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Different pathological conditions of mithun (*Bos frontalis*) in Papumpare and Lower Subansiri districts of Arunachal Pradesh

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Abstract

An observational pathological study was conducted on mithun disease conditions of Papumpare and Lower Subansiri districts of Arunachal Pradesh. For this purpose, thirty herds of mithun reared in various forest areas were visited and observed. The district veterinary hospital and the office of the District Veterinary Officer located in Hapoli and Siro regions respectively in Ziro valley, Lower Subansiri were visited. Evident results were produced. The overall incidence rate of various diseases affecting the population was found to be highest (40.59%) among the adult group (more than one year of age) of the population and the incidence rate of various diseases reduced along with the decline in the age of the animals and reached its minimum (27.85%) in animals group (up to 4 months of age). Among different seasons, the incidence rate of various diseases is the maximum (44.83%) during the winter season followed by (31.3%) in the summer season. It has been observed that mithun is most susceptible to stress and nutritional deficiency during winter, making it more predisposing to various infections during this season. It is evident that more deaths occurred in the Papumpare district owing to a larger population of mithun (57.3%) and in lower Subansiri district the population is less compared to Papumpare (42.7%) in Arunachal Pradesh.

Keywords: Pathology, mithun, disease, Papumpare, lower Subansiri, Arunachal Pradesh

Introduction

The North East region of our country is comprised of eight states, viz. Assam, Arunachal Pradesh, Manipur, Mizoram, Meghalaya, Nagaland, Sikkim and Tripura and cover around 2,62,379 sq. km of land of the Indian sub-continent. This region is unique geographically, representing the zone of transition between Indian, Indo-Malayan, Indo-Chinese regions as well as the meeting place of the Himalayan Mountains with the Peninsular India (Rao, 1994)^[17]. Under this geographic background, there is a unique bovine species called mithun (*Bos frontalis*) which is available only in four i.e. Arunachal Pradesh, Nagaland, Manipur and Mizoram out of eight North East states and plays a rather unique role in terms of social customs among the tribal inhabitants of these hilly terrains. Mithun, the pride animal called as 'Cattle of Hilly Region' of North-eastern hilly region of India. Mithuns are bovine species having hugely built bodies and strong horns. They prefer cool forested areas with temperatures ranging from 20-30 °C. The animal plays an important role in the day to day socio-economic life of the local tribal population. Arunachal Pradesh has 75% of the total population, Nagaland 24%, Manipur 0.8% and Mizoram 0.1%, having a total population of 0.29 million according to census (2012). The animal has got good potential for production of quality meat, milk and leather. A very meager population is found in Myanmar, Bhutan, Bangladesh and China. There are four defined mithun strains depending upon the geographical areas, namely the Arunachal, Nagaland, Manipur and Mizoram strains, respectively.

The tribal communities, those who possess the mithun, are considered to be the sign of prosperity in that society. Besides, this animal is also used as a marriage gift (Simoons, 1984)^[18] and sacrificed for different cultural ceremonies and ritual meat consumption. The meat of the Mithun is called beef. It is tender and delicious, has rapid growth like other livestock. Compare 300-600 gm/day as the concentration of growth hormone in plasma is 30- 90ng/ml and is much higher in mithun (Mondal *et al.*, 2006)^[13]. The tribal people sacrifice the meat during important social ceremonies and rituals.

The dressing percentage of mithun is 45-55% and varies according to age; it is advisable to slaughter the animal at the age of 4-5 years to receive a maximum dressing percentage. The consumption of mithun milk is not a routine practice; however the mithun produces around 1-1.5 kg of milk/day/animal (Nath and Verma, 2000) ^[14]. It contains 3.4 - 17% of fat, 6.8-22.2% SNF and 4.4-9.8% protein. Hence, mithun milk is superior from a nutrition point of view. Hence, used to prepare different delicious milk products (Annual report, 2003-04) ^[1]. Mithun hide and skin are of very good quality than cow leather, having more value in the tanning industry due to their toughness and longevity. Therefore, utilized for the production of goods like shoes, garments, bags, jackets, purses, attractive ornamental things and furniture covers etc. Like other bovine species, mithun reared in a semi-intensive system suffers from various infectious diseases like Tuberculosis, Para-Tuberculosis, Brucellosis, Foot and Mouth disease (FMD), Infectious Bovine Rhinotracheitis (IBR), and Bovine Viral Diarrhoea. (Rajkhowa *et al.*, 2003) ^[16] which has tremendous detrimental effect on profitable mithun husbandry practices. Regular deworming, vaccination and treatment of ailing animals is required to be undertaken to keep the animals healthy.

