

VOLUME 10 & 11.

1981-1982

REGD. NO. ZCBA 7/2025/26

Bulletin of

**Veterinary Science &
Animal Husbandry**

Nepal



Official Organ of Veterinary Association

Bulletin of Veterinary Science and Animal Husbandry, Nepal

Volume 10 & 11

1981-1982

Regd. No. ZCBA 7/2025/26

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Nepal Veterinary Association wants to wish its gratitude to the Scientific & Technological Publication sub-committee, **National Council of Science & Technology (NCST)** for their generous contribution of Rs. 2000/- for the publication of this Bulletin. (NCST Regd. No. 01/00/05-034)

Bulletin of Veterinary Science and Animal Husbandry, Nepal

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AN ATTEMPT TO IDENTIFY FASCIOLA SPECIES IN PALPA

Mahendra N. Lohani* and Monika Koehli Jaeckle**

Introduction

Fascioliasis, a parasitic disease of domestic ruminants, is caused by parasites of the fasciola species. This disease occurs in a chronic form in cattle, buffaloes, sheep and goats in the Tinau Watershed area, Palpa.

The penetration of the liver tissue by the immature flukes causes an acute hepatitis. Its severity and clinical symptoms depend on the number of parasites. The adult fluke lives in the bile ducts and causes a chronic inflammation. The intermediate hosts of this parasite belong to snails of the *Lymnaea* species.

Several authors have considered Fascioliasis as one of the most important parasitic disease in Nepal. Besides Haemorrhagic Septicaemia and Foot and Mouth Disease, Fascioliasis is responsible for the most serious economic loss in the livestock production of the country. A large percentage of domestic ruminants are infested with this parasite. According to different authors, fascioliasis causes up to 40% less milk production, up to 30% weight loss and render its host susceptible to all other diseases. Already in 1973 the annual loss of Nepal's economy had been estimated at N. C. 200 Millions.

Bommeli *et al* collected snails from different regions. They found *Lymnaea viridis* and *L. auricularia* rufescens widespread all over Nepal. Neff and Joshi collected for one year parasites from animals slaughtered in Kathmandu. All other authors limited their investigations to examination of faecal samples.

Materials and Methods

Liverfluke specimens were collected at all five slaughtering places of Tansen in the last week of July and beginning of August, 1981. They were extracted within 1 hour after slaughter-

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ing the animals. The sample washed with water, were preserved in a 4% Formaldehyde saline solution. Identification was done by Hoerning Institute of Parasitology, University of Bern and results were tabulated.

Results and Discussion

Liverfluke specimen of 24 animals have been identified, 22 of these animals were water buffaloes and 2 goats. The total number of specified flukes were 181. The number of Liverfluke collected from individual animal ranged from 2 to 13. (Table 1)

Table 1. Specification of investigated animals and type of infestation

S. N.	Species of Animal	Sex	Age (years)	<i>Fasciola gigantica</i>	<i>Fasciola hepatica</i>	Intermediate form	Number of Flukes
1.	Water buffalo	male	4	1	1	1	3
2.	" "	male	3	5	2		7
3.	Goat	male	1 1/2	5	2		7
4.	Water buffalo	female	2	8	2	2	12
5.	" "	female	8	7	1	1	9
6.	" "	female	6	1	1		2
7.	" "	female	14	2	4	1	7
8.	" "	female	8	2		3	5
9.	" "	male	1	1		1	2
10.	" "	female	6	7	1	1	9
11.	" "	male	9	2	1	1	4
12.	" "	male	11	7		3	10
13.	" "	male	3	6	1	2	9
14.	" "	male	6	5	1	1	7
15.	" "	male	3	8			8
16.	" "	female	8	5	5	3	13
17.	" "	female	6	5	1		6
18.	Goat	male	1	7	1	4	12
19.	Water buffalo	female	8	6		1	7
20.	" "	male	3	10			10
21.	" "	female	2	6	2		8
22.	" "	female	3	4	3	1	8
23.	" "	female	7	5			5
24.	" "	male	3	11			11

Table 2

Summary of Identified Flukes

Total number of specified flukes	181	100.0%
<i>Fasciola hepatica</i>	29	16.0%
<i>Fasciola gigantica</i>	126	69.6%
Intermediate form	26	14.4%

Infestation with *Fasciola hepatica* exclusively was discovered in no case. Infestation with *Fasciola gigantica* exclusively was found in 4 animals. Besides *Fasciola hepatica* and *gigantica*, in 15 animals an intermediate form has been found.

Mixed infestations appear in all age groups quite evenly distributed (Table 4). Exclusive infestation with *Fasciola gigantica* was found in 3 cases in age group 1 (1-3 years) and 1 case in age group 3 (7-10 years).

Intermediate forms have previously been described for Japan and North America. But nobody to authors knowledge have yet explained their appearance properly. It is suspected that this intermediate form occurs only in areas in which mixed infestation with *F. gigantica* and *F. hepatica* existed for a long time.

Table 3. Type of Infestation found in investigated animals

Total number of animals	24
Number of animals infested with <i>F. gigantica</i>	4
Number of animals infested with <i>F. gigantica</i> & <i>hepatica</i>	5
Number of animals infested with <i>F. gigantica</i> , <i>hepatica</i> and intermediate form	11
Number of animal infested with <i>F. gigantica</i> and intermediate form	4

Table 4.

Type of Infestation according to age groups

Age group	<i>F. gigantica</i>	<i>F. gigantica</i> & <i>hepatica</i>	<i>F. gigantica</i> , <i>hepatica</i> & intermediate form	<i>F. gigantica</i> & intermediate form
Age group 1 (1 - 3 years)	3	3	4	1
Age group 2 (4 - 6 years)		2	3	
Age group 3 (7 - 10 years)	1		3	2
Age group 4 (11 - 14 years)			1	1
Total	4	5	11	4

Table 5. Type of Infestation in different Panchayats.

Panchayats	Altitude m. a. s. l.	Number of animals	<i>Fasciola gigantica</i>	<i>F. gigantea</i> & <i>F. hepatica</i>	<i>F. gigantea</i> <i>F. hepatica</i> & intermedi- ate form	<i>F. gigantea</i> and interme- diate form
Baldengadhi	690-1070	1		1		
Bandipokhara	840-1860	2		1		1
Bhairabsthan	900-1510	1			1	
Chhahara	900-1790	3			2	1
Humin	690-1270	1		1		
Juthapaua	420-1790	6	3		2	1
Kaseni	670-1820	1			1	
Khasauli	840-1530	1			1	
Madanpokhara	680-1240	4			3	1
Mujhung	840-1530	3	1	1	1	
Pekh	420-1790	1		1		

According to table 2, *Fasciola gigantea* is the most important species in the Palpa region. Figure 1 shows the geographical location of the 12 panchayats from which the animals were brought. *Fasciola gigantea* was found exclusively also in the western region of palpa (Mujhung -1 case; Juthapaua 3 cases)

Results from table 5 indicate clearly that the different kind of mixed infestations are spread out evenly in the whole area. This could be due to the frequent inter-panchayat sale and purchase of animals. This fact has also been studied in the CNAS report of Tinau Watershed Project.

Conclusion

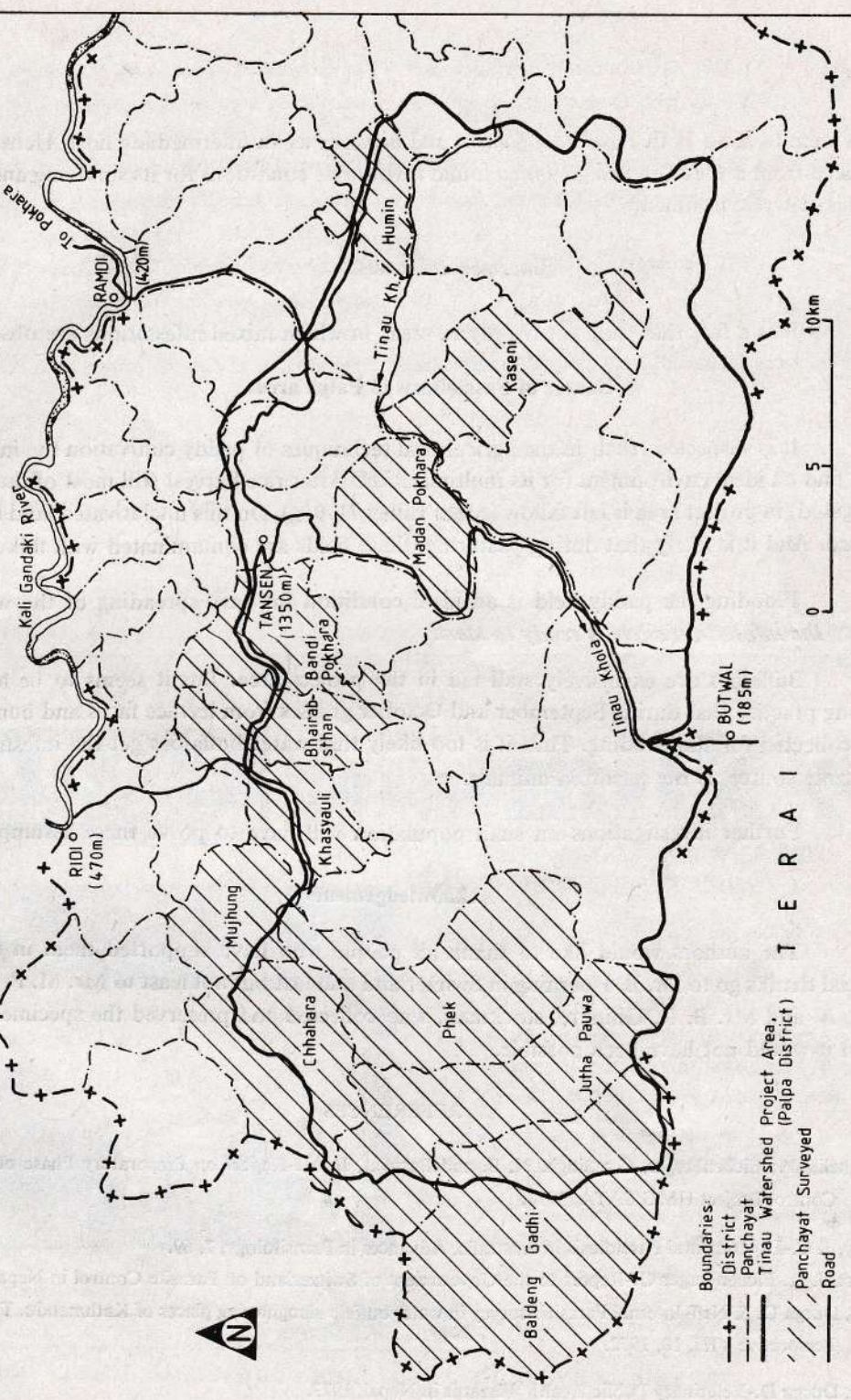
Origin of Mixed infestations

It was generally assumed so far that *Fasciola gigantea* occurs in the Terai belt (low land) whereas *Fasciola hepatica* is observed in the low and mid hills and the alpine belt.

