposed by Thomsen, *et al.*, (2006) as unaltered, slightly modified or severely modified. Flight distance was appreciated through observation and measuring the distance (in meters) to how close we could approach the cow before it retreats from us (Leeb *et al.*, 2004). In order to determine the avoidance distance, the cows were untied and moved outside the barn. The participant in the test waited at a distance of 3 to 4 m for the cow to look at him before approaching, i.e. more or less directly from the front, walking slowly looking at the cow without direct eye contact, and keeping arms and hands close to the body. We considered that the cows do not show fear when the avoidance distance is smaller or equal to 1 m and that the cows show fear when the avoidance distance was more than 1 m. We visited all the rangelands and forests (upto 500m inside) in Terai and Mid-hill region where they were taken to graze to know their conditions.

RESULTS

After assessment of 80 indigenous cattle, the lowest condition of major animal welfare parameters were found that 2 (2.5%) cows had lowest BCS between 1.5-2 which were considered as very thin cows; 15 (18.75%) had skin injuries with wound (major swelling on joints and neck region); 4 (5%) were moderately lame but unable to run and 10 (12.5%) showed fear at the approach of the examiner. The general condition of all the cattle 80 (100%) was found to be normal. Above data are shown in Figure 1.

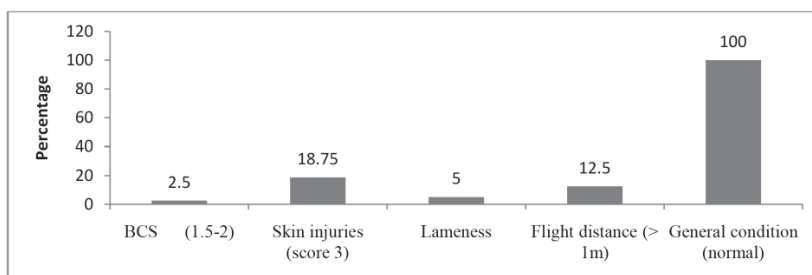


Fig. 1: Bar graph showing condition of major parameters of five freedoms of indigenous cattle in Central Terai and Mid-hill of Nepal.

The detailed distribution of the BCS scores has been shown in fig 2. It was found that 2 (2.5%) cows had BCS between 1.5-2 considered as very thin cows; 49 (61.25%) cows had BCS between 2.25-2.75, considered as thin cows; 29 (36.25%) had BCS between 3-3.75, considered as normal cows but no cattle had BCS more than 4, considered as fat cows.

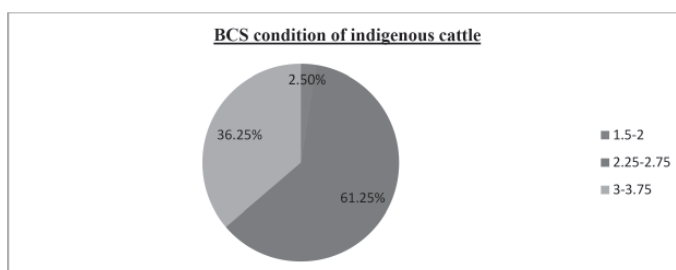


Fig. 2: Pie-chart showing detailed distribution of BCS of all assessed indigenous cattle.

The detailed condition of scores of body cleanliness at the level of three evaluated body areas was found that 69 (86.25%) had dirty lower legs, 60 (75%) had dirty flank and 9 (11.25%) had dirty udder region. The data are shown in fig.3

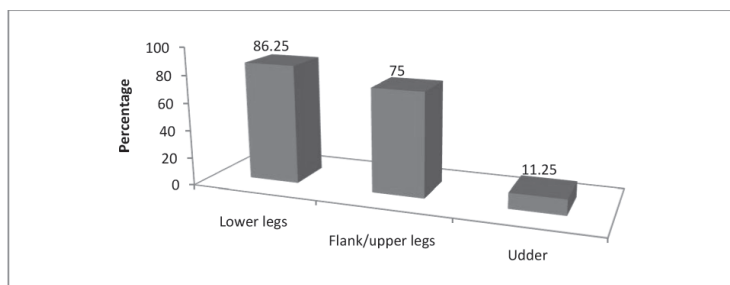


Fig.3: Bar graph showing scores of cleanliness of all assessed indigenous cattle.

The condition of injuries has been shown in fig 4. It was found that 27 (33.75%) cows had hair less spots or lesion on bald area of different body parts mainly at joints and neck, shoulder region; 15 (18.75%) had swelling joints with wound.

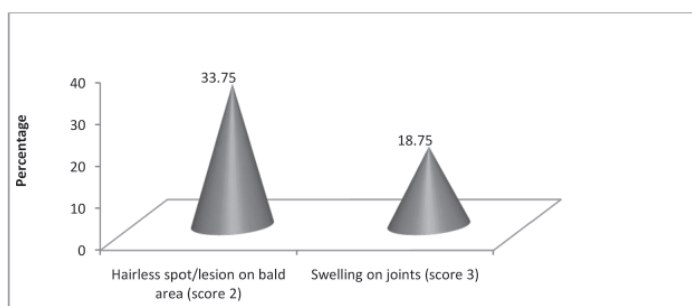


Fig. 4: Bar graph showing injuries condition in all assessed indigenous cattle.

The condition of rangelands and indigenous cattle in different regions of Terai and Mid-hill are presented in photos 1 to 8. It was found that most of their grazing land was occupied by alien species of plants like *Parthenium hysterophorus*, *Lantana camera*, *Eupatorium adenophorum*, and *Pteridium roxburghii*. While forests are invaded by *Michania micrantha*. At the same time those grazing areas as well as forests were also found full of human origin wastes like plastics and garments. All these conditions showed that they are deprived of nutrition, good housing and cleanliness which finally state their compromised welfare.

DISCUSSION

In our study, most of the cattle (61.25%) were found to have BCS 2.25-2.75 which were categorized as thin cows. While very thin cows having BCS ≤ 2 were found to be 2.5% which satisfies the welfare critical points criteria described by Grandin T. (2011). The similar findings were obtained by Gudaj, R.T. (2013) who found 4.37% and 4.19% cows with BCS ≤ 2 in 25 farms of Hungey in 2010 and 2011 respectively. While Popescu *et al.*, (2010) had found 56.45% cattle of BCS less than 2 in the farm of Romania. The higher number of thin cows in our study might be due to the forage deficiency in their grazing area which was found covered by alien species of plants and human origin plastic wastes.

In our study, the cattle with hair losses mainly in hock, knee joints were found to be 33.75%, the similar was found by whey *et al.*, 2003 in UK. i.e. 29.1%. Some lesser percent was found by Gudaj, R.T. (2013) in Hungary, who found 11.66% and 14.09% dry cow and lactating cow having hair losses in legs and Popescu *et al.*, (2010) found 14.38% cattle with hair loss or skin lesion in the farms of Romania. The more the hair losses condition in leg in cattle might be due improper bedding and floor type.

In our study, hock-knee lesions were found to be in 18.75% cows which was similar with the findings of Gudaj R.T. (2013) who found 15.28% and 16.75% dry cattle and 18.24% and 23.03% lactating cattle with hock and knee lesions. While Whey *et al.*, (2003) found 27.2% hock knee lesions in his research in UK. Hock- knee lesion might be arisen due to improper bedding and less-cleanliness of the shed, which favors the skin sloughing and then bacterial contamination in these parts.