Mithun (*Bos frontalis*) is intricately related to the mythology of Arunachal Pradesh. This bovine is found in Arunachal Pradesh and other North East states like Nagaland, Manipur and Mizoram besides Bhutan (Heli, 1994, 1995) ^[11, 12]. Arunachal Pradesh contributes about 82.84% (2,18,931 approx.) of the total Mithun population of 2,64279 (approx.) in the country. In Arunachal Pradesh, mithun is an essential part of festivals and dispute settlements in society. Mithun is sacrificed in religious ceremonies and rituals as an offering to the supernatural Gods and Goddesses (the saviour of human beings). The divine is supposed to bless the tribe to increase the productivity of the crops and livestock, and save the human race from unnatural catastrophes like famine, epidemics and diseases. Mithun is believed to be the symbolic representative of peace and communal harmony. Traditionally, the ownership of a mithun is considered to be a sign of prosperity and social status of an individual. Arunachal Pradesh is generally home to 28 major tribes and about 81 sub-tribes with 19 different districts, of which Papumpare district has the highest no. (44,286), according to the state report 2012 ^[21]. The Apatani, one of the major tribes, reside in the Ziro valley, the district headquarters of lower Subansiri in Arunachal Pradesh. The overall population of Mithun within this district is 36,001 and 6,540 nos. of Mithun falls under the Ziro valley alone, according to the State report 2012. Legend has it that the fore fathers of the present Apatanis had forefathers to their present place from the Tang Sang-Pho valley in Tibet. The tribe is officially known as "Apatani". Likewise, in other parts of Arunachal Pradesh, mithun holds the same number of traditional values in the Apatani society of Ziro Valley as well.

Materials and Methods

Mithuns reared in the tropical forest of Ziro valley under Lower Subansiri district of Arunachal Pradesh with cool climate, large undisturbed forest tract with hilly terrains and with moderate to heavy rainfall areas were approached between the conducted study period of September 2015 to June 2016 for materials and information. Thirty herds of mithuns reared at various forests area were visited and observed. District Veterinary hospital and Office of the

District Veterinary Officer located at Hapoli and Siro region respectively of Ziro Valley, Lower Subansiri were visited. Papumpare district of Arunachal Pradesh possesses the highest no. of mithun in the whole of Arunachal Pradesh. So, in this regard the District Investigation Laboratory situated at Nirjuli of Papumpare district was also visited. Post-mortem examination of dead animals was conducted and all the gross changes were noted in the suspected organs. Since mithun holds huge traditional value in the society of Arunachal Pradesh only a few nos. of post-mortem examination was possible. Suitable materials were collected for histopathological studies. Selection of materials was based on history, symptoms and gross changes.

Glassware's

During the study period, the glassware used was procured from Borosil, India. These were thoroughly washed and sterilized as per standard procedure.

Plastic Wares

During the study period, the plastic ware used was procured from Tarson, India. These were thoroughly washed and sterilized as per standard procedure.

Instruments

The instruments like scissors, forceps, and scalpel etc., used during the study period were thoroughly washed and sterilized as per standard procedure.

Collection and transport of material

Suspected samples were collected at the time of post-mortem. Organs like lungs, liver, kidney, intestine, skin, heart and spleen were also collected. Samples were then preserved in 10% formal saline for further histopathological study.