As it has been described by various authors for Thailand, Turkmenia and certain areas of Armenia, similarly the authors in the present investigation also found mixed infestations with predominance of *Fasciola gigantea* in Palpa district. A tentative explanation of this phenomenon can refer to the fairly new practice of keeping water buffaloes in the Tansen area. In the beginig they were all purchased from the Terai where *Fasciola gigantea* occurs normally. Hence the first water buffalo brought to the region took also *Fasciola gigantea* along.

The intermediate host of *Fasciola gigantea* is an aquatic snail, though this fluke species according to Boray is less specifically bound to a particular snail species. Some snail species

Location of Panchayats covered by the Liverfluke Survey; July - Aug. 1981



even seem to serve both *Fasciola gigantica* and *hepatica* as an intermediate host. Hence it can be deduced from it that *Fasciola gigantica* found favourable conditions for its surviving and spreading in this new environment.

Intermediate forms:

It is a fact that they occur only in areas in which mixed infestations are observed.

Source of Fascioliasis in Palpa area:

It is suspected that in the agricultural techniques of paddy cultivation the intermediate host find an ideal environment for its multiplication. After rice harvest still most of the khet land (irrigated) in project area is left fallow (Madi valley 71.9%). On this unclutivated land livestock is grazed. And it is likely that during pasturing, these fields are contaminated with fluke eggs.

Flooding the paddy field is an ideal condition for easy spreading of the water snail. Hence the infestation circle is ready to start.

Buffaloes are exclusively stall fed in the project area. But it seems to be a common feeding practice that during September and October grasses from terrace faces and bunds are cut and collected for stall feeding. Thus it is too likely that water buffaloes get the infestation from the same source as the pastured animals.

Further investigations on snail population will have to prove these assumptions.

Acknowledgements

The authors would like to thank all people who have supported them in their task. Special thanks go to Dr. B. Hoerning in Switzerland and last but not least to Mr. M. P. Kayastha, J. T. A. and Mr. B. R. Ghimire, stockman, who collected and preserved the specimen, without them it would not have been possible.

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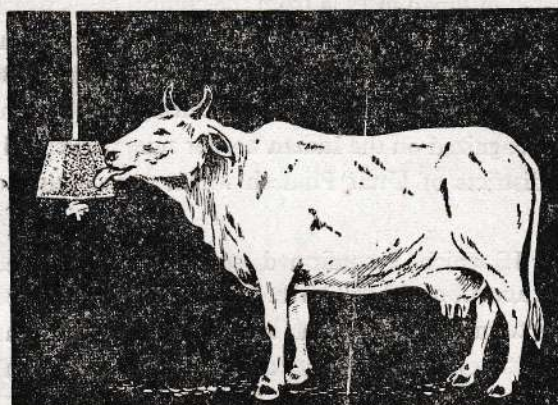
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SEROLOGICAL SURVEILLANCE OF VIRUS ENCEPHALITIS IN NEPAL

II. Serological survey of pigs, birds, and other animal population for JE in the epidemic areas following the outbreak in 1979 & 1980.

D. D. Joshi* and S. Gaidamovich**

Introduction:

Virus encephalitis is an infection of the central nervous system caused by arthropod-borne viruses transmitted to man through arthropod bites. JE virus is one of the member of the arthropod arbovirus group B which is now known as flavivirus. Most of the arboviral infections are zoonotic diseases having their natural epidemiological cycle in domestic and wild vertebrates and blood sucking arthropods. These viruses are generally maintained in enzootic forms and appear as focal outbreak under specific ecological conditions (WHO Report 1979). JE is also a zoonotic disease which infects mainly animals and incidentally man. When humans are infected it is a dead end for the virus. Among vertebrate hosts other than man are mainly pigs (both wild and domestic pigs), horses, birds and to some extent cattle and buffaloes.

Japanese Encephalitis (JE) is an important disease of humans in all south and south-east asian countries. It was first studied and isolated in Japan in 1934 from the clinical cases which were involved up to 8000 cases annually. In Nepal, the first outbreak was found in Rupandehi district in 1978 and later affected in all 23 terai districts. From 1978 to 1980 there were about 1200 human cases recorded and mortality rate was 30% (Joshi 1981 a. & b.). During the months September and October 1978, JE outbreak was recognized on the Indian border side, particularly in Gorakhpur, Deoria and other surrounding districts of Uttar Pradesh which was confirmed serologically (Bhardwaj et al 1981).

Pigs are important amplifying hosts for JE which is confirmed serologically. Infected pigs do not develop any clinical symptoms except abortion and still birth. Mosquitoes get infected by biting the infected pigs and transmit the virus to man. In Nepal, no serological investigations were so far attempted to trace out the antibodies titre on pigs and other animal sera. This was the

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first serological study carried out in the country with close collaboration between WHO and HMG Nepal.

Materials and Methods

Serum samples: A total of 146 blood samples (91 piglets, 40 ducks, 6 poultry, 4 pigeons, 2 buffaloes, 2 cattle and 1 horse) were collected randomly during the month of March 1980 and in March to mid June 1981. The samples collected in the most endemic areas of Morang, Sunsari, Saptari, Sirha, Bara, Parsa, Nabalparasi, Rupandehi, Kapilbastu, Kailali and Kanchanpur. The serum were separated and transported to Viral Zoonotic Lab. Unit of Central Health Laboratory, Kathmandu.

Antigens and Antisera: Antigens used were:

Arboviruses group B, JE.

Dengue - 2 and Dengue - 4 antigens.

All the sera were treated twice with acetone to remove non-specific inhibitors and then absorbed with goose red blood cells to remove non-specific agglutinins. Three units of antigen-JE, Dengue- 2 and Dengue - 4 were used and known positive and negative serum was included in each test to avoid the possibilities of cross antigenic reaction. The sera were tested by haemagglutination inhibition (HI) test on micro U plates as per the method of Clarke and Casals (1958).

After testing in Kathmandu, these all sera were absorbed in the special filter paper discs by a standard procedure and sent to Virus Research Institute, Bangkok as well as to Ivanovsky Institute of Virology, Moscow for further confirmation. Results received from both the places were similar to the author's findings.

The lowest dilution of serum tested was 1:10 in HI test.

Results :

Out of 146 serum samples tested, 41 samples (28.1 %) were found to be positive against JE, Dengue- 2 and Dengue- 4 antigens. Detail results of positive sera is presented in Table 1. Among these 41 positive samples all were positive against JE, 11 against Dengue- 2 and 10 against Dengue- 4. 30 of these sera were positive only against JE.

Table 1 Serological test result of animal serum for JE

S. N.	Date of blood collection	Type of animals	Sex	Age in months	HI antibody titre result		
					JE	DEN-2	DEN-4
1.	10/3/80	Pig landrace	F	8	10	<10	<10
2.	" "	" "	M	5	10	<10	<10
3.	4/3/80	" "	F	8	10	<10	<10
4.	" "	" "	F	8	80	20	20
5.	" "	" "	M	8	10	<10	<10
6.	" "	" "	F	6	10	<10	<10
7.	16/3/80	" "	F	5	10	<10	<10
8.	" "	" "	M	6	20	20	40
9.	17/3/80	Wild Pig	F	8	10	<10	<10
10.	" "	Pig local	F	3	20	<10	20
11.	" "	" "	M	3	10	<10	<10
12.	18/3/81	" "	F	4	40	10	40
13.	21/3/81	" "	F	6	80	40	80
14.	31/5/81	Duck	M	6	20	<10	<10
15.	" "	Pig cross breed	M	4	20	10	<10
16.	" "	" "	M	3	20	<10	<10
17.	1/6/81	Duck	F	3	20	10	<10
18.	" "	Pig local	M	4	20	<10	<10
19.	3/6/81	Duck	M	3	20	<10	10
20.	" "	" "	F	4	20	20	<10
21.	" "	" "	F	3	20	10	10
22.	5/6/81	" "	M	5	20	<10	<10
23.	8/6/81	" "	F	5	20	20	10
24.	10/6/81	Pig local	M	3	80	20	40
25.	2/7/81	Pig landrace	M	3	10	<10	<10
26.	" "	" "	M	4	10	<10	<10
27.	" "	" "	F	5	10	<10	<10
28.	" "	" "	M	6	10	<10	<10
29.	" "	" "	F	6	10	<10	<10
30.	3/7/81	" "	M	4	10	<10	<10
31.	" "	Duck	F	4	40	20	20
32.	7/7/81	Pig yorkshire	F	3	10	<10	<10
33.	" "	" "	F	2	20	<10	<10
34.	" "	" "	F	7	10	<10	<10
35.	" "	" "	F	2	20	<10	<10
36.	" "	" "	M	3	20	<10	<10
37.	" "	" "	F	8	20	<10	<10
38.	" "	" "	F	18	20	<10	<10
39.	10/7/81	Pig landrace	F	2	10	<10	<10
40.	" "	" "	M	2	10	<10	<10
41.	12/7/81	Pig local	M	3	10	<10	<10

Out of 91 pig sera tested, 33 samples (36.3%) were found to be positive and 40 ducks sera tested, 8 samples (20%) were found to be positive for JE (Table 2.). Rest 105 samples were negative for JE, DEN-2 and DEN-4 antigens

Table 2. Total number of pigs and ducks sera found positive and different HI antibody titre for JE.

S. Type of N. animals	No. of sera tested	Positive for JE	Sera shown HI antibody titre for JE				
			10	20	40	80	%
1. Pigs	91	33	19	10	1	3	36.3
2. Ducks	40	8	—	7	1	—	20.0
Total	131	41	19	17	2	3	31.3

Discussion and Conclusions:

In Nepal, JE in human has been confirmed clinically and serologically in the month of October 1978. Thus the recognition of the prevalence of this disease in Nepal posed many public health problems, like diagnosis of the disease and understanding the control measure with an adequate understanding of the complex ecology of the JE virus. Hence a study was proposed and conducted as an especial programme under WHO/NEPAL-ESD 001/E.

The present serological survey on animals and birds was carried out only in highly endemic areas of JE where many human cases were recorded. This was the Pre and post epidemic JE sero-survey done on animal sera of different localities.

In this study, pigs and ducks sera were found positive for JE. The HI titre of JE antibody observed was higher than DEN-2 and DEN-4 antibody in all the positive sera which indicates that this is a response to JE infection in pigs and ducks and not merely a cross reaction to Dengue viruses.

The mosquito vectors culicine species for JE were also collected, identified and recorded in the JE epidemic areas of Nepal (S. Pradhan 1981).

Examination of serological evidence in this study indicates that positive serological evidence of JE virus is widely distributed in local, improved and cross breed pigs and also in ducks population of the endemic areas in the country.

Although present study indicates that enzootic foci of JE virus are likely to be more widely distributed in both rural and urban pig and bird population, further studies are needed to bring out a clear picture and determine such foci in rural and urban animal and bird population of the country.

Acknowledgements:

The authors are grateful to Dr. I. B. Khatri, Chief Epidemiologist and Dr. V. L. Gurubacharya, Chief, Central Health Laboratory for permission to carryout this work, and to Director, Ivanovsky Institute of Virology, WHO collaborating centre, Moscow, USSR for the supply of

antigens, antisera and special filter discs. The study was conducted with the financial and technical support of the WHO/SEARO, New Delhi. The authors wish to express the appreciation to Director, Virus Research Institute, Bangkok, for running the serological tests. Thanks are also due to Mr. Puskal Regmi and Mr. Nirmal Acharya for their help in blood collections and serological investigations.