In our study 5% cattle were found to be moderately lame which is similar to the findings of Popescu *et al.*, (2010) who found 4.86% lame dairy cattle in 52 dairy farms of Romania. But disagrees with Haskell *et al.*, (2006), Huxley *et al.*, (2004), Rutherford *et al.*, (2009) and Gudaj R.T. (2013) who found 24.3%,19%, 34% and 35.39% clinically lame respectively in dairy farm of Hungery. Lameness is caused by poor management; low quality feed supplement and genetic combination of the animal. The lesser no. of lame cattle in our study might be due to hardy nature of indigenous cattle which produce less milk and so need less energy and minerals for their body maintenance.

Our study found 86.25%, 75% and 11.25% dirty cattle at lower legs, flank region and at udder region which was more than the findings of Gudaj, R.T. (2013) who found 37.38%, 21.85% and 21.26% dirty dry cattle at lower legs, flank region and udder region respectively in the farm of Hungery. Whay *et al.*, (2003) found 47.4%, 10.7% and 22.2% respectively in UK. And Popescu *et al.*, (2010) found 11%, 19% and 14% respectively in the farms of Romania. The higher number of dirty cows might be due to less number of manpower in these religious farms to clean the shed and groom the cattle regularly and were kept more in a single shed.

In our study only 12.5% of the assessed cattle showed fear at the approach of examiner. Similar was found by Popescu *et al.*, (2010) i.e. 14.59% cattle showed fear in the farm of Romania. The smaller number of cattle showing fear indicates good human-animal relationship and which was due to religious concept of owners toward these cattle in our context. The general condition of these cattle was found as unaltered which is like the findings of Popescu *et al.*, (2010).

CONCLUSION

The greatest and the most frequent deviation from normality was found in BCS and cleanliness. So, feed deficit and poor housing and sanitation system was analyzed as the major factor or parameter leading to compromised welfare condition of these indigenous cattle, causing the decrease in their number and production. Their grazing area were found largely invaded by alien species of plants like *Parthenium hysterophorum* in Bageswari region of Central Terai, by *Lantana camara* and *Pteridium aquilinum* in Mrighsthali region and by little *Eupatorium adenophorum* in Ramdi region of Mid-hill of Nepal. At the same time human origin wastes particles mostly, the plastics were also found to be thrown in the grazing area of these cattle

in all three region which all cause feed deficit and increase chances of swallowing foreign materials leading to the decreased welfare.

RECOMMENDATION

Appropriate fund, knowledge and awareness toward health and sanitation should be given to the Ashram/hermitage by Government to conserve these indigenous cattle. Appropriate housing and appropriate stocking density should be maintained in the shed. Medicinal value of milk, milk fat, urine of these indigenous cattle should be made well known to all. Appropriate strategies should be made to control the invasion of alien species of plants and rules should be made to throw plastics and other waste only in the safe place.

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REFERENCES

- Broom, D.M., (1991). Animal Welfare: Concepts and Measurement. *J. Anim. Sci.*, **69**: 4167-4175.
- Capdeville J., Veissier I., (2001). A method of assessing welfare in loose housed dairy cows at farm level, focusing on animal observations. *Acta Agric Scand A Anim Sci* 2001, **30**: 62-8.
- Definition adapted from Farm Animal Welfare Council: Farm Animal Welfare in GB: Past, present and future. October 2009.
- Dixit, R., (2015). https://www.youtube.com/watch?v=23djTM_h064.
- Farm Animal Welfare Council 1992. FAWC updates the five freedoms. *Vet. Rec.* **17**: 357.
- Grandin, T. (2011): Outline of cow welfare critical points for dairies. [www.document] <http://www.grandin.com/cow.welfare.ccp.html> (accessed 29 January 2012).
- Gudaj, R.T. (2013). Study of animal welfare status and lameness in dairy cow herds in Hungery.
- Guru, S., (2014). Through personal communication.
- Haskell, M.J., Rennie, L.J., Bowell, V.A., Bell, M. J., Lawrence, A.B. (2006). Housing system, milk production, and zero-grazing effects on lameness and leg injury in dairy cows. *Journal of Dairy Science*, **89**: 4259-4266.
- Huxley, J.N., Burke, J., Roderick, S., Main, D.C.J., Whay, H.R. (2004). Animal

- welfare assessment benchmarking as a tool for health and welfare planning in organic dairy herds. *Veterinary Record*, **155**: 237-239.
- Leeb C.H., Main D.C.J., Whay H.R., Webster, A.J.F., (2004). Bristol welfare assurance programme – cattle assessment. *University of Bristol, UK* 2004. [http:// www.vetschool.bris.ac.uk](http://www.vetschool.bris.ac.uk).
- Pathak, M.L. and Kumar, A., (2003). Cow praising and importance of Panchyagavya as medicine. *Sachitra Ayurveda*, **5**: 56-59.
- Popescu, S., Borda, C., Dana, C., Razvan, S., Eva, S., (2010). Lazar University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Manastur street, no. 3-5, 400372, Cluj Napoca, Romania.
- Rutherford, K.M.D., Langford, F.M., Jack, M.C., Sherwood, L., Lawrence, A.J., Haskell, M.J. (2009): Lameness prevalence and risk factors in organic and non-organic dairy herds in the United Kingdom. *The Veterinary Journal*, **180**: 95-105.
- Thomsen, P.T., Baadsgaard, N.P., (2006). Intra- and inter-observer agreement of a protocol for clinical examination of dairy cows. Short communication. *Preventive Veterinary Medicine*, 2006, **75**:133-139.
- Vasseur E., Gibbons J., Rushen J., Passille A.M., 2012. Development and implementation of a training program to ensure high repeatability of lameness score of dairy cow in animal welfare assessments.
- Vasseur E., Gibbons J., Rushen J., Passille A.M., 2012. Development and implementation of a training program to ensure high repeatability of skin injury score of dairy cow in animal welfare assessments.
- Vasseur E., Gibbons J., Rushen J., Passille A.M., 2013. Development and implementation of a training program to ensure high repeatability of body condition score of dairy cows in animal welfare assessments. *J. Dairy Sci.* **96**:4725-4737.
- Vasseur E., Gibbons J., Rushen J., Passille A.M., 2013. Development and implementation of a training program to ensure high repeatability of cleanliness score of dairy cows in animal welfare assessments.
- Whay, H.R., Main, D.C.J., Green, L.E., Webster, A.J.F. (2003): Assessment of the welfare of dairy cattle using animal-based measurements: direct observations and investigation of farm records. *Veterinary Record*, **153**: 197-202.
- Winckler C., Bühnemann A. (2002). Social behaviour of commercial dairy herds as a parameter for on-farm welfare assessment. *Proc 36th Int Cong ISAE*, 2002.

Effect of Music on Animal Behavior: A Review

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ABSTRACT

Music is an expression of moods and emotions, which has a history of physical and emotional healings. It is thought to have both analgesic and anxiolytic properties. Various effects of music therapy on the physiology and psychology of human have been documented. The effect of music on physiology and behavior have been studied in animals too. Many of these studies claim that even animals are affected by the music. The potential benefits of music in animals might be through auditory enrichment which modifies the behavior of animals. The milking behavior and milk yield of farm animals including cattle and buffalo are affected by music. The objective of this study was to review the influence of music in animal behavior and discuss its usefulness for stress relief. The available literatures indicated that there is a variation among animals for music preference and their behavior is affected depending upon the animal species.