Gross study

Clinical signs and symptoms were observed and, accordingly, different pathological conditions of the mithun were observed. Post-mortem examination of the few dead animals found as possible was conducted. Routine post-mortem technique was adopted. Gross changes were noted and suspected lesions were photographed and collected for future studies.

Histopathological technique

The specimens were preserved in 10% formalin. After fixation, tissues were cut into pieces of about 2-4 mm in thickness and kept in running tap water for overnight. The tissues are then dehydrated through ascending grades of alcohol (50%, 70%, 80%, 90%, 95% and absolute alcohol) for 1 hour each. The clearing was done in cedar wood oil till the tissue was transparent, then the tissue was kept in paraffin (no. 1, no. 2, no. 3 and no. 4) each for 1 hour at 56 °C temperature. Then the paraffin blocks were made with tissue, sections were cut into 4 μ thickness in rotator microtome. Glass slides were mounted on the smeared glass slides over a hot water bath, and then the mounted slides were air dried. After that, the dried slides were put into xylene (no.1 and no.2) to remove paraffin. The slides were passed through absolute alcohol no.1 and no.2 for 1 minute each and then the slides were washed in running tap water for 2 minutes to remove the alcohol. The slides were then put in haematoxylin solution for about 10 to 15 minutes and washed in running water to remove the excess of haematoxylin. Then the slides were given a quick dip in acid alcohol and washed thoroughly

with water. After washing, the slides were dipped in ammonia water quickly and washed in water. Then slides were put in eosin solution for 40 seconds. After that, the slides were passed through two absolute alcohols (no.1 and no.2). Then the blotting of the slides was passed through xylene (no.1 and no.2). Finally, the section was mounted on the cover slip with the help of Canadian balsam and examined under a microscope (Bancroft and Stevens, 1990) [3].

Morphological study of parasites

Specimens of different infected organs were collected with worms at random for specific identification of the worm. The parasites were carefully taken out and flattened in between two slides and bound by thread. Then the slides were placed in 5% formalin for 12 hours. Then washed in running tap water for 24 hours and stained in Borax Carmine. The worms were dehydrated at ascending grades of alcohol and cleared in clove oil and xylene, mounted permanently in a DPX mountant. The specific identification of the worms was done on the basis of their size and morphology and the findings were confirmed by the Parasitology department of this university.

Egg identification

Faecal samples were collected from Mithun in separate vials with proper identification and preserved in 5% formalin. The faecal samples were examined under a microscope for detection of eggs by direct smear method, sedimentation and floatation technique (Soulsby, 1982) [19] using a saturated solution of common salt.

Result and Discussion

A. Incidence of Diseases

Incidence of diseases in Mithun has been investigated according to the age of animals and season of incidence. According to age, animals have been grouped into three age groups viz., up to 4 months, more than 4 months to 1 year and more than 1 year of age. Three seasons have been identified, viz., summer, rainy, and winter. It is evident from (Table-1) that the incidence rate of various pathological conditions was highest (40.59%) among the adult group (more than 1 year) of age.

The incidence rate of various pathological conditions reduces along with the decline in the age of animals and reaches a minimum (27.85%) in animals of up to 4 months of age. Among different seasons (Table-2), conditions of various pathological conditions they are maximum (44.83%) during the winter season, as this season offers the most congenial environment for the propagation of different causative organisms, as they seem to share the pastures with local cattle who seem to be the transmitter of diseases to mithun. Incidence rate of various pathological conditions was (31.3%) during summer, which is second in order. It has been observed that mithun was most susceptible to stress and nutritional deficiency. During winter, making it more predisposing to various infections. It is evident from (Table-3) that more deaths occurred in the Papumpare district owing to a larger population of mithun i.e. (57.3%) and in Lower Subansiri district the population was lesser compared to Papumpare i.e. (42.7%) in Arunachal Pradesh.