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A KILLER DISEASE IN DOGS

N. B. Singh*

A killer disease of dogs and young pups, clinically similar to canine parvovirus disease, described by several workers, is spreading as a new dog disease in Nepal.

A review of literature on the work on similar disease in many countries has clearly shown a virus known as canine parvovirus (CPV) as the etiological agent of this type of disease. Australian workers, Johnson and Spradbrow (1979) isolated CPV, a virus closely related to feline Panleukopenia virus (FPV) from dogs with severe enteritis, Robinson et al (1979) found CPV to be the cause of severe myocarditis in puppies, Lenghans and Studdert (1980) isolated CPV from 10 dogs with enteritis and 8 dogs with myocarditis, on comparative study author observed that all the strains of CPV isolated and feline panleukopenia were identical in serum neutralization test. In Canada, Hayes et al (1979) studied cases of sudden death with myocarditis in four to six week old pups, CPV was found to be the cause of myocarditis. They succeeded to reproduce experimentally CPV enteritis in dogs by injecting the homogenates from myocarditis cases.

Appel et al (1978) isolated CPV like virus from dogs with haemorrhagic enteritis in UK, the virus was similar to canine and feline panleukopenia virus and agglutinated pig and rhesus monkey RBC. In UK, McCandlish et al (1979) isolated and identified CPV from faeces of young dog with symptom of vomition and diarrhoea and cases of fatal myocarditis in 3 to 4 week old puppies, 5 to 6 week old puppies had histological evidence of myocarditis but no active myocarditis, Whereas dogs above eight week had only gastroenteritis.

Nelson et al (1979) observed outbreak of CPV disease in seven kennels of USA with death occurring in 2 to 3 days, morbidity found to be 20 - 100 % and mortality slightly lower. Virus isolated from these cases reacted with the antibody to feline panleukopenia virus in immunoelectron and fluorescentmicroscopy. Jezyk et al (1979) studied causes of sudden death in 4 to 8 week old pups from 10 litters, authors described myocarditis with intracellulalr inclusion bodies and mono-nuclear infiltration.

It is now believed by some workers that this CPV is a mutant of feline panleukopenia virus which changed or went wild or began infecting dogs, others believe that CPA-1 complemented by the widespread use of modified live canine adenovirus vaccine over past two years resulted in stable infective parvovirus CPV-2.

* Chief, Animal Diseases and Parasite Control Division, Tripureswar Kathmandu.

Observation

The disease has been grouped under two groups depending on the symptoms observed.

Group I. (Dogs above 10 weeks old)

Common clinical symptoms:-

Gastroenteritis
 Nausia and frequent vomition (lasting 2 to 12 days)
 Nature of vomit:- frothy, watery with or without bile.
 Off feed
 Temperature; sub normal (98-100°F.)
 Pulse; feeble
 Respiration; shallow
 Onset of diarrhoea; from the day the dog found sick or 2 to 3 days after onset of symptoms

severe dehydration, depression
 death or recovery mostly within 7 days

Blood Examination - Leucopenia (6000-3000)

Postmortem findings of a 2 year old dog:-

Haemorrhagic ulcers in the body of stomach.
 Catarrhal gastritis
 Petechial or echimotic haemorrhagic patches in the mucosa of pyloras.
 Small intestine with necrotic patches covered with brown foetid faecal material or thick bile, skeletal muscles dehydrated,
 No change in heart, lung, liver and kidney, Visceral lymphnode swollen.

Group II. (pups under 5 weeks)

Sudden death (cardio-pulmonary symptom)

Common clinical symptoms:-

Sudden death - death within hours without noticeable symptom.
 Depression, huddle in corner, grunting sound produced, completely off feed, shrunken eyes with tears, nose dry or with watery discharge, comma
 Pulse; feeble
 Heart beat; slow
 Temperature; sub normal (98-100°F.)
 Termination; in most of the cases fatal on second or third day.

Postmortem findings of two, 4 weeks old pups:-

Cardiomegaly, pulmonary congestion

No other significant gross changes

Observation of two infected litters:

Case A. A bitch, 3 week after whelping become sick with symptoms of vomition and bloody diarrhoea. She recovered after 7 days of symptomatic treatment. All 4 pups in the litter become sick with symptoms of acute myocarditis after recovery of the bitch and died on second and third day of sickness. The morbidity in 4 weeks old pups was 100% and mortality was also 100%.

Case B. A bitch with 7 pups started showing symptoms of vomition and diarrhoea 4 week after whelping but recovered after 5 days. Except 2 pups which were weaned and taken away from mother before the mother showed symptoms all 5 pups developed symptoms of myocarditis of which 3 died within 5 days and 2 recovered after intensive care. The morbidity in this case was 100% and mortality 60%.

The average of both the cases was morbidity 100% and mortality 80%.

Table 1. Cases of gastroenteritis reported at Central Veterinary Hospital, Kathmandu shows that there is no strict seasonal pattern of gastroenteritis cases and also GE represents 17.8 to 20.17% of the total dog patient brought for treatment, many of which may be the cases of parvovirus enteritis.

Table 2. Mortality in young pup is 26.3% whereas it is 11.6% in adult cases. The non reported cases not being considered.

Conclusion

The disease is fatal in young pups, as they develop acute myocarditis, It seems likely that myocarditis and gastroenteritis symptoms in dogs represent different manifestation of the same parvovirus infection as has been described by several authors else where. As believed by McCandlish et al (1978) there seem some tropism of canine parvovirus to rapidly growing and dividing cells. The multiplication and growth rates of the heart muscle and intestinal mucosa at different age of dogs may be a deciding factor in the pathogenesis of the different form of parvovirus infection. The review of the work from several workers indicate that canine parvovirus is antigenically similar to the feline panleukopenia virus. Feline pan leukopenia virus vaccine has been used as alternative protective vaccine against parvovirus infection.

As experienced by author the percentage of death in young pup can go as far as 100% and a few pups can respond positively to proper care and treatment. In two litters under study morbidity was 100% in both but 100% (4/4) mortality was observed in pups which were nearing

4 week of age where as 60% (3/5) mortality was observed in pups above 4 week of age. It showed that chances of survival is more when the pup grows older even by few weeks or days.

Table 1 show that the total cases of dogs brought in Central Veterinary Hospital, Kathmandu, gastroenteritis cases represent 17.8 to 20.17% which is a significant number. Many of these cases may be the cases of parvovirus enteritis.

Table 2 shows that the mortality in pups of all age included is 26.3% where as mortality in adult reported cases is 11.6% which also indicate the tendency of the disease to cause more damage in young age group.

As the virus is shed in the faeces of the infected dogs, gastroenteritis cases are a great source for epidemiological spread of the disease.

Detailed study of this disease with emphasis to its virological and pathological aspects needs to be under taken to gain more information on this fatal disease of canine.

Summary

A fatal disease of pups and adult dogs has been spreading in Nepal since last few year. The symptoms of the disease are similar to a new disease, canine parvovirus enteritis or canine parvovirus myocarditis (sudden death syndrome) described by several workers else where. The symptoms, lesions morbidity and mortality of the disease in pups and adult is described.

Acknowledgements

The author is thankful to Dr. Uday Singh, Central Veterinary Hospital for permitting the Collection of data. Thanks are also due to Dr. Pawan K. Agrawal for his help during the study and data collection and to Mr. Dipak Malla and Mr. Raju Munkarmi for their assistance.

Table 1. Total dog patients registered at Central Veterinary Hospital, Kathmandu compared to gastroenteritis cases from January 1980 to December 1982.

Months	1980			1981			1982		
	total cases registered	gastroenteritis cases			total cases registered	gastroenteritis cases			total cases registered
		pup	adult	total		pup	adult	total	
Jan	519	89	83	172	219	29	40	69	382
Feb	308	37	27	64	312	37	26	63	377
Mar	211	22	17	39	386	52	66	119	351
Apr	423	29	31	60	342	37	37	74	275
May	256	16	30	46	306	24	38	62	315
Jun	302	17	31	48	310	22	31	53	296
Jul	279	10	17	27	287	21	47	68	284
Aug	253	7	21	28	281	14	24	38	297
Sep	276	9	20	29	240	11	14	25	176
Oct	57	7	19	26	320	20	22	42	145
Nov	183	13	44	57	441	30	51	81	241
Dec	338	16	23	39	297	29	30	59	281
Total	3405	272	363	635	3741	326	426	753	3320
									220
									389
									609

Table 2. Parvovirus like cases of pups and adult dogs from August 1981 to December 1982*

Year	Months	Total cases	Pups			adult dogs			
			Total cases	R	D	NR	Total cases	R	D
1981	Aug.	4	—	—	—	—	4	2	2
"	Sep.	5	3	2	1	—	2	1	—
"	Oct.	3	2	1	—	1	1	1	—
"	Nov.	2	1	—	—	1	1	—	—
"	Dec.	3	—	—	—	—	3	2	—
1982	Jan.	3	2	2	—	—	1	1	—
"	Feb.	15	5	3	—	2	10	4	2
"	Mar.	11	5	4	1	—	6	2	—
"	Apr.	9	8	3	1	4	1	1	—
"	May.	8	5	1	2	2	3	3	—
"	Jun.	9	9	4	4	1	—	—	—
"	Jul.	3	2	—	1	1	1	1	—
"	Aug.	3	2	2	—	—	1	1	—
"	Sep.	3	2	1	1	—	1	1	—
"	Oct.	3	2	2	—	—	1	1	—
"	Nov.	4	2	1	1	—	2	2	—
"	Dec.	12	7	3	3	1	5	3	1
Total		100	57	29	15	13	43	29	5
									11

R=Recovered, D=dead, NR=Not reported

* Source Kathmandu Veterinary Clinic, Maharajganj, Kathmandu.

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STUDIES ON ACUARIA SPIRALIS, THE PROVENTRICULAR WORM OF POULTRY

Pawan K. Agrawal*

Introduction:

Acuaria spiralis (Molin, 1858), a nematode parasite of chicken, turkey, pigeon, guinea-fowl etc, belong to the family acuariidae. Because this parasite mostly occurs in the proventriculus and due to its spiral shape, it is commonly known as proventricular or spiral worm.

The life cycle of the parasite involves an intermediate host, a species of isopod i. e. pill-bug (*Armadillidium vulgare*) or sowbug (*Porcellio scaber*). The birds become infected with this nematode by swallowing infected intermediate host with food and water.

A. spiralis (Synonyms: *Dispharynx nasuta*, Rudolphi: 1819) had never been a problem to poultry farmers in Nepal, but many cases had been recorded during the last two years. Since there is no satisfactory treatment against this parasite, it will be wise to adopt proper control measures before an outbreak occur and causes heavy losses to the poultry farmers.

In November 1981, an outbreak of *A. spiralis* was observed in a flock of 74 birds raised at Biological Products Division. A few more outbreaks of *A. spiralis* in poultry farms had been recorded in Kathmandu valley.

Nematodes of this genus caused heavy loss in swan (Mognihan and Stovell 1955), pigeon (Pattern), in domestic geese and ducks (Gibson and Barnes 1957). Pathology of *A. spiralis* on experimentally infected fowls was described by Nagy et al in 1977. This parasite is most prevalent in Cuba between December and February, Birova et al (1977) described the seasonal dynamics of this parasite. Factors responsible for the outbreak and rapid propagation of intermediate host species was described by Macko et al. In Nepal, occurrence, gross and histopathology of the *A. spiralis* was described by Singh et al in 1975.