Keywords: *Animals; Behaviour; Effect; Music*

INTRODUCTION

Music is the expression of moods and emotions, which has a history of physical and emotional healings (Trehan, 2004). Any unwanted sound is noise (Broucek, 2014) and music is also a kind of sound. Whether or not a music is to be described as noise depends on the subjective notion depending upon whether hearing brings positive and negative feelings (Broucek, 2014). The effects of any kind of sound including music on animal behavior and productivity depends not only on its frequency, intensity (dB), pattern and duration, but also on the ability of the animal species to perceive. It also depends on the sound exposure history of the animal and to the stimuli of music (Castelhano-Carlos and Baumans, 2009). Human behavior in many ways have been influenced by music depending on the genre of music where classical music is found to have soothing effect but fast rock music has exciting effect (Dalton and

Behm, 2007). Since years, music has been successfully used to reduce the stress, depression, pain, and anxiety (McCaffrey and Good, 2000; Hilliard, 2001 and Vickers and Cassileth, 2001) as well as to improve body immunity (Gangrade, 2012 and Alsworth, 2013). This indicates that the music has both analgesic and anxiolytic properties. The music therapist usually generates music either from flute or piano or from other source that pleases the mind and can be used as an alternative form of therapy (Lipe, 1987 and Christie, 1992). The effects of music therapy on the physiology and psychology of humans have been well documented. The comparison of music to the white noise, that has been composed specifically to enhance mood in humans, found that both of them increased the rates of socialising behaviors such as play (Graves, 2011), but only the music decreased abnormal behavior (Robbins, L., & Margulis, S.W.; 2014).

The effect of music on physiology and behavior has been a matter of study on various animal species too. Many authors have reviewed the sensitivity of birds, baboons, monkeys, gibbons, dogs, cats and many other species to different types and levels of sound (Broucek, 2014). Auditory enrichment through music has improved the welfare of animals by masking noises and decreasing anxiety and stress (Patterson-Kane & Farnworth, 2006). The objective of this review was to understand effects of music on different aspects of animal behaviors.

MATERIALS AND METHODS

Various literatures including journals, proceedings, research reports and master's thesis research regarding music, and animal behavior were reviewed, and inferences were drawn mainly based on the findings of the past studies carried out.

RESULTS AND DISCUSSION

Effects of music in different animal species

Birds

Head bobbing and foot lifting in response to music was found in Sulphur-crested cockatoos and African gray parrots (Patel, A. D., Iversen, J. R., 2009; Schachner, A., Brady, T. F., 2009). Young Songbirds learn how to sing properly only after hearing adult songs. Naturally, the young deaf and isolated birds develop abnormal songs (Brainard, M.S., & Doupe, A. J., 2002; Waser, M. S., & Marler, P., 1977). Birds have the highest increase of auditory threshold in the higher frequency ranges after exposure to sound of 95-100 decibel (dB) (Marler *et al.*, 1973, Algers *et al.*, 1978). Disturbance from animals or human can be avoided with background sound level of 50 dB (Clough, 1999).

Baboons and Gibbons

Radio music has been found to decrease the heartrate of laboratory housed baboons (Brent and Weaver, 1996), but abnormal behavior was observed in rhesus macaques (*Macaca mulatta*) (O'Neill, 1989). This indicates that same music can have different effects depending upon animal species.

Chimpanzees

The use of sound system for example playing compact discs (CD) or MP3 music could increase socialization in laboratory housed chimpanzees (Howell *et al.*, 2003). Classical instrumental music could effectively increase amicable behavior in laboratory housed chimpanzees than vocal music (Videan *et al.*, 2007).

Elephants

Loud sound is well known for its adverse effects on heart rate and blood pressure in both humans and animals (Geverink *et al.*, 1998; Morgan, Tromborg, 2007). Wells and Irwin, 2008 analysed valuable effect of music of classical type on stereotypy in zoo housed Asian elephants (*Elephas maximus*), with elephants showing less abnormal behavior on exposure to classical music.

Rats

Many studies have been performed to understand the effect of music in laboratory rats. As their usual social communication occurs in ultrasonic range (Patterson-Kane, E. G., & Farnworth, M. J., 2006), it is difficult to believe that they enjoy music for humans (Panksepp, J., & Bernatzky, G., 2002). Da Cruz, *et al.*, (2011) found that exposure to chronic treatment with simvastatin (1 or 10 mg/kg/day) reduced anxiety levels in rats when Mozart music was played in an elevated plus-maze.

Dog

Kogan (2012) investigated the impact of classical, heavy metal, and specially designed or altered classical music on vocalization, activity level, and shaking of body in kenneled dogs. Vocalization and body shaking was lowered while sleep duration was increased in dogs after classical music was played in the kennel. The fast and loud music like heavy metal increased both vocalizations and body shaking.

Cats

Snowdon *et al.*, (2015) created species-appropriate music for domestic cats and compared with music that has similar affective content composed for humans. Two examples of cat music in counterbalanced order with two examples of human music were presented, and the behavior and response latencies of cats to each piece were evaluated. It was found that cats showed a significant preference for and interest in species-appropriate music compared with the other type.

Pigs

The researches carried out in pigs found no significant difference on the number of vocalizations between two conditions: one during playing music and another during stressful procedures in the farm (Cloutier *et al.*, 2000).

Equine

Classical, jazz or rock music was found to have non-significantly less calming effect in ponies than country music (Houpt *et al.*, 2000). Very few researches have been carried out on equine music preference.

Cattle

Lanier *et al.*, 2000 found dairy breeds to be more sensitive to musical sound than beef breeds. Algers and Jensen (1991) exposed dairy cows to 80-100 dB of sound for 1.4 hour twice daily instead of their preferred musical sound. This resulted in reduced milk yield in dairy cows and even interrupted ejection in progress (Algers *et al.*, 1978). The physiological responses of dairy cows to noise were reported by Broucek *et al.*, (1983). Loud cry causes stress responses in farm animals (Hemsworth *et al.*, 2003). Shouting on dairy cows appears to be very aversive (Pajor *et al.*, 2000). Sound made by human shouting and slamming of metal gates increases heart rate and activity in cattle (Waynert *et al.*, 1999). Abrupt high intensity sound like low altitude jet aircraft flights at milking time could stimulate increased peripheral or mammary release of catecholamines (Albright and Arave, 1997). Excessive sound has an influence on behaviour and coordination. This further tells that music which is also a sound also has effect depending on its intensity. Many of these studies claim that even animals cannot remain unaffected by the music. Slow music is found to increase daily milk yield in cows by 3% while 2% decrease by fast music (Nold, 2007). Wells, 2009 found classical and country music to have more positive effects on behavior than rock music increasing stress. In stress, milk cortisol concentrations increase (Gygax *et al.*, 2006). Results from playing the classical music for an extended period of time produced a steady increase in milk production (Kenison, L., 2016).

Effects of music on Buffalo

Buffaloes behavior and their production may be affected by music. This is supported by the research carried out by S.M.M. Abuzead and A.M. Khalil, 2007 who presented slow music to buffalo cows and noticed that they spent ample time displaying behaviors like absence of rumination and standing still with great attention and directing the ears towards the source of sound indicating relaxation. The slow music

led the buffaloes to be physically and psychologically prepared to milking spending less time showing stress behavior like increase in milk latency period than the control ones. Moreover, slow music could make nervous buffalo cows to respond and they could easily be manipulated compared to control buffalo group. Soothing noise is stimulating and relaxing which resulted in increased milk production (McCowan, *et al.*, 2002). Higher levels of stress lead to decreases in total milk production (Hopster, 2002).