Table 1: Common incidence of pathological conditions in mithun

Sl. No	Pathological condition /disease	0-4 Months (no.)	5-12 Months (no.)	Above 1 Year (no.)	Total (no.)
1.	Diarrhoea	15	10	12	37
2.	Foot and Mouth disease	34	22	18	74
3.	Pneumonia	0	5	7	12
4.	Pyrexia/fever	10	15	22	47
5.	Tuberculosis	5	8	12	25
6.	Weak/debilitated	2	4	5	11
7.	Corneal opacity	2	5	7	14
8.	Ectoparasites	13	17	25	55
9.	Endoparasites	21	28	32	81
10.	Black quarter	3	5	13	21
Total		105	119	153	377
Percentage (%)		27.85	31.56	40.59	100

Table 2: Seasonal prevalence of pathological condition in mithuns

Sl. No.	Disease/Pathological condition	Season					
		Summer		Rainy		Winter	
		No. of Cases	Percentage (%)	No. Cases	Percentage (%)	No. of Cases	Percentage (%)
1.	Diarrhoea	23	62.2	2	5.4	12	32.4
2.	Foot and Mouth disease	5	41.7	3	25.0	4	33.3
3.	Pneumonia	9	12.2	15	20.3	50	67.5
4.	Pyrexia/fever	33	70.2	9	19.1	5	10.7
5.	Tuberculosis	9	36.0	9	36.0	7	28.0
6.	Weak/debilitated	1	9.1	3	27.3	7	63.6
7.	Corneal opacity	6	42.9	4	28.6	4	28.6
8.	Ectoparasites	21	38.2	4	7.3	30	54.5
9.	Endoparasites	5	6.2	36	44.4	40	49.4
10.	Black quarter	6	28.6	5	23.8	10	47.6
Total		118	31.3	90	23.87	169	44.83

Table 3: Recorded causes of death of mithun in Papumpare and Lower Subansiri districts of Arunachal Pradesh during the study period

Sl. No	Disease/Pathological condition	Papumpare		Lower Subansiri		%
		Total No. of Cases	No. of Cases	%	No. of Cases	
1.	Diarrhoea	37	23	62.2	14	37.8
2.	Foot and Mouth disease	74	49	66.2	25	33.8
3.	Pneumonia	25	13	52	12	48
4.	Pyrexia/fever	47	31	66	16	34
5.	Tuberculosis	12	7	58.3	5	41.7
6.	Weak/debilitated	11	4	36.4	7	63.6
7.	Corneal opacity	55	27	49.1	28	50.9
8.	Ectoparasites	81	47	58	34	42
9.	Endoparasites	14	8	57.1	6	42.9
10.	Black quarter	21	7	33.3	14	66.7
	Total	377	216	57.3	161	42.7

B. Parasitic Diseases

To determine infestation of gastrointestinal parasites faecal eggs were examined, by means of centrifugal flotation method. Identification of each helminth species were performed according to the descriptions of Soulsby (1982) [19].

Helminth eggs encountered in the faecal samples collected from mithun in investigated areas i.e. *Fasciola* spp., *Toxocara* spp., *Monezia* spp., *Gross Fasciola* spp., *Gross Setaria* spp., *Gross Paramphistomum* spp., *Gross Toxocara* spp., *Gross Monezia* spp. (Fig 1, 2, 3, 4, 5, 6, 7, 8).

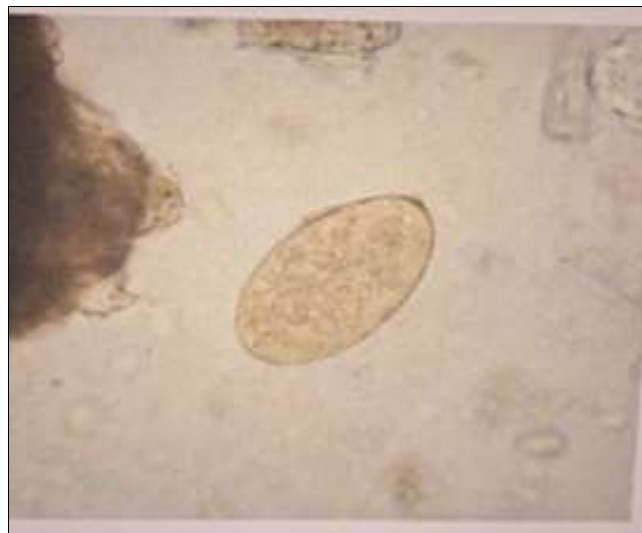


Fig 1: Egg of *Fasciola* spp.