Materials and Methods :

In a flock of 74 birds, 24 died due to heavy infestation with *A. spiralis*. Postmortem of all the dead birds was carried out after recording clinical symptoms of the sick birds. Lesions and total number of worms from each proventriculus were noted. Twenty worms taken randomly from proventriculus of five birds and length of each worm was measured. A few worms were

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collected for morphological study. Six heavily damaged proventriculus were collected in 10% formaline for histopathological examination. Selected specimens were processed for histopathological slide preparation. Sections were cut and stained with routine H & E technique.

Observations:

Clinical Symptoms:-

Infestation with proventricular worm caused emaciation anaemia and weight loss. Ruffled feather, droopiness, weakness, diarrhoea and unthriftiness were the main clinical symptoms observed. Hind part of the birds was soiled with foetid faeces. Heavy infestation caused death of the sick birds. Mortality was 34%. The parasite caused detrimental effects on the growth and weight gain.

Necropsy finding:-

Postmortem of the dead birds revealed emaciation and anaemia. Numerous worms had penetrated into the wall of the proventriculus (Fig. 1). Mucous membrane of the glandular stomach was tremendously thickened and macerated. Ulcerated patch, nodules, healed scar of ulcer, petechial haemorrhage and necrosed area were the common lesions on the proventriculus. Oesophagus, gizzard and intestine were quite normal, except in a few cases, a red patch was observed just anterior to the proventriculus (Fig. 1). The extensive damage in the proventriculus was probably the cause of death of the birds.

The infected birds harboured between 20 to 60 (average 35) of the nematodes.

Histopathological findings:-

Haematoxylin-eosine stained histological slides revealed extensive destruction of the proventricular glands and marked cellular infiltration of the underlying tissue. The simple columnar epithelial layer was degenerated with area of necrosis. The tunica mucosa and tunica submucosa was highly congested and infiltrated with lymphocytes (Fig. 2). The lamina propria revealed oedematous fluid infiltration.

Morphology of the worm:-

The worms were white to pale cream in colour. The length of the worms ranges from 7.5 - 10 mm. The female was found to be slightly longer than the male worm. The cordons were present on the anterior end, originating at the base of the lips and were recurrent, the distal extremity of the cordons turning forward and extending anteriorly a short distance (Fig. 3). The worm mostly were rolled in a spiral form.

In male, five pairs of postanal and four pairs of preanal papillae were present. In female, vulva was situated in the posterior part of the body. The tail of the male was spirally coiled. The embryonated ova was elliptical in shape and brown in colour.

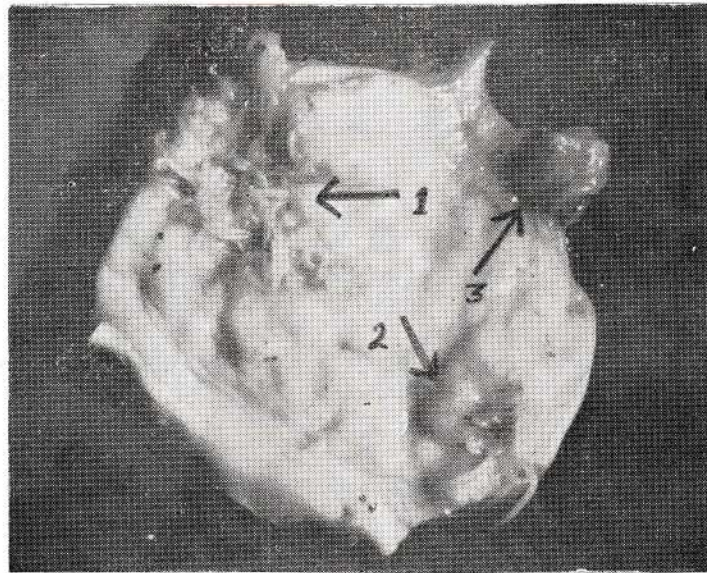


Fig. 1. Proventriculus of a dead bird with worms.

1. Worms penetrated into the wall of proventriculus.
2. Healed scar (nodule)
3. Heamorrhagic patch.

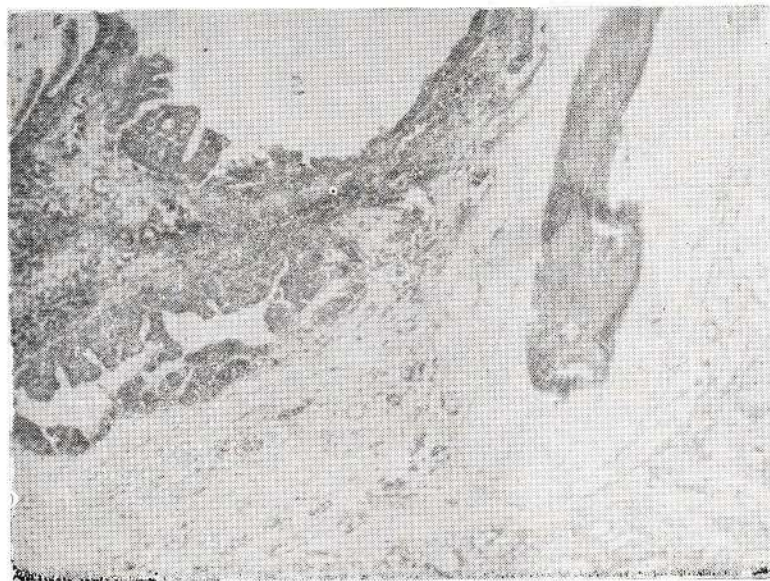


Fig. 2. A section of proventriculus showing lymphocytic infiltration

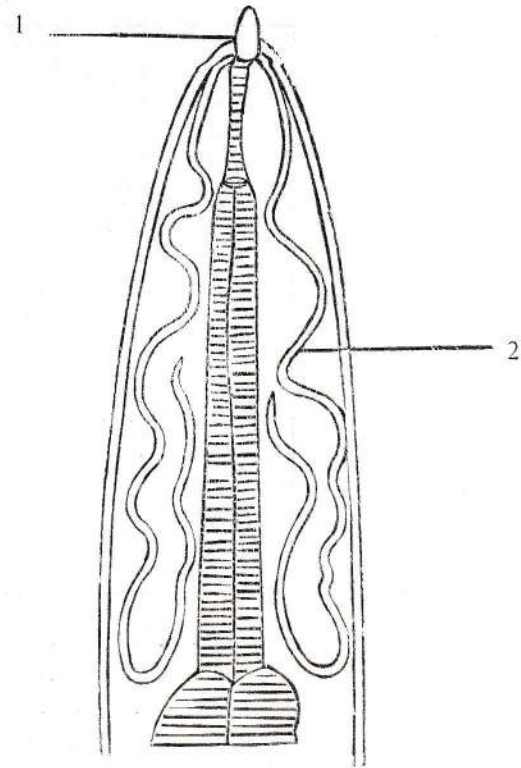


Fig. 3. a. Head end of *Acuaria spiralis*

1. Mouth part, 2. Recurrent cordon

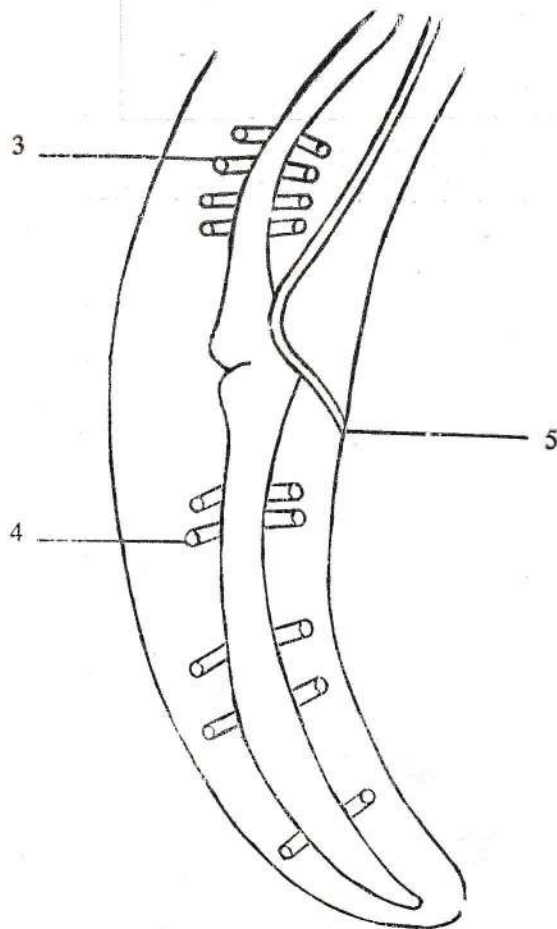


Fig. 3. b. Tail of a Male worm

3. Freanal Papillae, 4. Postanal papillae, 5. Anus

Conclusion:

The lesions observed in this study suggested that this parasite causes severe damage to the proventriculus of the infested birds resulting in decreased digestive power and weight loss. The lesions in heavily infested birds were extensive enough to cause death of the effected birds.

In Nepal, this parasite is not so common in the commercial poultry farms but it occurs frequently in the back-yard farms where people raise few birds in free range.

Prevalence of the infection depends on overcrowding, poor hygiene, management, high rain fall and warm climate which favoured reproduction of the intermediate host. Possibility of an outbreak in poorly managed poultry farms can not be ruled out. Since there is no satisfactory treatment available against this parasite, proper control measures should be undertaken.

Further investigation on country wide incidence, life cycle, its economic impact and selection of effective anthelmintic is yet to be worked out.

Summary

Infestation of chicks with *Acuaria spiralis* was studied. Numerous nematodes seen with their head burried deep into the wall of the proventriculus causing an extensive damage to the host. Out of 74 birds, 24 died due to heavy infestation with *A. spiralis*.

The morphology of the worm, gross and microscopical changes has been described. Total numbers of worms found in each proventriculus varied between 20 to 60 (average 35)

Acknowledgements :

The author is grateful to Dr. M. N. Pandey, Chief, Biological Products Division and Dr. N. B. Singh, Chief, Animal Diseases and Parasite Control Division for their valuable guidance and encouragement during this study. The author expresses thanks to Dr. B. K. P. Shah and his staff for the help in histopathological work.

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THE USE OF XYLAZINE AND KETAMINE HYDROCHLORIDE IN COMBINATION IN ZOO ANIMALS

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Introduction

Xylazine in combination with other tranquilliser have been chosen as a potential restraint because of its successful usage in the chemical restraint of a number of domestic and wild species. It has provided good sedation, muscle relaxation plus a wide margin of safety. It causes a few changes in blood parameters and is quickly administered. A combination of xylazine and ketamine hydrochloride has been used in different species of zoo animals. The purpose of this combination was to reduce the drug volume to fill the dart syringe as well as to study the efficacy, compatibility and induction time of xylazine for quick sleep.

Materials and Methods

10% xylazine and 10% ketamine hydrochloride, ready to use solution were used in different zoo animals to restraint them for minor surgery, treatment and for reintroduction and translocation to the natural habitat. The required volume of drugs, after the rough estimation of body weight of the animal, were filled in the same dart syringe and injected intramuscular with the help of blow gun. Immediately after immobilization each was weighed and then necessary procedures followed.