CONCLUSION

The effect of music on animals is found to be species specific varying from positive to negative or no effect. It also depends on the type of music. Music influences the animal behavior as well as helps to increase the milk yield of farm animals like buffaloes and cows. Listening of preferred music has a calming effect that reduces stress and finally improves the milk production in milk producing animals. On contrary, unpreferred music has a noising effect that increases stress and finally reduces milk yield. The auditory enrichment through music reduces the stress of the animals and helps to ensure a normal or calm behavior. Using species-appropriate music helps to lower stress in animals.

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REFERENCES

- Abuzead, S.M.M. and Khalil, A.M. (2007). Behavioral and Physiological Influences of Listening Slow and Fast Music on Milking Buffaloes. *Assiut Vet. Med. J.* Vol. 53: 114
- Albright, J. L., & Arave, C. W. (1997). *The behaviour of cattle*. CAB international.
- Algers, B., & Jensen, P. (1991). Teat stimulation and milk production during early lactation in sows: effects of continuous noise. *Canadian Journal of Animal Science*, 71(1), 51-60.
- Algers, B., Ekesbo, I., & Strömberg, S. (1978). The impact of continuous noise on animal health. *Acta veterinaria Scandinavica. Supplementum*, 68: 1-26.

- Algers, B.; Ekesbo, I. and Sromberg, S. (1978): The impact of continuous noise on animal health. *Acta Vet. Scand.* 19 (suppl. 67), 1-26.
- Alworth, L. C., & Buerkle, S. C. (2013). The effects of music on animal physiology, behavior and welfare. *Lab animal*, **42**(2): 54.
- Brainard, M. S., & Doupe, A. J. (2002). What songbirds teach us about learning. *Nature*, **417**(6886), 351.
- Brent, L., & Weaver, O. (1996). The physiological and behavioral effects of radio music on singly housed baboons. *Journal of Medical Primatology*, **25**(5): 370-374.
- Broucek, J. (2014). Effect of noise on performance, stress, and behaviour of animals. *Slovak Journal of Animal Science*, **47**(2): 111-123.
- Castelhano-Carlos, M. J. – Baumans, V. 2009. The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory Animals*, vol. 43, 2009, p. 311-327.
- Cloutier, S., Weary, D. M., & Fraser, D. (2000). Can ambient sound reduce distress in piglets during weaning and restraint? *Journal of Applied Animal Welfare Science*, **3**(2): 107-116.
- Da Cruz, J. N., de Lima, D. D., Dal Magro, D. D., & da Cruz, J. G. P. (2011). The power of classic music to reduce anxiety in rats treated with simvastatin. *Basic and Clinical Neuroscience*, **2**(4): 5.
- Dalton, B. H., & Behm, D. G. (2007). Effects of noise and music on human and task performance: A systematic review. *Occupational Ergonomics*, **7**(3): 143-152.
- Graves LM. 2011. The effect of auditory enrichment on abnormal, affiliative, and aggressive behavior in laboratory housed rhesus macaques (*Macaca mulatta*) [Master's thesis]. San Marcos, TX: Texas State University.
- Gygax, S. E., Schuyler, J. A., Kimmel, L. E., Trama, J. P., Mordechai, E., & Adelson, M. E. (2006). Erythromycin and clindamycin resistance in group B streptococcal clinical isolates. *Antimicrobial Agents and Chemotherapy*, **50**(5): 1875-1877.
- Hopster, H. R. M. B., Bruckmaier, R. M., Van der Werf, J. T. N., Korte, S. M., Macuhova, J., Korte-Bouws, G., & Van Reenen, C. G. (2002). Stress responses during milking; comparing conventional and automatic milking in primiparous dairy cows. *Journal of Dairy Science*, **85**(12): 3206-3216.

- Houpt, K., Marrow, M., & Seeliger, M. (2000). A preliminary study of the effect of music on equine behavior. *Journal of Equine Veterinary Science*, 20(11), 691-737.
- Kenison, L. (2016). *The Effects of Classical Music on Dairy Cattle* (Doctoral dissertation).
- Kogan, L. R., Schoenfeld-Tacher, R., & Simon, A. A. (2012). Behavioral effects of auditory stimulation on kennel dogs. *Journal of Veterinary Behavior: Clinical Applications and Research*, 7(5): 268-275.
- Marler, P. (1973). A Comparison of Vocalizations of Red-tailed Monkeys and Blue Monkeys, *Cercopithecus ascanius* and *C. mitis*, in Uganda. *Ethology*, 33(3-4): 223-247.
- McCaffrey, R. G., & Good, M. (2000). The lived experience of listening to music while recovering from surgery. *Journal of Holistic Nursing*, 18(4): 378-390.
- Morgan, K. N., & Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*, 102(3): 262-302.
- Panksepp, J., & Bernatzky, G. (2002). Emotional sounds and the brain: the neuro-affective foundations of musical appreciation. *Behavioural Processes*, 60(2): 133-155.
- Patel, A. D., Iversen, J. R., Bregman, M. R., & Schulz, I. (2009). Experimental evidence for synchronization to a musical beat in a nonhuman animal. *Current biology*, 19(10): 827-830.
- Patterson-Kane, E. G., & Farnworth, M. J. (2006). Noise exposure, music, and animals in the laboratory: a commentary based on Laboratory Animal Refinement and Enrichment Forum (LAREF) discussions. *Journal of Applied Animal Welfare Science*, 9(4): 327-332.
- Robbins, L., & Margulis, S. W. (2014). The effects of auditory enrichment on gorillas. *Zoo biology*, 33(3): 197-203.
- S.M.M. Abuzead and A.M. Khalil. (2007) Behavioral and Physiological Influences of Listening Slow and Fast Music on Milking Buffaloes. *Assiut Vet. Med. J. Vol. 53:114 July 2007*
- Schachner, A., Brady, T. F., Pepperberg, I. M., & Hauser, M. D. (2009). Spontaneous motor entrainment to music in multiple vocal mimicking species. *Current Biology*, 19(10): 831-836.
- Snowdon, C. T., Teie, D., & Savage, M. (2015). Cats prefer species-appropriate music. *Applied Animal Behaviour Science*, 166, 106-111.

- Trehan, S. (2004). Music to my ears. *Journal of PMedicine*, 7(6): 868-869.
- Videan, E. N., Fritz, J., Howell, S., & Murphy, J. (2007). Effects of two types and two genre of music on social behavior in captive chimpanzees (*Pan troglodytes*). *Journal of the American Association for Laboratory Animal Science*, 46(1): 66-70.
- Waser, M. S., & Marler, P. (1977). Song learning in Canaries. *Journal of Comparative and Physiological Psychology*, 91(1): 1.
- Waynert, D. F., Stookey, J. M., Schwartzkopf-Genswein, K. S., Watts, J. M., & Waltz, C. S. (1999). The response of beef cattle to noise during handling. *Applied Animal Behaviour Science*, 62(1): 27-42.
- Wells, D. L., Coleman, D., & Challis, M. G. (2006). A note on the effect of auditory stimulation on the behaviour and welfare of zoo-housed gorillas. *Applied Animal Behaviour Science*, 100(3): 327-332.