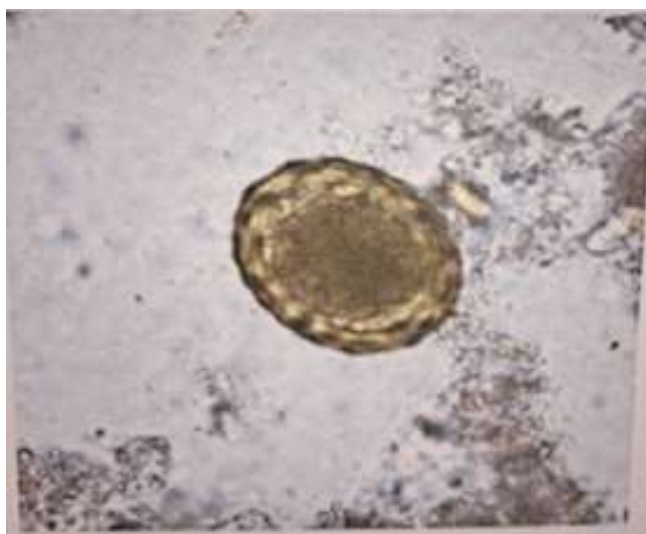


Fig 2: Egg of *Toxocara* spp.

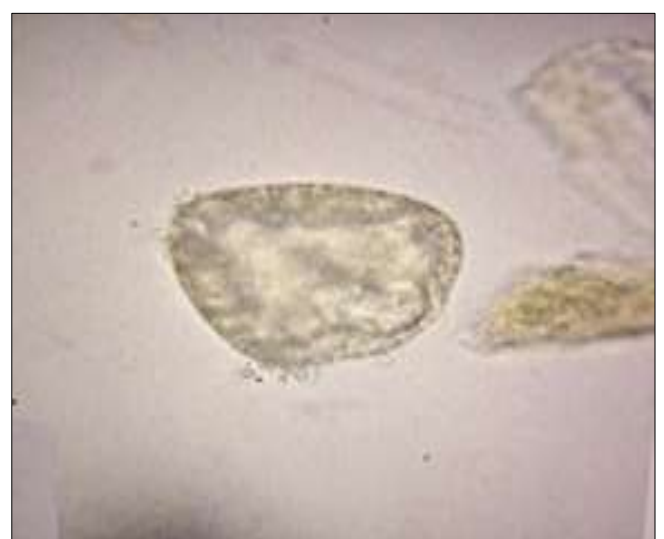


Fig 3: Egg of *Monezia* spp.

Endoparasites encountered in the gastrointestinal tract of mithuns in investigated areas



Fig 4: Gross *Fasciola* spp.



Fig 7: Gross *Toxocara* spp.



Fig 5: Gross *Setaria* spp.



Fig 8: Gross *Monezia* spp.



Fig 6: Gross *Paramphistomum* spp.

Different pathological conditions/diseases encountered mithun during the study period

Fascioliasis

Fluke infestation in livestock animal is an acute problem and mainly caused by *Fasciola hepatica*, *F. gigantica*, *F. magna* and *Dicrocoelium dendriticum*. The causative agent for fascioliasis in mithun has been identified as *Fasciola gigantica* (Chamuah J.K., et al., 2005) [6]. Fascioliasis is most prevalent during the monsoon season as there is marked increase in the reproduction of snails. The affected animal was dull, depressed and emaciated. There was sub-mandibular edema (bottle jaw) (Fig 9), debility and anaemia. Mucous membrane was pale (Chakraborty A, 2001) [5]. The lesions and symptoms were similarity with the researchers (Rajkhowa, S. et al., 2004, Chamuah, J.K., et al., 2009 & 2014) [9, 7-8].