Results and Discussion

Big cats:— Doses from 1.2 to 2.0 mg/kg body weight of xylazine and 3.0 to 5.1 mg/kg ketamine were used (Table). Those combination of dose levels were sufficient to induce sleep in leopard. The sleep induced was deep where no pain reflex could be elicited. By the observation of duration of sleep, it was estimated that minor surgical operations could be performed at this stage. In all the cases of leopard, tiger and lion nausea was noted after 4–5 minutes of injection (Philo, L. Michael) but vomition occurred only in two cases (all were fed 16 hour prior to injection). Though the doses of xylazine used during this study were lower than the dose recommended for wild cats, sedation with sleep was achieved with the stated dose level in combination with ketamine. (Recommended dose of xylazine alone for wild cats is 8–10 mg/kg). Though the dose rate in leopard

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was lower (1.2 – 2.0 mg/kg) than in lion and tiger (2.7 – 2.9 mg/kg), the depth of sleep was seen to be very good in leopard than in lion and tiger. The doses of ketamine was also slightly lower than the recommended. The results also indicated that high doses of xylazine in combination anaesthetised the animals quickly for a longer duration. Whereas with high dose of ketamine, the initiation was slower, anaesthesia late but recovery was earlier. After recovery one leopard became furious for 3–4 days and was reluctant to eat for 8 hours, but in other cases no deviation was noted.

Antelope: In barking deer, dose rate of xylazine and ketamine ranges from 1.2–2.6 mg/kg and 4–5 mg/kg respectively. The recommended dose of xylazine is 2–3 mg/kg which is slightly higher than the doses used in this trial. The initiation of action in one case was too early i. e. complete sedation was achieved within 4 minutes of injection, whereas in other cases, sedation was achieved in about 10 minutes. In first case, though the dose rate of xylazine was normal, duration of sleep was quite short (55 minutes) and pain reflex could also be elicited in later half stage of sedation. This may be due to individual variation or may be due to the fact that less tractable animals require higher doses than that of more tractable ones. (Himes & Jones). It was noted that xylazine even in slightly low dose rate than the recommendation can be safely used in combination with ketamine in barking deer.

Black bucks: The dose rate of xylazine and ketamine used in this trial varies from 4.5–5.5 mg/kg and 6–8.3 mg/kg respectively. Though the dose rate used for black bucks was slightly higher than the recommendation, no complications or toxic effect was noted except a premature birth from a female black buck occurred after injection (the female being not known to be pregnant). Slight tympany developed in a few cases which subsided after the animal began to move.

recovery in all the cases was smooth, uncomplicated and no undesirable effects or post operative complications were observed after recovery.

Important side effects noted: 1. strong mydriasis 2. Nausea and vomition 3. Transient slight rise in temperature and 4. Retarded ruminal tone.

Conclusion

Xylazine plus ketamine hydrochloride is an excellent combination for the induction of sedation and sleep in zoo animals. Their action is not similar in all animals but in most of the cases, the combination causes sleep like action during which transportation, translocation and minor surgical operations can be performed in the zoo animals.

Table : The effect of xylazine and ketamine in combination in zoo animals

Animals	drugs mg/kg body weight		begin of sleep	sleep level	begin of awakening
	xylazine	ketamine			
1. Leopard (<i>Panthera pardus</i>)	1.28	5.1	11 min.	deep	1 hr. 50 min.
2. " "	1.6	4.3	8 1/2 min.	deep	2 hr. 29 min.
3. " "	2.0	3.0	7 min.	deep	2 hr. 15 min.
4. Lion (<i>Panthera leo</i>)	2.9	0.35	8 min.	deep	2 hr. 11 min.
5. " "	2.7	0.35	10 min.	deep	2 hr. 20 min.
6. Tiger (<i>Panthera tigris</i>)	2.7	0.38	11 min.	deep	1 hr. 56 min.
7. " "	2.7	0.38	12 min.	deep	2 hr.
8. Barking deer (<i>Muntiacus muntjak</i>)	2.3	5.0	4 min.	light	55 min.
9. " "	1.2	4.0	8 1/2 min.	deep	1 hr. 22 min.
10. " "	1.4	4.3	13 min.	deep	2 hr. 10 min.
11. " "	2.6	5.0	10 min.	deep	2 hr. 40 min.
12. Black bucks (<i>Antelope cervepara</i>)	5.5	8.3	8 min.	deep	1 hr. 32 min.
13. " "	5.4	8.0	6 min.	deep	1 hr. 40 min.
14. " "	5.2	7.5	9 min.	deep	1 hr. 45 min.
15. " "	5.2	7.5	10 min.	deep	1 hr. 32 min.
16. " "	5.0	7.2	7 min.	deep	1 hr. 30 min.
17. " "	5.0	7.0	7 min.	deep	1 hr. 40 min.
18. " "	4.5	6.0	12 min.	midium	1 hr. 15 min.
19. " "	4.5	6.0	15 min.	medium	1 hr.

Summary

The efficacy of xylazine and ketamine hydrochloride in combination was studied in leopard, tiger, lion, barking deer and black bucks. It was observed that in big cats, xylazine and ketamine in combination (1.2–2.9mg/kg and 0.35–5.1 mg/kg respectively) intramuscular could induce sleep like stage. The same stage in barking deer could be achieved with xylazine and ketamine at the dose rate of 1.2–2.6 mg/kg and 4–5 mg/kg respectively. In black bucks the dose rate of xylazine and ketamine was 4.5–5.5 mg/kg and 6–8.3 mg/kg respectively. In one case of barking deer, the animal was under light sleep, the sedation was sufficient to allow further administration of anaesthetics easily and could serve the zoo purpose.

The main side effects were mydriasis, nausea, vomition and ruminal atony.

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CONTROL OF GID IN GOATS

B. K. P. Shah*

Tape worms need two or more hosts to complete their life cycle. *Multiceps multiceps*, a common tape worm of dog, fox, jackal etc. is one of the important tape worm. The larval stage in ruminant host (intermediate host) cause a disease called gid. An infected carnivorous sheds a large number of tape worm ova (oncosphere) on the ground in its stool and contaminates the pasture. When the ova are swallowed by the intermediate host (ruminants) while grazing the infected pasture, the shell of the ova is dissolved by the action of pancreatin and bile in the small intestine. The embryo penetrates the wall of the intestine and enters circulation and get lodged in suitable tissue where they encyst and produce a bladder like structure containing fluid. The cells of the wall of the cyst give rise to scolex which then is known as *Coenurus*. The carnivorous get infected by eating such cysts from the ruminants. The scolex present in the cyst develop into tape worms in the gut of dogs, thus the life cycle goes on.

The coenurus have preference for the brain tissue for which the name *coenurus cerebralis* has been given. The presence of *coenurus cerebralis* in or on the surface of brain cause different degree of damage to that part of the brain through the constant pressure of the developing cyst. This damage leads to the clinical symptoms of the gid disease.

The only available method of treatment is to remove the cyst surgically where ever possible. The control of gid in animals depends on breaking the life cycle of the tape worm. This is possible only if pasture is protected from contamination with infective ova or the tape worm. An attempt had been made to see the effectiveness of gid control by keeping the dog population free from the tape worm by deworming the dogs in a pocket area.

Materials and Methods:

Stool samples of the dog in the study area were examined prior to treatment and those found infected were treated with 500 mg. of Mansonil¹ powder in 10 ml of water on three occasions at monthly interval i. e. September, October and November, 1980. The non infected dogs were also treated during the last course (the third treatment). Villagers were advised not to feed cysts to the

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¹ Registered Trade Mark - BAYER GERMANY.

dogs and to burn or bury the cysts. The data on the gid situation prior to and after treatment of dogs were collected from the study area.

Table : Incidence of tape worm in dogs after treatment with Mansonil from November, 1980 to May, 1981.

Month/year	Total No. of stool sample examined	No. of samples found negative for tape worm	No. of samples found positive for tape worm
Nov. 1980	24	22	2
Dec. 1980	24	23	1
Jan. 1981	24	24	0
Feb. 1981	38	37	1
Mar. 1981	40	39	1
Apr. 1981	26	26	0
May. 1981	26	25	1

Results:

Table. 1 represents the result of faecal examination for the tape worm infestation from November, 1980 to May, 1981. Marked reduction in tape worm infestation was noted. In one dog the infestation reoccured because it was found to visit buture's shop frequently.

Number of gid cases between October, 1979 and May 1980 was 65 in goat population of 552, an incidence rate of 11.7%. A total of 23 cases were recorded in a population of 380 goats in November, 1980, an incidence of 6%. The over all incidence of gid in the goat population was 4.3% in the observation period.

Discussion:

Niclosamide (Mansonil/Yomesan) had been tested for efficiency in removing the dog tape worm, *Dipylidium caninum* and *Taenia pisiformis* (Poole et al 1973). The result of faecal examination before and after treatment (Table) clearly indicate that the Mansonil powder is effective against *Multiceps multiceps*. The study clearly showed that the incidence of gid in goats could be effectively lowered by clearing the dogs with suitable anthelmintics.

Further work in this direction would be necessary to evaluate the use of Mansonil in tape worm control over a wide area.

Acknowledgements:

The author is grateful to Dr. N. B. Singh, Chief, Animal Diseases and Parasite Control Division for his kind help in supplying Mansonil. The author expresses his sincer appreciation to the villagers of Mohanpur (Saptari) for their co-operation and enthusiasm during the study.

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A STUDY OF GROWTH RATE IN GOATS IN THE TERAI OF NEPAL

R. M. McTaggart* and F. C. Wilkinson**

Introduction

There is little information available on the growth rate and the effects of nutrition and parasitism on growth of young goats in the Terai of Nepal.

A trial using twin goats was undertaken between February and October 1982 and the results are reported.

Materials and Methods

20 pairs of twin goats, 3 – 6 months of age were randomly purchased from the villages of the Janakpur area of the Terai and taken to the Project Resource Farm in February 1982.

One of each twin was allocated to a separate group. Group 'A' was not given any routine anthelmintic treatment whereas group 'B' was drenched monthly with an anthelmintic albendazole. The two groups were kept in separate fenced areas of 140 square metres for the first 66 days and rotated every 2 weeks. The animals were fed ad lib green fresh cut berseem clover by placing in the self feeders. Fresh water was always available. All the goats were housed overnight.

During the next 140 days the two groups were run together and herded during the day for grazing around the resource farm as is the local custom. The trial terminated in October after running for 206 days.

All goats were treated for external parasite on two occasions and for coccidiosis on 3 occasions. Faecal samples were collected at monthly intervals for total worm egg counts. The berseem was weighed before placing in the feeders as was any wastage left. The goats were weighed every 14 days.

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Results:

Total berseem fed in kgs over the 66 days:

	Berseem offered	wastage	Consumed
Group 'A'	5035	520	4515
Group 'B'	5035	524	4511

The combined liveweights in kgs of the two groups:

	Group 'A'	Group 'B'
Initial weight	161	156
66 days	276	299
206 days	357	370

Average growth per goat per day was 98 grams during the first 66 days and 29 grams over the next 140 days.