Effects of Ketamine and Xylazine-Ketamine Combination in Clinical and Haemato-biochemical Parameters in Captive Rhesus Monkey (*Macaca mulatta*)

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ABSTRACT

This study was carried to compare the effects of ketamine and xylazine-ketamine combination on clinical and haematobiochemical parameters in captive rhesus monkey. The animals were divided into two groups; one ketamine (K) group and other xylazine-ketamine (XK) group. In ketamine group (n=6) atropine sulphate and ketamine was used intramuscularly at the rate of 0.04 mg/kg body weight and 10 mg/kg body weight respectively. In xylazine-ketamine group (n=3) xylazine and ketamine was used intramuscularly at the rate of 1 mg/kg body weight and 5 mg/kg body weight respectively. Blood samples were collected immediately after induction and 30 min after induction in both groups. Effects of anaesthetics on haematological and biochemical parameters such as TEC, TLC, Hb, PCV, DLC, serum cholesterol, serum triglyceride, HDL and LDL and clinical parameters such as rectal temperature, heart rate, respiration rate, muscle relaxation, jaw tone, pedal reflex, palpebral reflex and position of eye ball were studied. Rectal temperature was significantly lower ($P<0.01$) in group XK than in group K. Heart rate was significantly higher ($P<0.01$) in group K than in group XK. Respiratory rate was lower in group K than in group XK but it was nonsignificant ($P>0.05$). Hb and PCV level was significantly lower ($P<0.05$) in group XK than group K. TEC and TLC was higher in group K than in group XK but it was nonsignificant ($P>0.05$). Neutrophil and Eosinophil was higher in group XK than in group K but it was nonsignificant ($P>0.05$). Lymphocyte was lower in group XK than in group K but it was nonsignificant ($P>0.05$). Monocyte was significantly lower ($P<0.05$) in group XK than in group K. Serum cholesterol was higher in group XK than in group K but it was nonsignificant ($P>0.05$). Serum triglyceride and LDL was significantly higher ($P<0.05$) in group XK than in group K. HDL was lower in group XK than in group K but it was nonsignificant ($P>0.05$). Smooth induction was

found in group XK and duration of anaesthesia was longer in group XK whereas smooth recovery was found in group K but duration of anaesthesia was shorter. Muscle relaxation was excellent in group XK in comparison to group K. It can be suggested from the results of this study that for shorter duration of anaesthesia only ketamine can be used but for longer duration, xylazine-ketamine combination will be appropriate.

Keywords: Anesthesia; Monkey; Surgery; Wildlife

INTRODUCTION

Monkeys are wild animals that belong to order primates. They are abundantly found in forests all over the world. Besides forest, they are also found in temple areas and human residents where plenty of trees are present. They are also kept captive in zoo as an important zoo animal. They are said to be ancestor of human beings. Their genetic property is very close to human beings, chimpanzee and gorilla (Patterson *et al.*, 2006). In Hindu religion monkey is regarded as symbol of god Hanuman (Jones and Ryan, 2006).

Ketamine hydrochloride is widely used anaesthetic in wild animals. It is used alone or in combination with various sedatives and tranquilizers such as xylazine hydrochloride, diazepam, medetomidine (Naccarato and Hunter, 1979). The addition of xylazine increases the duration of anaesthesia. Ketamine is the drug of choice when animal has to be sedated prior to fasting in emergency cases such as accidents and animal bites. Other anaesthetics such as thiopentone and propofol are used intravenously once the animal becomes calm/ sedated. Once the animal is sedated, inhalant anaesthetics such as methoxyflurane, halothane, isoflurane and sevoflurane are used for maintenance. Xylazine is used as a sole agent for minor diagnostic procedures or manipulative procedures. Xylazine is used for short procedures to make the animal calm and safe. It is used as preanaesthetic sedative prior to local, regional or epidural anaesthesia. It is combined with opioids to produce profound sedation and analgesia (Paddleford, 1999).

Common surgical affections found in monkeys are wounds (incised wounds, biting wounds, lacerated wounds, and traumatic wounds), fracture (closed or open), lameness due to several causes. Sedation are used for various purposes like; to stitch or dress the wound, to correct fracture, lameness or for other procedures such as physical examination, blood collection, transportation from one place to another. For safety of handlers also, anaesthesia is required. Monkey is difficult to handle and may cause severe injury to the handler and other persons around. When used under laboratory conditions, these animals produce unique hazards to handlers, particularly zoonotic infection (Young *et al.*, 1999). Proper anaesthesia reduces the physiological stress of

the animals also. Keeping all these things in mind, this study was undertaken to find out a good anaesthetic or anaesthetic combinations and their effects on monkey to carry out minor or major surgical/ clinical procedures safely. The objectives of this study include to compare the effects of ketamine and xylazine-ketamine combination for anaesthesia in monkeys and to know the effects of these anesthetics on haemato-biochemical parameters such as complete blood cell count (CBC), serum cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) and clinical parameters such as rectal temperature, heart rate, respiration, pedal reflex, palpebral reflex, jaw tone and muscle relaxation.

MATERIALS AND METHODS

The experiment was done in Bangladesh National Zoo, Mirpur-1, Dhaka during the period of January to May 2017. Nine apparently healthy monkeys were chosen for experiment. In one group there were six monkeys and in other group there were 3 monkeys. Their age ranged from 2-8 years and weight ranged from 4-12 kg. Monkeys of either sex (male or female) were used. They were housed in well ventilated tin shed having concrete floor. They had ad libitum access to food and water. In zoo, they were given bread, fruits, eggs, vegetables, ground nuts and grams about 1.5 kg food/ animal/ day. The experimental animals were divided into 2 groups.

a) Ketamine (K) Group

In this group there were 6 animals. Atropine sulphate was used as preanaesthetic at the rate of 0.04 mg/kg body weight and ketamine was used as anaesthetic agent at the rate of 10 mg/kg body weight intramuscularly (IM).

b) Xylazine-Ketamine (XK) Group

In this group there were 3 animals. Xylazine was used as sedative at the rate of 1mg/ kg body weight and ketamine was used as anaesthetic at the rate of 5 mg/kg body weight intramuscularly (IM).

Experimental procedure

The monkeys in squeeze cage were used in ketamine group (K) and the monkeys in shed were used for xylazine-ketamine group (XK). In ketamine (K) group, ketamine HCl (G-Ketamine[®], 50mg/ ml, Gonoshasthaya Pharmaceuticals Ltd, Bangladesh) and atropine sulphate (Atrovet, 1mg/ ml, Techno Drugs Ltd., Bangladesh) was given intramuscularly by squeezing the animal in squeeze cage. Animal was taken out from the cage when they were recumbent on floor. In xylazine-ketamine (XK) group xylazine and ketamine was drawn in a single syringe and monkeys were darted in their shed by using blow pipe. The animals were taken out of the shed when they

were recumbent on the floor.

In both the groups after the animals were anaesthetized and safe to handle, clinical parameters were taken. Rectal temperature was taken with mercury thermometer; heart rate was taken by auscultating with stethoscope and respiration rate was taken by observing chest rise and fall. Pedal reflex was noted by pinching the digits, palpebral reflex was noted by touching the eyelid with finger, jaw tone was felt by opening the mouth with finger, muscle relaxation was felt by extending and flexing the hand of animal and position of eye ball was noted by observing eye. These parameters were noted immediately after induction when the animal was brought out of cage/ shed and then 30 min after induction.