Fig 9: Bottle jaw condition in Fascioliasis in mithun.

Foot and Mouth disease

This animal suffers from many infectious and non-infectious diseases (Bhattacharyya H.K. *et al.*, 2016) [4]. FMD is one of the most acute, highly contagious viral diseases of all cloven footed domestic as well as wild animals but mithun is mostly

affected by FMD than other livestock species. In Arunachal Pradesh so far serotype O has been detected. FMD most commonly occurred among the young calves i.e., below 1 year of age. Animal affected had high fever (105-107°F), loss of appetite, dull, depressed and frothy salivation (Fig 10). The animal also had marked vesicle formation on its hoof region (Fig 11a & 11b) (Dutta P.K., *et al.*, 1979, Verma ND and Sarma DK. 1997, Rajkhowa S. *et al.*, 2003) [10, 22, 16].



Fig 10: Drooling of saliva in mithun suffering from FMD.



11a



11b

Fig 11: (a & b): Vesicles developed in the hoof region of the animal

Tuberculosis

TB is a chronic contagious wasting disease of animals which is clinically manifested by gradual emaciation in spite of having good appetite. TB is caused by an acid fast bacterium namely *Mycobacterium tuberculosis*. And since, Arunachal Pradesh has got sub-tropical climate the disease was more

prevalent. The carcass of the affected animal was emaciated with rough body Coat (Fig 12). On conducting post-mortem examination of the animal haemorrhages were observed in the lung surface and massive formation of tubercles were also seen in the lungs (Fig 13).



Fig 12: Carcass of a Mithun



Fig 13: Lung surface showing massive tubercle formation

Black Quarter

It is a fatal infectious disease of mithun aged 6 months-2 years old. This disease is caused by a gram positive bacteria i.e. *Clostridium chauvoei*. The history of the animal given was high temperature (105-106°F), laboured respiration, dullness, depression, rough body coat and swollen hind limb. Upon conduction of the post-mortem, the affected muscles were found to be swollen and dark. There was foul smelling fluid exudate. The affected muscles became porous and gangrenous (Fig 14).



Fig 14: Necrosis of muscle of hind leg.

Tick infestation

Tick infestation is a very common and major problem in the mithun population. It is recorded throughout the year but most commonly occurs during winter followed by summer. Ticks are localized in various parts of the body viz., eyelids, inner surface of ear, sub-mandibular region, and ventral part of neck and in the inguinal region where the density of hair is less. The affected animal was restless, catechetic (Fig 15) anaemic owing to blood loss due to the sucking of blood by the tick (*Boophilus microplus*) (Fig 16) which are similarity with findings of researchers (Chamuah, J.K., *et al.*, 2009 & 2014) [9, 7-8].



Fig 15: A weak and emaciated mithun suffering from diarrhoea



Fig 16: Gross *Boophilus microplus* ticks found from the mithun body

Eye worm

The parasite responsible, *Thelazia* spp. inhabits in the eye of the animal and it is manifested by lacrymation, keratitis, conjunctivitis and photophobia. The adult worms lay ova in the ocular discharge of host. The muscid flies, *Musca domestica* were infected ingestion of larvae and transmit to the healthy eye. The animal brought was showing lacrymation with mucopurulent discharge. Adult worms were seen moving during the examination of the eye (Soulsby, 1986) [19]. Symptoms also include keratoconjunctivitis, corneal opacity, fibrosis and ulceration in the eye resulting into photophobia (Fig 17).



Fig 17: Eye showing adult worm with ocular discharge.

Histopathological changes observed in different organs of the diseased mithun

Liver: Marked congestion was found in the central portal and sinusoidal vessels (Fig 18). Coagulative necroses of hepatocytes were also evident. Haemorrhagic lesions were prominent throughout the liver parenchyma. Apart from the above changes focal areas of hepatocytes associated with the presence of necrosis Polymorphonuclear leucocytes were also observed (Fig 19).