The range of weights in kgs:

	Lowest	Highest	Average
Start	4.2	10.2	8.0
End	10.0	24.0	18.5

The faecal worm egg counts indicated 1/6 had round worms at the start but 60 days later there were no worm eggs in faecal from either groups and only occasional low egg counts were recovered until the end of the trial.

Discussion

The growth rate of goats when fed ad lib berseem was 98 grams per day. A body weight increase of 285 kgs over 66 days was obtained from feeding approximately 9,000 kgs of berseem. This is a conversion of 1:32 wet weight or 1:3.2 if berseem is 10% dry weight.

The growth rate during the subsequent 140 days when the goats grazed natural pasture was only 29 grams per day. The animals were at a different stage of growth and other factors affecting growth rate such as disease preclude a direct comparison. However the fall in growth rate was immediate and the slower rate of growth persisted until the end of the trial. There was no parasitism or obvious infectious disease. It appears that the natural pasture could not sustain the potential growth rate of the goats.

The anthelmintic treated group gained an average 1.4 kgs. more than the untreated group. This gain occurred in the first 30 days. The animals had some round worms at the start and it appears drenching removed these worms and led to increased weight gains. However self cure occurred in the infected untreated group during the 66 days of berseem feeding and under the system

of management reinfestation did not take place. A possibility is that berseem had some anthelmintic effect.

This observation should be investigated further as parasitism with round worms in similar aged goats kept in villages has been found to be a major disease problem.

Conclusions

This study showed that the growth rate of young terai goats fed berseem and kept relatively worm free was nearly 100 grams a day. This was about three times the growth rate when the same goats later grazed natural pasture. The terai goats thus do have a potential for higher growth rates if nutrition can be improved.

The effects of parasitism could not be defined. An initial 12% increase in liveweight response was obtained by treatment to remove a light infestation of worms. However self cure occurred in the untreated goats and there was no reinfestation.

Acknowledgements

This trial was undertaken using resources provided by the Project Coordinator of the Livestock Development Project. The assistance of the manager Janakpur Resource Centre and his staff is appreciated. The worm egg counts undertaken by Disease Investigation Laboratory, Tripureswar is acknowledged with thanks.

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THE EFFECT OF FUMIGATION AND HOLDING TIME ON EMBRYONIC MORTALITY IN CHICKEN EGGS OF HIGH OR LOW SHELL QUALITY AS MEASURED BY SPECIFIC GRAVITY*

Mrs. Rama Bhurtel,**

Introduction

Fumigation of hatching eggs with formaldehyde gas has been recommended by Stove (1960) to control microbes on the exterior of the shell. Research by Graham and Mitchel (1932) showed that formaldehyde fumigation properly accomplished was effective in killing bacteria on eggs and equipments. However, investigators and hatchery managers have been concerned that fumigation may be a direct cause of embryonic mortality.

To confirm this point, the following studies were conducted on different shell quality eggs held for two different periods of time prior to incubation.

Materials and Method

Experiment I

Eggs were collected from artificially inseminated hens (Cobb female x Arboracher male) and egg's specific gravity determined and separated into two groups; one with specific gravity above 1.080 and another with a specific gravity below 1.080.

One hundred twenty eggs from each group were subdivided into group "A" and "B" for a control and fumigated prior to incubation. Fumigation was done in an empty hatcher. Formalin with commercial grade (2.5 fl. oz.) combined with a Potassium permanganate (1.5 oz) per 100 cubic feet of space at 90-92°F with a humidity of 85-92%. A measured amount of the fumigant potassium was poured into a metal container placed on the floor of the empty hatchery. The formalin was poured into the potassium. The hatcher exhaust was closed for an hour. Fumigated and control eggs were then incubated together.

* Experiment conducted at Alabama University, U.S.A.

** Assistant Poultry Development Officer, Poultry Section, Khumaltar.

Table 1. Comparison of embryonic mortality between control and fumigated groups after 18 day candling

Expt. No.	Specific gravity	CONTROL GROUP					FUMIGATED GROUP				
		Total eggs	Unfertilized number	Fertile number	Embryonic mortality	% E. mortality	Total eggs	Unfertilized number	Fertile number	Embryonic mortality	% E. mortality
I	above 1.080	60	3	57	8	14.0	60	3	57	7	12.28
	below 1.080	60	5*	55	9	16.3	60	4	56	23	41.079
II	above 1.080	60	3	57	3	5.2	60	2	58	8	13.70
	below 1.080	60	3	57	5	8.7	60	3	57	12	21.06
III	above 1.080	60	5	55	3	5.4	60	5	55	6	10.80
	below 1.080	39	6	33	10	30.3	39	7	32	13	40.60

* 2 broken

High embryonic mortality in all fumigated groups.

High embryonic mortality in control group of Expt. No. I (above 1.080 sp. gr.) which is insignificant.

Table 2. 21 st. day observation

Sp. gr.	Expt. No.	CONTROL GROUP			FUMIGATED GROUP		
		Total chick hatched	Late mortality	% Late mortality	Total chick hatched	Late mortality	% Late mortality
above 1.080	I	47	2	4.2	50	—	—
	II	46	4	8.6	48	2	4.1
	III	41	1	2.1	46	3	6.5
below 1.080	I	38	8	21.05	30	2	6.6
	II	49	3	6.12	42	3	7.1
	III	23	—	—	16	3	18.75

High late mortality in fumigated group.

Experiment II

Repeat of Experiment I.

Experiment III

Eggs were held 15 days and the same procedure repeated as in experiment I and II. After 18 days, eggs were candled and early embryonic mortality recorded (Table 1). True fertility was determined in all experiments by candling and carefully breaking-out all eggs which appeared clear or contained dead embryos. At hatch, the number of chicks and unhatched eggs were examined by break-out. Chicks were maintained for five weeks to observe their growth.

Results and discussion

In experiment I, fumigated poor-shell quality eggs had a significantly higher percent embryonic mortality rate when compared to the control group (Table 1 & 2). However, eggs of high shell quality when fumigated suffered less embryonic mortality than the controls.

In experiment II, higher embryonic mortality was found in fumigated high and low specific gravity eggs when compared to the controls.

In experiment III, eggs 15 days old suffered greater embryonic mortality in both control and fumigated groups in low specific gravity eggs. Late embryonic mortality was also found greater in the eggs held 15 days. There was no significant difference in chick weight among treatments.

Results of these experiments demonstrated that fumigation may increase the incidence of early embryonic mortality in eggs of low specific gravity or eggs held 15 days prior to incubation. Increased holding time affected eggs of either specific gravity range with regard to early and late embryonic mortality.

Summary

Three experiments were conducted to study the effects of fumigation on embryonic mortality in poor and high shell quality eggs stored for different lengths of time. The results indicated that fumigated eggs of poor shell quality had higher early mortality than controls. Eggs held 15 days had higher embryonic mortality in both poor and high shell quality groups compared to fresh fumigated eggs.

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EGG LAYING PERFORMANCE OF LOCAL HEN*

Tulsi Prasad Shrestha**

Introduction

The indigenous poultry are mostly maintained in all the villages of Nepal. They are seen scavenging everywhere in search of food. They eat insects, waste grains, green vegetables, kitchen waste, grasses etc. In addition they are sometimes provided with some grains such as maize, millet, wheat, rice and pulses.

The economics of such poultry keeping is not much known. It is believed that they lay few eggs and have low potentiality for meat. Attempts have never been made to exploit their prolificacy and production potentiality by maintaining them intensively or sub-intensively and feeding balance diet.

An experiment was therefore designed to determine the laying performance of local hens, housing them in individual cage and feeding locally made concentrate ration.

Materials and Methods.

A portable bamboo battery house, sufficient to keep 12 hens, (six individual cages on each side) was constructed. The cage were raised up about 4 ft. from the ground on the bamboo poles. The size of each cage was 12 inches x 15 inches, which provided 1.25 sq. ft. floor space for individual bird. The floor was inclined 15° externally to facilitate rolling of eggs in front of individual cage in egg collecting troughs attached externally. Again the split-bamboo floor kept the floor clean as the droppings dropped down on the ground immediately. The feed and water troughs were attached outside the cage. The birds ate and drank by pushing head and neck through bamboo splits.

Twelve indigenous birds were picked up randomly irrespective of age, size and housed after deworming in individual cage for an year. Daily 800 gm. of locally made concentrate feed

* Experiment Conducted at Pakhribas Agriculture Centre, Dhankuta, from September 1975 to August 1976.

** Senior Livestock Development Specialist, Agricultural Project Service Centre (APROSC), Kathmandu.



1
2
3

View of the bamboo battery house.
1. Water trough, 2. Feed trough, 3. Egg trough

and sufficient water were provided. Each cage was numbered and daily egg production from individual cage was recorded for statistical analysis.

Table 1. *The composition and cost of feed*

Ingredients	Parts	Cost per kg at the rate of Rs.	Total Cost Rs.
Maize crushed	66.0	2.50	156.00
Soyabean roasted crushed	29.0	3.00	87.00
Bone meal	3.0	1.00	3.00
Lime stone	1.5	1.06	1.60
Salt	0.5	0.80	0.40
Total	100		248.00

One kg. of feed did cost N. Rs. 2.48 and contained about 20 percent crude protein.

Results and Discussion

Table 2. *Annual egg production by experimental birds in individual cage*

Months	Cage No.												Total Eggs.
	1	2	3	4	5	6	7	8	9	10	11	12	
September	4	2	9	3	8	5	8	7	4	6	10	9	75
October	4	4	10	1	4	7	12	—	13	11	6	10	82
November	10	8	10	2	7	9	8	2	7	9	11	12	95
December	17	1	8	3	15	12	2	1	14	7	17	15	112
January	15	4	15	10	20	14	1	2	7	5	14	7	114
February	16	15	10	4	19	7	4	6	12	7	10	9	119
March	13	9	9	9	14	10	16	9	13	11	17	14	144
April	15	10	10	2	9	10	17	18	7	9	15	9	131
May	19	16	15	4	11	9	12	10	11	8	7	15	137
June	11	11	16	5	3	8	12	9	4	5	13	15	112
July	5	16	17	2	3	14	14	13	14	6	5	11	120
August	—	12	16	7	—	15	13	2	14	2	5	18	104
Total	129	108	145	52	113	120	119	79	120	86	130	144	1345

Average annual egg production = 112 eggs per bird

Table 3. *Food intake by birds and their cost:*

Month	Food intake (kg)	Cost at the rate of Rs. 2.48 per kg.
September	21.600	53.56
October	24.800	61.50
November	24.000	59.52
December	24.800	61.50
January	24.800	61.50
February	23.200	57.53
March	24.800	61.50
April	24.000	59.52
May	24.800	61.50
June	24.000	59.52
July	24.800	61.50
August	24.800	61.50
Total	290.400	720.19

Food cost per dozen eggs = Rs. 6.42

It would therefore be appreciated that the laying performance was almost four times than that was expected. Stress factor due to change of environment, energy loss due to scavenging and poor diet must have been responsible in decreased laying in local birds. Statistical analysis of egg production data indicated that the egg laid by birds in individual cage was highly significant (PZ 0.1%). Hence, selection of local birds for egg laying performance should result better egg production.

Summary and Conclusion

The experiment which was conducted for knowing the actual egg laying potentiality of indigenous birds indicated that the potentiality for egg laying was lowered due to insufficient feed, energy loss during scavenging and stress factors in the environment. Egg production could be raised up by confining the birds in a small room and feeding concentrates. Selection of good layers among local birds for more egg production would be a possibility to add more eggs.