In both groups, blood was collected from either radial or saphanous vein immediately after induction and then 30 min after induction. Collected blood was kept in K₂EDTA 3 ml tube (Cure Medical Tech Co., Ltd., USA) for complete blood cell count (CBC) and serum clot activator 4 ml tube (Cure Medical Tech Co., Ltd., USA) for serum collection. Collected blood was brought in cool box to Bangladesh Agriculture University (BAU) and was manually examined in Physiology Laboratory, Department of Physiology, BAU for total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb), packed cell volume (PCV) and differential leucocyte count (DLC) by the method described by Shastry (Sastry, 1989). In lipid profile, parameters such as cholesterol, triglyceride, high density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined by using 3000 evolution semiautomatic biochemistry analyser. Detailed procedure is illustrated in the diagram (Fig 1). The data obtained in the present investigation were analysed by repeated measures analysis of variance and students' "t" test.

RESULTS

Effects of anaesthetics on clinical parameters in monkey

Rectal temperature

The normal temperature in rhesus monkey is 98.06-102.02 °F (Bourne 1975; Bush *et al.*, 1977; Liu and Delauter, 1977). Rectal temperature was within normal range in both groups K and XK immediately after induction and 30 min after induction. Rectal temperature in group XK was significantly lower ($P < 0.01$) 30 min after induction in comparison to that of group K. In macaques, a similar decrease in body temperature was observed during anesthesia with ketamine, but this was followed by post-anesthetic hyperthermia (Lopez *et al.*, 2002). These findings are in agreement with the present study where temperature slightly increased with ketamine but decreased by approximately 1°F with xylazine-ketamine anaesthesia. The effects of

anaesthetics on rectal temperature is given in Table 1.

Heart rate

The normal value of heart rate in rhesus monkey is 100-120/ min (Bourne, 1975; Johnson-Delaney, 1994; Haruo, 1995). The heart rate in group K was significantly higher ($P<0.05$) immediately after induction and 30 minutes after induction in comparison to that of group XK. Xylazine has cardiodepressant action on heart rate (Grub *et al.*, 2008).

Thurmon (1996) reported that ketamine had cardiovascular depression in rhesus monkey up to 30 min. This is in agreement with the findings of the present study where heart rate decreased after 30 min while using ketamine only and increased after 30 min while using xylazine-ketamine combination. The effects of anaesthetics on heart rate is given in Table 1.

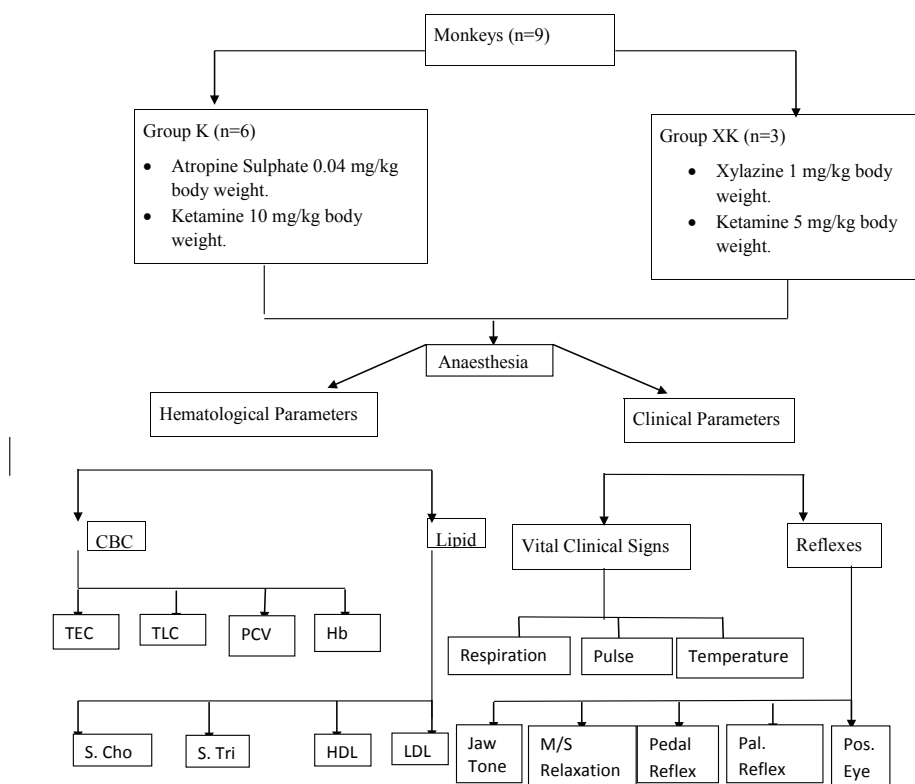


Figure 1. Layout of the experiment.

Table 1: Effects of anaesthetics on clinical parameters in monkey

Parameters	Anaesthetic agents	Immediately after induction	30 min after induction
Rectal temperature	K(n=6)	101±0.6	101.45±1.05
	XK(n=3)	100.86±0.61	98.93±0.31**
Heart rate	K(n=6)	182.66±25.88	170.66±23.14
	XK(n=3)	90.66±10.06**	97.33±6.11**
Respiration rate	K(n=6)	42.33±9.99	46.66±21.86
	XK(n=3)	45.33±6.11	32

± = Standard deviation ** = Significant at 1% level of probability between the group

K = Ketamine XK = Xylazine-ketamine

Respiration rate

The normal respiration rate in rhesus monkey is 20-50/ min (Bush *et al.*, 1977; Liu and DeLauter, 1977). Respiration rate increased in group K and decrease in group XK after 30 min of induction which was nonsignificant ($P>0.05$). Xylazine may cause respiratory depression (Paddleford, 1999). Ketamine, in contrast to most of the anesthetic drugs, has shown to possess incremental effects on the heart rate, blood pressure and respiratory rate due to increase in sympathetic activation (Von Ungern-Sternberg *et al.*, 2007). The effects of anaesthetics on respiration rate is given in Table 1.

Effects of anaesthetics on haematological parameters in monkey

Haemoglobin

The normal value of haemoglobin in rhesus monkey is 12.5±1.5 gm/dl (Fowler, 1986). In group XK, mean value of haemoglobin was recorded as 8.13±0.83 which decreased to 7.8±0.72 after 30 min of induction and was significant ($P<0.05$) within the group. Mean value of haemoglobin was significantly higher ($P<0.05$) in group K after 30 min of induction in comparison to that of group XK. The effects of anaesthetics on haemoglobin is given in Table 2.

Packed cell volume (PCV)

The normal packed cell volume in rhesus monkey is 40.3±3.5 % (Fowler, 1986). The mean value of packed cell volume immediately after induction was significantly higher ($P<0.05$) in group K in comparison to that of group XK. The mean value of

packed cell volume was significantly higher ($P<0.01$) after 30 min of induction in group K in comparison to that of group XK. The effects of anaesthetics on packed cell volume is given in Table 2.

Total erythrocyte count (TEC)

The normal value of total erythrocyte count in rhesus monkey is 4.48 ± 0.63 mm/cm³ (Fowler, 1986). The mean values of total erythrocyte count were nonsignificant ($P>0.05$) both within and between groups K and XK immediately after induction and 30 min after induction. The effects of anaesthetics on total erythrocyte count is given in Table 2.