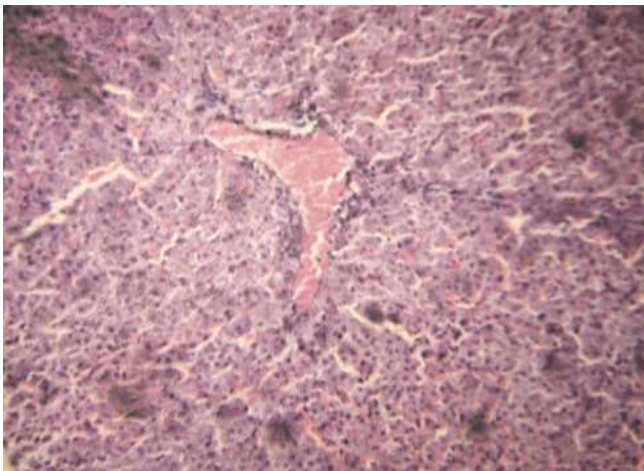


Fig 18: Congested central portal and sinusoidal vessels in Liver. (H & E X 100)

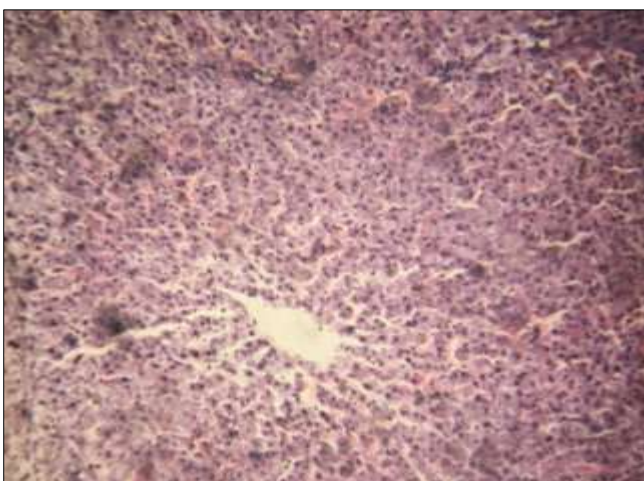


Fig 19: Necrosis of hepatocytes with infiltration of Polymorphonucleocyte in liver (H&E X 100)

Lungs: Focal lesions of edema, emphysema and pneumonia are the features of infected animal. The pneumonic lesion is characterised by infiltration of polymorphonuclear leucocytes, lymphocytes and emphysema. Mono-nuclear infiltration with peri-arteriolitis and peri-bronchiolitis were also observed (Fig 20).

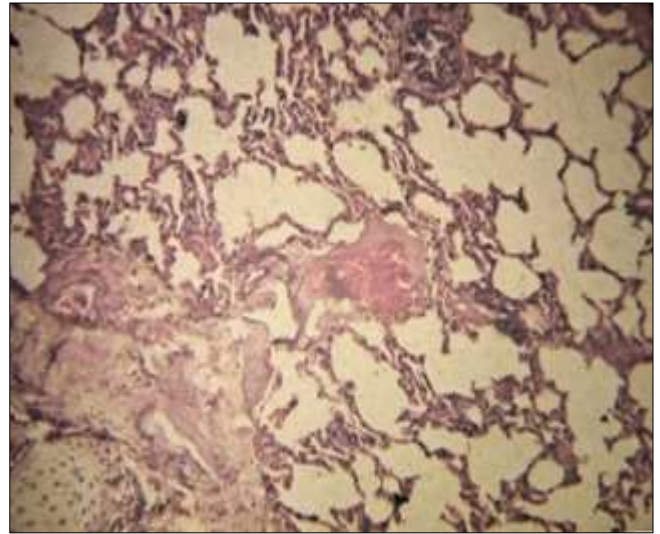


Fig 20: Focal lesions of edema, infiltration of polymorphonuclear leucocytes, lymphocytes and emphysema in Lung. (H & E X 100)

Intestine: Section of intestine showed coagulative necrosis of the villi with congestion of the blood vessels. Focal area of mono-nuclear cell infiltration was also evident (Fig 21).