BUFFALO PRODUCTION IN NEPAL

Shree G. Shah*

Adaptation and Population

Livestock farming is an integral part of the farming system and buffalo contributes substantially in the livestock sector in Nepal. Buffalo, in Nepal, has been acclimatized and adapted to a wide range of environmental conditions. It is widely distributed from subhumid regions of subtropics in Terai to cool temperate regions in the hills and mountains.

Nepal shared 2.8% of the world population in 1970 (FAO 1971). The ruminant livestock population in Nepal in the year 1980 are: cattle 5.99 million, buffalo 2.7 million, goat 3.65 million, and sheep 0.56 million (Rajbhandari and Shah 1981). The number of buffaloes in 1966-67 was estimated to be 3.28 million (UNDP/FAO 1971). The accelerating market price of goat and sheep meat has led to the increased use of buffalo meat, and this has considerably increased the culling rate in the recent years. The increased culling rate might have taken off the best animals for slaughter leaving behind comparatively low producing animals.

Acute shortage of forage, prolongation of first breeding age, long calving intervals, forced mortality of young male calves, high incidence of parasitic infestation and poor management practices have all combined to produce inferior types of animals and this all could be the major factors for declining population trend and the total productivity. However, the projection of buffalo population has been made assuming to have an annual increment of 0.5%, and thus the total buffalo population will reach 2,773,000 and 2,843,000 in 1985 and 1990 respectively. There are 5.9% adult males, 53.3% adult females and 40.8% youngstock in the total population (Rajbhandari and Shah 1981).

Table 1. Ecological Distribution of Buffalo Population in 1980 (Rajbhandari and Shah 1981).

	High hills	Mid-hills	Terai	(in Thousand) Total
Adult males	21	100	38	159
Adult Females	190	904	348	1442
Young stock	146	692	266	1104
Total	357	1698	652	2705

* Assistant Livestock Development Officer, Livestock Development Farm, Khumaltar

Terai region (upto 500 metre a. s. l.) is having warm subhumid climate with a decreasing monsoonal rain of about 3000 mm annually in the east to about 1500 mm in the west. Mid-hills (500–1800 metre a. s. l.) have warm subhumid climate at the valley floors to warm temperate environments at higher elevations. High hills for buffalo adaptation are the cool temperate regions between 1800 to 2900 m. a. s. l.

The hills occupy about 85 percent of the total area and this has influenced the disbalanced distribution of buffalo number in the hills. The distribution of land area and human population as reported by Rajbhandari and Shah (1981) is as follows:

			(In Thousand)
	Hills	Terai	Total
Land area (ha)	11,926	2,180	14,106
Population	8,544	5,466	14,010

Dairy breeds of river buffalo in India are best adapted to warm subhumid to warm dry with seasonal rain. Field observations made in India indicates 60–70°F as the zone of thermoneutrality (Fahimuddin 1975), and buffaloes in physical stress was observed above 74.48°F by Goswami and Narayan (1962). However, Mishra et al (1963) found 97°F as the critical temperature of thermoregulatory mechanism in river buffaloes. Climatic stress adversely affects feed intake and productivity in humid regions. However, the effect of cooler climate in the Himalayas on morphological and physical changes is not clear.

Thus the wide range of adaption and naturalisation indicate the occurrence of some variation in the phenotypes and the genotypes of the buffalo population in Nepal which could be due to random variability or inherent character.

Breeds and Strain

Epstein (1977) has classified Nepalese buffaloes into three regional types: Terai, Hilly Midland and Himalayan Mountain. These types are distinguished by the size, shape, curvature and length of their horns, and the colour of their coats.

Native buffaloes are mostly of River type, although Cockrill (1974) observed a few buffaloes in the midhills and the Terai having Swamp ancestry. Good milch buffaloes in eastern Terai are sometime called Lahure and Gujrati which indicates presence of blood of Murrah and Surti breeds of India. Epstein (1977) observed a greater resemblance of the Nepalese Terai buffaloes with the Indian Terai buffaloes than to either Surti or Murrah. However the indiscriminate crossbreeding for generations has led to a non discript character (Cockrill 1974; Leue 1971).

Epstein (1977) observed heavier and back curving horns in the eastern Terai buffaloes identical with the types bred in West Bengal, India. These buffaloes differ in horn characteristics from the ones reared in the central and western terai. Hill buffaloes have a long head with a slender nasal part and the horns are long curving gradually backward and inward in a wide. The

Himalayan mountain buffaloes are extremely hairy with more or less circular type of horns varying in length and degree of curvature. Epstein (1977) recorded the following measurements of the three types of buffalo cows in Nepal.

	Terai buffalo	Hill buffalo	Himalayan/mountain buffalo
Height at withers (in cm)	120	126	124
Length of body (shoulder point to pinbone) (in cm)	127	147	142
Girth (in cm)	174	183	191
Length of horn (in cm)	35-40	60-80	53

However, Schulthes (1965) at Jiri, about 1800 meter a. s. l. recorded 118.8 cm and 132.5 cm height at withers and length of body from shoulder point to pinbone in the hill buffalo cows respectively.

Prevalent Practices

Buffaloes are considered as the main source of milk and meat and their dung being fully utilized for compost since they are stallfed more usually than cattle.

Management is still traditionally oriented and is not encouraging for high milk and meat production although they are better cared and managed compared to other ruminant animals. In general, management has been influenced by type of breed and feed availability.

In general, ruminant animals are facing an acute shortage of feeds and forages in the country. At present the total feed available as a percentage of requirement in the hills ranges from 48 to 56 percent (Shah 1980; Rajbhandari and Shah 1981). It has been observed that buffalo gets most of its requirements at the cost of low producing cattle. It could be assumed that buffaloes in Nepal are at best 70 to 80 percent fed and the cattle are nearly halfstarved although the quality of feed is of quite inferior type. In Terai and valley floors, buffaloes are mostly fed on crop residues and byproducts, whereas in hills, crop residues are relatively less available and have to depend much more on grazinglands. Native buffaloes mostly graze in the depleted forest areas in the midhills, and move upto 4300 meter a. s. l. in subalpine meadows in the high hills which could probably be the highest elevation for buffalo grazing anywhere in the world.

Back crossing in buffaloes is a common practice, even imported buffalo cows from India and their crossbreds are frequently mated to native bulls of inferior genotype mainly due to unavailability of improved sires in the locality. Bulls are seldom selected for their reproductive traits and are generally used for 1-2 service years. Wide spread of forced culling upto 45% of young male calves, leaves little chance for bull selection. It has been observed that except in Terai the calves of highly productive cows are forced to die just after their birth merely for short term economic value of milk being consumed by calves.

Parasitic infestation is very high compared to other diseases and causes heavy losses. Unhealthy barn condition grazing in swampy areas, drinking stagnant muddy water could be the main causes of parasitic infestation. Foot and Mouth disease, mastitis and haemorrhagic septicaemia are the other diseases affecting buffalo production.

The effect of environmental influences such as the nutritional and management level has led to most unsatisfactory results in the Murrah buffaloes which have been imported from India and distributed to farmers in recent years.

Reproduction traits

(i) Age at first calving

The average age at first calving in Murrah buffalo at Pokhara and Jiri are 55.3 and 63.1 months respectively, whereas in the Indian studies age at first conception has been reported to be between 42 and 44.3 months (Venkaya and Anantakrishnan 1957; Dutta et al 1965 and others).

Table 2. Age at first calving in months of Murrah, Local and their crossbreds

Type	Location	Range	Sample size	Mean	SD	Source
Murrah	Pokhara	46-58	6	55.3	4.59	Shah (1975)
Murrah	Jiri	47.5-73.3	8	63.1	12.07	Farm Reports 1969-74
1/2Murrah	Jiri	46.0-82.1	16	57.5	11.03	" " "
1/4Murrah	Jiri	46.6-57.8	4	52.3	5.13	" " "
Local	Phewa area	46.3-70.3	180	56.1	6.07	Shah (1980)

There is significant effect of elevation and cooler climate on the age at first calving in Murrah breed. The age at first calving should be the reasonable measure for selecting a good herd replacer. Sexual maturity could be enhanced through better feeding and management although there will probably be some environmental and heritability effects.

(ii) Calving Season

Majority of the native buffalo in the midhills calve in a definite season. Shah (1980) reported 88 percent of the hill buffaloes in Phewa area are calving from July to mid-December and the remaining 12 percent in the rest of the year. Whereas 84.2 percent of the Murrah buffaloes in Pokhara farm calved between July and October (Shah 1975). Indian studies have shown a definite seasonal sex-periodicity in respect of oestrus and conception in Murrah buffaloes (Yadav and Kushwaha 1965; Sharma and Sarkar 1968 and others). Yadav and Sarkar (1965) reported 82 percent of the services taking place during september to January.

(iii) The calving Interval.

The average calving intervals in Murrah buffalo at Pokhara and Jiri are 433 and 594 days respectively. Nutrition and management level has significantly reduced the calving interval

in local hill buffaloes from an average of 582 to 433 days. A distinct climatic effect on calving interval has been observed at Jiri, although it could mainly be influenced by nutrition and management

Table 3. *The calving Interval in days of Murrah, Local and their crossbreds.*

Type	Location	Range	Sample size	Mean	SD	Source
Murrah	Pokhara	345-605	10	443.6	94.23	Shah (1975)
Local	"	357-607	11	432.8	71.43	"
Local	Phewa Area	365-830	180	581.5	136.71	Shah (1980)
Local	Jiri	353-613	16	457.2	97.93	Farm Reports (1969-74)
Murrah	"	490-650	24	593.4	49.8	" " "
1/2Murrah	"	366-709	43	604	108.35	" " "

Usually the farmers do not maintain the breeding bull which they feel most uneconomic. In its absence the buffalo can not be mated in time resulting longer calving interval, reducing proficacy and loosing milk yileld.

(iv) Lactation Days and Milk Production.

The average lactation length of Murrah buffalo are 314.5, 259.1 and 407.1 days in Pokhara, Khumaltar and Jiri Farms respectively. Whereas the local buffaloes in these locations have 254.8, 188, and 237.5 days respectively. Local buffaloes have comparatively shorter lactation length (Table 4).

The average lactation yield of local buffalo at Pokhara (midhills) and Jiri Farms (highhills) in better management and feeding practices are 622 and 308 litres respectively. (Table-5). Lactation yields of local buffalo under village condition in the Phewa area (midhills) and Janakpur (Terai) have been reported to be 460 and 510 litres respectively (Shah 1980; LDPN Study 1979)

Improved management, feeding and deworming practices have increased the daily milk yield of local hill type buffalo at Pokhara farm by 46.1 percent. About 54% of the population produced 501 to 900 litres per lactation. This indicates that the genetic potentiality of the local buffalo are not fully exploited.

The average lactation yield of Murrah buffalo was significantly higher at Pokhara-farm. There is a big variability in milk yield at all the locations. However 50% of the population at Pokhara farm had a lactation yield of 1101 to 1806 litres and about 55% and 67% of the population at Khumaltar and Jiri had 901 to 1400 litres.