Table 2: Effects of anaesthetics on haematological parameters in monkey

Parameters		Anaesthetic agents	Immediately after induction	30 min after induction
Haemoglobin (gm/dl) XK(n=3)		K(n=6)	9.18±1.31	9.4±1.14
		8.13±0.83 ^a	7.8±0.72*	
Packed cell volume (%) XK(n=3)		K(n=6)	38.08±3.54	39.58±5.01
		32.33±3.05*	31.33±0.57**	
Total erythrocyte count (mm/cm ³) XK(n=3)		K(n=6)	5.45±0.74	5.65±0.88
		5.49±0.89	5.15±0.89	
Total leucocyte count (thou/cm ³) XK(n=3)		K(n=6)	15.86±13.70	8.97±2.96
		8.63±0.42	8.26±0.33	
Differential leucocyte count (%)	Neutrophil	K(n=6)	59.5±8.47	60.16±12.71
		XK(n=3)	57.67±20.50	72.66±1.15
	Lymphocyte	K(n=6)	35.33±8.61	35±13.78
		XK(n=3)	37.33±17.90	22.66±1.15
	Monocyte	K(n=6)	2.66±1.3	2.66±1.2
		XK(n=3)	1±0	1.66±0.57
	Eosinophil	K(n=6)	2.5±1.97	2.16±2.13
		XK(n=3)	2.64±0.47	3±1
	Basophil	K(n=6)	0	0
		XK(n=3)	0	0

* = Significant at 5% level of probability between the group

‡ = significant at 5% level of probability within the group

** = Significant at 1% level of probability between the groups

Total leucocyte count (TLC)

The normal value of total leucocyte count in rhesus monkey is 11.5 ± 4.3 thou/cm³ (Fowler, 1986). The mean values of total leucocyte count were nonsignificant ($P > 0.05$) both within and between groups K and XK immediately after induction and 30 min after induction. The effects of anaesthetics on total leucocyte count is given in the Table 2.

Effects of anaesthetics on differential leucocyte count (DLC)

The normal value of neutrophil, lymphocyte, monocyte, eosinophil and basophil count in rhesus monkey are 39.09 ± 11.97 %, 57.40 ± 12.61 %, 2.29 ± 1.23 %, 0.43 ± 0.61 %, and 0.06 ± 0.24 % respectively (Chen *et al.*, 2009). The mean value of monocyte immediately after induction was significantly higher ($P < 0.05$) in group K in comparison to that of group XK. The mean value of neutrophil, lymphocyte, eosinophil and basophil was nonsignificant ($P > 0.05$) both within and between the groups K and XK immediately after induction and 30 min after induction. The effects of anaesthetics on differential leucocyte count is given in the Table 2.

In a study on the haematology of vervet monkeys, Wall *et al.*, (1985) found that PCV decreased in ketamine anaesthetized groups which may be due to sequestration of erythrocytes in the capillary beds. A similar decrease in PCV has been noted in ketamine anaesthesia in rhesus monkeys (Loomis *et al.*, 1980) as well as in other species with other anaesthesia (Kocan *et al.*, 1981) which is dissimilar to the results obtained in this study.

According to Bennet *et al.*, (1992) decrease in total erythrocyte count (TEC), hemoglobin (Hb) concentration, total leucocyte count (TLC) and lymphocyte count, following ketamine injection in rhesus monkeys (*Macaca mulatta*), can be attributed to a reversal of stress or “alarm reaction”. Loomis *et al.*, (1980) and Wall *et al.*, (1985) working with rhesus and vervet monkeys, also observed a decrease in peripheral erythrocytes and lymphocytes.

This observation is similar to results of the present study where TLC has decreased whereas TEC and Hb concentration has increased with ketamine anaesthesia. In case of XK combination TEC, TLC, Hb concentration and lymphocyte count has decreased which is similar to the results obtained by Loomis *et al.*, (1980), Wall *et al.*, (1985) and Bennet *et al.*, (1992). The decrease in blood cellular elements may

be due to haemodilution by infiltration of intestinal fluids during immobilization (Drevemo and Karstad, 1974). The fall in PCV and TEC may be due to lytic effects of xylazine (Moor and Desmet, 1971).

Effects of anaesthetics on lipid parameters in monkey

Serum cholesterol

The normal value of serum cholesterol in rhesus monkey is 32.48 ± 8.51 mg/dl (Chen *et al.*, 2009). The mean value of serum cholesterol was much higher than normal in both groups K and XK immediately after induction and 30 min after induction but nonsignificant ($P > 0.05$). The effects of anaesthetics on serum cholesterol is given in Table 3.

Serum triglyceride

The normal value of serum triglyceride in rhesus monkey is 261.29 ± 46.05 mg/dl (Chen *et al.*, 2009). The level of serum triglyceride was significantly higher ($P < 0.05$) 30 min after induction in group XK in comparison to that of group K. The effects of anaesthetics on serum triglyceride is given in Table 3.

Table 3: Effects of anaesthetics on lipid parameters in monkey

Parameters	Anaesthetic agents	Immediately after induction	30 min after induction
Serum cholesterol (mg/dl)	K(n=6)	125.33 ± 47.70	123.66 ± 43.84
	XK(n=3)	137.66 ± 30.98	136.33 ± 26.10
Serum Triglyceride (mg/dl)	K(n=6)	107.66 ± 72.84	97.83 ± 63.41
	XK(n=3)	136.66 ± 77.41	130.66 ± 74.01^a
High Density lipoprotein (mg/dl)	K(n=6)	60.20 ± 20.33	56.5 ± 11.22
	XK(n=3)	54.66 ± 12.42	54.33 ± 16.44
Low density lipoprotein (mg/dl)	K(n=6)	45.56 ± 31.54	47.43 ± 32.13
	XK(n=3)	60.20 ± 20.33	$64.53 \pm 18.72^*$

^a = Significant at 5% level of probability within the group

* = Significant at 5% level of probability between the group

High density lipoprotein (HDL)

The normal value of high-density lipoprotein is 49.49 ± 11.60 mg/dl (Chen *et al.*, 2009). The mean values of high-density lipoprotein immediately after induction and 30 min after induction was nonsignificant ($P > 0.05$) within and between the groups K and XK. The effects of anaesthetics on high density lipoprotein is given in Table 3.

Low density lipoprotein (LDL)

The normal value of LDL in rhesus monkey is 78.86 ± 15.08 mg/dl (Chen *et al.*, 2009). The mean value of low-density lipoprotein was significantly higher ($P < 0.05$) after 30 min of induction in group XK in comparison to that of group K. The effects of anaesthetics on low density lipoprotein is given in Table 3.

DISCUSSION

A study on serum lipid parameters in adult bonnet monkeys (*Macaca radiata*) by Perumal *et al.*, (2007) showed that ketamine anesthesia caused significant decreases in serum cholesterol and HDL which is dissimilar to the results obtained in my study. Studies on ketamine anesthesia in aged bonnet monkeys caused no significant alteration in serum cholesterol level; this apparently contradictory result might reflect the age of the animals (Venkatesan *et al.*, 2006). Plasma cholesterol level is under the direct influence of the liver and the stress response (Gelhot *et al.*, 1997). Lipolysis increases under the influence of catecholamines and corticosteroids and contributes to fat metabolism.

Many studies reported age-related changes in lipid concentrations in nonhuman primates. HDL tended to decline with the progression of age in normal rhesus monkeys, whereas serum triglycerides, cholesterol, and LDL remained unchanged (Tigno *et al.*, 2004). In another study of rhesus monkeys, only triglyceride level is found to increase with the advancement of age (Smucny *et al.*, 2001). In Tibetan macaques, it is found that cholesterol, HDL, and apolipoprotein A₁ decreased with age, but triglyceride and LDL levels remained the same (Wu *et al.*, 2014).