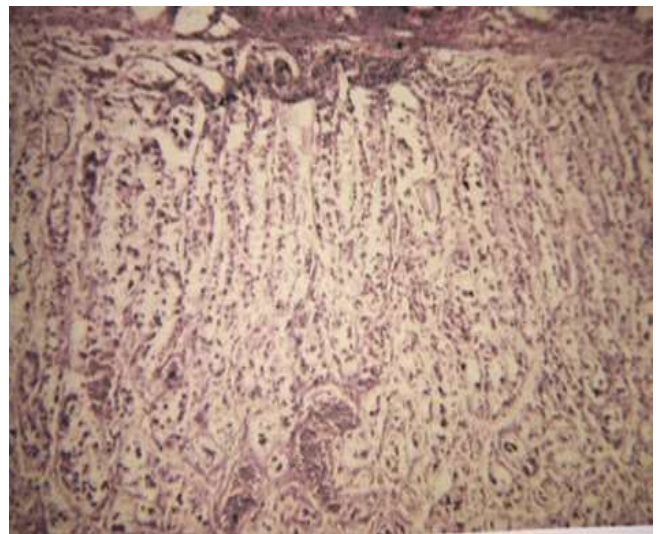


Fig 21: Coagulative necrosis of the villi with congestion of blood vessels in intestine. (H & E X 100)

Spleen: Section of spleen showed congestion and haemorrhages of blood vessels, lympho-follicular hyperplasia in the white pulp. Diffuse lymphocytic proliferation infiltrating the red pulp was also observed. Haemosiderin deposition was seen all over the splenic tissue. Other organs did not showing significant histopathological changes (Fig 22).

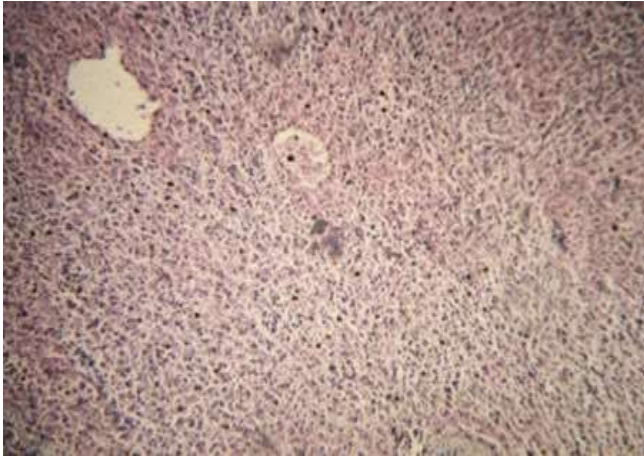


Fig 22: Congestion and haemorrhages of blood vessels with presence of haemosiderin in spleen. (H & E X 100)

Conclusion

Increasing urbanization with unplanned strategies is depleting the natural environment of the mithuns. More numbers of mithun are being affected by various diseases and pathological conditions. Arunachal Pradesh being one of the biodiversity hot spots in India, mithun, one of its unique species, remains to be vulnerable. Education and Awareness Programme awareness can play the greatest role in understanding the significance of biological diversity. Lack of proper awareness programmes is one of the limiting factors for biological conservation in the state today. Through campaigns, local beliefs like unending races of Mithuns can be replaced by their vulnerable status. Complete domestication could be one of the alternatives for propagation of the mithun population. Mithun, being closely associated with the tradition and culture of the tribes of Arunachal Pradesh, are scarified for rituals and matrimonial functions. Public awareness and government should take proper steps for a sustainable population of mithun. Mithun, being a semi-wild animal, is difficult to reach to for proper deworming and vaccination. So, by imparting the importance of deworming and vaccination among the mithun rearers will prove in handy for the conduction of performing effective deworming and vaccination for the mithuns. There are more such steps which can be undertaken to restore the vulnerable status of mithun in the society of Arunachal Pradesh. To maintain the population of mithun, the Govt. should implement the mentioned steps to motivate the common mass of people, the importance and value of mithun and to prevent the hazardous and fast declining rate of the mithun population.

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