Crossbreeding with Murrah breed has considerably increased milk yield at both the locations. The introduction of 50% exotic inheritance in local stock alone has produced 20.1% and 50% more milk per day at Pokhara and Jiri farms respectively. Observations at Pokhara clearly

indicates that improved management, feeding and crossbreeding local hill buffalo with Murrah breed (50% level), could considerably increase the milk yield by 75.5% over local population.

Average higher lactation yield at lower elevations could be the influence of better management and feeding practices. However, it indicates the occurrence of better genotypes for milk production at warmer climate or the Himalayan mountain type has low milk production potentiality.

Table 4. *Lactation Days of Murrah, Local and Crossbreds at various locations.*

Type	Location	Range	Lactation number	Mean	SD	Source
Murrah	Pokhara	198-442	14	314.5	121.82	Shah (1975)
Local	Pokhara	176-359	13	254.8	64.27	" "
Murrah	Pokhara	NA	NA	272.0	NA	Farm Report (1978-80)
1/2Murrah	Pokhara	NA	NA	250	NA	" "
Local	Phewa Area	180-420	180	275.4	40.1	Shah (1980)
Murrah	Jiri	307-508	24	407.1	148.38	Farm Reports (1969-74)
Local	Jiri	176-358	16	237.5	78.0	" "
1/2Murrah	Jiri	238-411	43	346.6	63.25	" "
Murrah	Khumaltar	178-334	29	259.1	37.66	Farm report (1969-1971)

Table 5. *Lactation yield in litre of Murrah, Local and crossbreds at various locations*

Type	Location	Range	Lactation Number	Mean	SD	Milk/day*	source
Murrah	Pokhara	605-1806.3	14	1057.9	349.55	3.36	Shah (1975)
Local	"	303-1009.8	13	622.1	212.13	2.44	" "
Murrah	"	NA		967	NA		Report 1978-80
1/2Murrah	"	NA		733	NA	2.93	" "
Local	Phewa Area	128-785	180	459.9	145.73	1.67	Shah (1980)
Murrah	Jiri	543.5-1348.2	24	983.5	246.58	2.42	Report 1969-74
Local	"	198.5-538.3	16	308.2	156.48	1.3	" "
1/2Murrah	"	437.7-903.6	43	674.7	142.98	1.95	" "
Murrah	Khumaltar	596.5-1379	29	968.4	266.16	3.74	Report 1969-71
Murrah	Tarhara	NA		960	NA		Report 1979
Local	Janakpur	NA		510	NA		LDPN Study 1979

* Milk yield/day: an average based on total lactation days.

Suggestions and Recommendations

Classification of the types and the variability present in different biotypes of naturalised or adapted native buffaloes should be done. There is a big variability in the production parameters within the local types adapted to various environments and the prevailing culling practice would make selection easier. Crossbreeding up to 50% exotic level has shown considerable increase in milk production and greatly influenced other reproduction traits.

The effect of cooler climate on morphology and physiology of different crossbreds at various hill locations have to be studied. Research Stations for buffalo breeding have to be established in the hills and inner Terai. A detailed review of work in buffalo breeding and production has to be immediately carried out.

A nucleus herd of Murrah buffalo has to be maintained in the farm. Selection should be based on reproduction traits and buffaloes producing less than 900 litres per lactation has to be immediately culled off.

Conclusion

Elevation and cooler climate along with other environmental factors such as nutrition and management affected first calving age, calving interval and lactation yield in both local and Murrah buffaloes.

Observation on reproductive traits, however, indicate the occurrence of better genotypes for milk production at warmer climate or the Himalayan mountain type as classified by Epstein (1977) has low milk production potentiality.

Acknowledgements

The author expresses his sincere thanks to Mr. N. D. Joshi, Chief, Livestock Division; Dr. T. P. Shrestha, Senior Livestock Specialist, APROSC and Mr. L. Sherchand, Livestock Officer, for their help and valuable suggestions in results interpretations.

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MANUFACTURING PROCESS OF HARD CHEESE FROM YAK MILK IN NEPAL

L. B. Nembang*

Milk for cheese making

To produce high quality yak cheese, the acidity and fat content of the cleanly produced milk should be 7-9 SH° and 3.5 - 4.5 percent respectively. Reduction time of the milk should be atleast four hours. The morning and evening milk is pasteurized at 63°C and kept in cooling pan. 1-2 percent of starter is added when milk reaches a temperature of 32-36°C.

Renneting time and temperature

Five teaspoonful of salt and one measure spoon of rennet powder (2.5 gm.) should be dissolved in a quarter litre of clean and cold water and added to 150 litres (33 gallons) of milk to bring about firm coagulation. Meanwhile, the milk is stirred thoroughly to ensure uniform distribution. Then allow to settle without having wave in the milk by covering the cheese milk with planks and jute cloths. Every time freshly prepared solution should be used. If coloured cheese is desired, the cheese color should be mixed in the milk before the addition of rennet. The curd will be hard enough to cut within about 25-35 minutes.

Cutting the curd

After a firm curd is formed, it is cut with cheese harp. To expose the cheese to uniform temperature, the cheese is turned slowly with the help of cheese shovel. While cutting the curd for the second time, the cheese curd should be stirred properly, when the curd particle reaches the size of hemp seed, the cheese curd is stirred with cheese stirrer.

Heating the curd

While cooking the curd cheese or green cheese, continuous stirring is necessary. The green cheese can be cooked at the temperature of 44-48°C. During the heating process, the curd particles contract to about one-half their original size, eliminating considerable water or whey. The cheese

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makers determines the point at which to stop heating by the feel and appearance of the curd particles.

Pressing the cheese

After heating is completed the cheese curd should be placed into the hoops and wrapped with jute bags. The hooped curd, then is pressed firmly. Due to pressure the whey or water out comes from the cheese.

Turning the cheese

Turning is necessary during the pressing process. Meanwhile, smooth out all the wrinkles that may be formed all around the cheese block. First turning should be done with one cheese cloth whereas two dry cloths are required for second turning. Third turning should be done after 2 hours of the second turning. Third turning is done without using any cheese cloths on the sides (cheese cloths are placed on top and bottom of the cheese). Sides of the cheese are cut to bring the cheese in good shape.

Maturing and salting

For maturing, cheese should be kept under the best possible condition. The cheese are immersed in the build brine bath for 2-3 days. Then the cheese are turned after spraying salt water daily for a month. During the next month, the cheese are turned at the interval of 2-3 days.

The high humidity in the maturing room brings the desired smear on the cheese which prevent it from drying. The cheese is stored at 8-12°C for 6 months.

Transportation of the cheese

The cheese should be properly wrapped in wet jute bags and turned once or twice a day in the transit. If possible the cheese should be washed daily in the transit. The cheese should not be exposed to direct heat or sun light during the summer season.

Proper wrapping and packing is essential if cheese is to be exported.

Composition of yak cheese*

1. Fat	30.6% (45.7% in dry matter basis)
2. Protein	33.8%
3. Salt	2.6%
4. Water	33.0%

(* Analysed at D. D. C. Laboratory, Lainchour)

PROFESSIONAL NEWS

1. XXII World Veterinary Congress will be held in Perth, Australia from August 21, 1983 to August 27, 1983. If any Veterinarian wishes to register his name, forms are available at NVA office on request.
2. Dr. S. N. Pyakural, Chief Veterinary Division has got WHO consultancy for two years in Virology in Dhacca, Bangladesh.
3. Dr. D. D. Joshi, life member and General Secretary of NVA and Chief, Zoonotic Diseases Control Section, Dept. of Health, has been nominated as a Member-Secretary of the Nepal Medical Research Committee, HMG, Nepal.
4. Dr. N. B. Singh, President of NVA and Chief, Animal Diseases and Parasite Control Division has gone to Australia for three months observation tour in Animal Production and Animal Health.
5. Dr. D. D. Joshi, WHO-Member of Advisory Panel on Zoonoses, is leaving on April, 10, 1983 for Washington, D. C., U.S.A. to attend consultancy meeting on "Veterinary Public Health Participation in Primary Health Care" organised by WHO/H. Q., Geneva.
6. Dr. S. M. S. Pradhan has joined UN Volunteer service as a Veterinary Officer in Bhutan.
7. Dr. P. N. Sharma, Dr. M. S. Sharma, Dr. P. M. S. Pradhan and Dr. M. N. Lohani have gone to U.S.A., India, U.K. and Philippines respectively for their Post Graduate Course in Veterinary Sciences.
8. Dr. K. B. Shrestha, Dr. S. R. Bhandari and Dr. C. M. Lamichhane are attending P. G. Training Courses in Parasitology, Serology and Virology respectively in Perth, W. Australia.
9. Dr. Harsha R. Shakya has joined Gloxo Laboratories (India) as a Veterinary Representative for Nepal.
10. Dr. K. N. Upadhyay has returned back to Department after completing his training in Veterinary Epidemiology and Economics (University of Reading U. K.) and F. M. D. Virus typing and diagnosis (Animal Virus Research Institute, U. K.)

THE NEPAL VETERINARY ASSOCIATION

BUILDING FUND

We are highly anxious to have our own Office Building. Since we have a limited resources, We are requesting to all those who are engaged in Animal Health activities to donate generously toward the building fund.

Amount can be deposited directly in the Saving Account No. 19117, Nepal Bank Ltd. New Road, Kathmandu or send your Cheque/Draft/ Money Order or Cash to Nepal Veterinary Association, P. O. Box No. 1885, Kathmandu.

Name and address of the contributors will be published in the annual issue of "Bulletin of Veterinary Sc. & Animal Husbandry, Nepal.

LIST OF CONTRIBUTORS

(Until Feb. 1983)

1. Dr. A. C. Gupta, Chief, A. I. Project, Tripureswar	Rs. 100/-
2. Dr. P. Pathak, Asst. Virologist, Biological Products Division, Tripureswar	Rs. 120/-
3. Dr. S. L. Shrestha, Vet. Coordinator, Livestock Development Project, Harihar Bhawan	Rs. 100/-
4. Dr. Adarsha Pradhan, Asst. Bacteriologist, Animal Diseases & Parasite Control Div. Tripureswar	Rs. 100/-
5. Dr. B. K. P. Shah, Asst, Pathologist, Animal Diseases & Parasite Control Div. Tripureswar	Rs. 100/-
6. Dr. D. D. Joshi, Chief, Zoonotic Diseases Control Section, Dept. of Health Services, Teku.	Rs. 100/-
7. Dr. S. R. Bhandari, Vet. Officer, Animal Diseases & Parasite Control Division, Tripureswar	Rs. 100/-
8. Dr. K. B. Shrestha, Vet Officer, Animal Diseases & Parasite Control Division, Tripureswar	Rs. 100/-
9. Dr. C. M. Lamichhane, Vet. Officer, Animal Diseases & Parasite Control Division, Tripureswar	Rs. 100/-
10. Dr. P. N. Sharma, Senior Vet. Officer, Biological Products Division, Tripureswar	Rs. 100/-
11. Dr. N. B. Singh, Chief, Animal Diseases and Parasite Control Division, Tripureswar	Rs. 100/-
12. Dr. M. N. Lohani, Vet. Officer, Vet. Hospital, Palpa	Rs. 100/-

Life Members of Nepal Veterinary Association

1. Dr. D. D. Joshi: Chief: Zoonotic Diseases Control Section, Dept of Health Services Teku.
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