CONCLUSION

The following conclusions can be made from the findings of the present study. Rectal temperature and respiration rate decreased in both groups ketamine and xylazine-ketamine combination. Heart rate remained much above normal when only ketamine was used but remained almost stable when used in combination with xylazine. TEC, Hb and PCV increased and TLC decreased in ketamine group and TEC, TLC, Hb and PCV decreased in xylazine-ketamine combination group. In DLC only neutrophil increased in ketamine group and only lymphocyte decreased in xylazine-ketamine combination group. In lipid profile serum cholesterol, serum triglyceride and HDL decreased in both ketamine group and xylazine-ketamine combination group, whereas

LDL increased in both groups, ketamine and xylazine-ketamine combination.

REFERENCES

- Bennet JS, Gosset KA, McCartht MP and Simpson ED. 1992. Effects of ketamine hydrochloride on serum biochemical and hematologic variables in rhesus monkeys (*Macaca mulatta*). *Veterinary Clinical Pathology*. **21**(1): 15-18.
- Bourne GH. 1975. *The Rhesus Monkey*. London: Academic Press.
- Bush M, Custer R, Smeller J. and Bush LM. 1977. Physiologic measures of non-human primates during physical and chemical immobilization. *Journal of the American Veterinary Medical Association*. **171**(9): 866-869.
- Chen Y, Qin S, Ding Y, Wei L, Zhang J, Li H, Bu H, Lu Y and Cheng J. 2009. Reference values of clinical chemistry and hematology parameters in rhesus monkeys (*Macaca mulatta*). *Xenotransplantation*. **16**(6): 496-501.
- DeMoor A. 1971. Effect of rompun on acid-base-equilibrium and arterial O₂ pressure in cattle. *Veterinary Medical Review*. **213**: 163-169.
- Drevemo S and Karstad L. 1974. The effect of xylazine and xylazine-etorphine-acepromazine combinations on some clinical and hematological parameters in impala and eland. *Journal of Wildlife Diseases*. **10**(4): 377-383.
- Fowler ME. 1986. Zoo and wild animal medicine. 2nd edn. W.B. Saunders Company. pp. 706-707.
- Gehlot A, Godhwani JL, Godhwani S, Aseri ML, Jain P and Vyas MCR. 1997. Sound stress-induced changes and their modification by drugs in albino rats: an experimental study. *Indian Journal of Pharmacology*. **29**(3): 187-189.
- Grubb TL, Benson GJ, Thurmon JC, Olson WO, Tranquilli WJ, Foreman JH, Constable PD and Davis LE. 2008. Effects of xylazine/ketamine on cardiac function and serum ionised calcium in horses. *Veterinary Anaesthesia and Analgesia*. **24**(2): 6-11.
- Haruo M. 1995. Ventricular wall thickness and blood pressure values in normal cynomolgus monkeys. *Journal of Veterinary Medical Science*, **57**(6):1045-1048.
- Johnson-Delaney C. 1994. Primates. *The Veterinary Clinics of North America. Small Animal Practice*. **24**(1): 121-156.
- Jones AC and Ryan JD. 2006. Encyclopaedia of hinduism. Infobase publishing. pp.

177-178.

- Kocan A, Glenn B, Thedford T, Doyle R, Waldrup K, Kubar G and Shaw MO. 1981. Effects of chemical immobilization on haematologic and serum chemical values in captive white-tailed deer. *Journal of the American veterinary Medical Association*. **179**(11): 1153-1156.
- Liu C and DeLauter R. 1977. Pulmonary functions in conscious and anesthetized rhesus macaques. *American Journal of Veterinary Research* **38**(11): 1843–1848.
- Loomis MR, Henrickson RV and Anderson JH. 1980. Effects of ketamine hydrochloride on the haemogram of rhesus monkey (*Macaca mulatta*). *Laboratory animal science*. **30**(5): 851-853.
- Lopez KR, Gibbs PH and Reed DS. 2002. A comparison of body temperature changes due to the administration of ketamine-acepromazine and tiletamine-zolazepam anesthetic in cynomolgus macaques. *Journal of the American Association for Laboratory Animal Science*. **41**(2): 47–50.
- Naccarato EF and Hunter WS. 1979. Anaesthetic effects of various ratios of ketamine and xylazine in rhesus monkeys (*Macaca mulatta*). *Laboratory Animals*. **13**(4): 317-319.
- Paddleford RR. 1999. Anaesthetic agents. In: *Manual of Small Animal Anaesthesia*. 2nd edn. W. B. Saunders Company. pp. 22-47.
- Paddleford RR. 1999. Preanaesthetic agents. In: *Manual of Small Animal Anaesthesia*. 2nd edn. W. B. Saunders Company. pp. 12-24.
- Patterson N, Ritcher DJ, Gnerre S, Landr ES and Reich D. 2006. Genetic evidence for complex speciation of humans and chimpanzees. *Nature*. **441**(7097): 1103-1108.
- Perumal N, Ramasamy V, Kumar MM and Majumdar SS. 2007. Effects of ketamine and thiopentone anesthesia on serum lipid parameters in adult bonnet monkeys (*Macaca radiata*). *Journal of the American Association for Laboratory Animal Science*. **46**(3): 21-3.
- Sastry GA. 1989. *Veterinary clinical Pathology*. 3rd edn. CBS Publishers and Distributors P Ltd, New Delhi. pp. 1-25.
- Smucny DA, Allison DB, Ingram DK, Roth GS, Kemnitz JW, Kohama SG and Lane MA. 2001. Changes in blood chemistry and hematology variables during

- aging in captive rhesus macaques (*Macaca mulatta*). *Journal of Medical Primatology*. **30**: 161–173.
- Thurman JC, Tranquilli WJ and Benson GJ. 1996. Anesthesia of wild, exotic and laboratory animals. Lumb & Jones' veterinary anesthesia. pp. 686-735.
- Tigno XT, Gerzanich G and Hansen BC. 2004. Age-related changes in metabolic parameters of nonhuman primates. *The journals of gerontology series a: biological sciences and medical sciences*. **59** (11): 1081-1088.
- Venkatesan R, Nagarajan P, Ravishankar R and Majundar SS. 2006. Hematologic and serum biochemical values in aged bonnet macaques anesthetized with ketamine hydrochloride. *Journal of American Association of Laboratory Animal Science*. **45**: 45–48.
- Von Ungern Sternberg BS, Regli A, Frei FJ, Ritz E, Jordi M, Hammer J, Schibler A and Erb TO. 2007. A deeper level of ketamine anesthesia does not affect functional residual capacity and ventilation distribution in healthy preschool children. *Pediatric Anesthesia*. **17**(12): 1150-1155.
- Wall HS, Worthman C and Else JG. 1985. Effects of ketamine anesthesia, stress and repeated bleeding on the haematology of vervet monkeys. *Laboratory animals*. **19**(2): 138-144.
- Wu D, Yi Y, Sun F, Zhou L, Yang F, Wang H, Zhang G, Zhang YA and Yue F. 2014. Effects of age and sex on the haematology and blood chemistry of tibetan macaques (*Macaca thibetana*). *Journal of the American Association Laboratory Animal Science*. **53**(1): 12-17.
- Young SS, Schilling AM, Skens S and Ritacco G. 1999. Short duration anaesthetic with medetomidine and ketamine in cynomolgus monkeys. *Laboratory Animals*. **33**(2): 162-168.