



Pathological Studies on Specimens Collected from Slaughtered Bovines in Aizawl District of Mizoram

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ABSTRACT

Background: A slaughter house study helps in the assessment of the disease status of herds and contain valuable informations about the incidence and epidemiology of animal diseases. Abattoir study also plays a significant role to know about the extent of exposure of the public to certain zoonotic diseases and helps in estimation of the financial losses due to condemnation of affected organs. Even in India, there is a lack of data from a proper slaughterhouse study of cows in Mizoram to determine the prevalence of pathological lesions affecting the organs and corpses.

Methods: Total of 90 samples of each organ i.e. liver, lungs, kidneys, heart, spleen and 35 intestine samples were collected during the period from March 2019 to March 2020 for the present study from the Govt. slaughterhouse, Aizawl, Mizoram. Representative tissue samples were collected in 10% neutral buffered formalin (NBF) for further histopathological analysis. Fixed tissue samples processed through paraffin embedding technique and sections were cut at a 4-5 μ thickness, stained with H and E and special staining like Masson's trichrome, Luna-Ishak, Von Kossa and Perl's.

Result: Among all the organs studied, liver (65.55%) showed the highest prevalence of pathological lesions followed by intestine (42.86%), lungs (38.88), kidneys (35.55%), heart (31.11%) and spleen (20%). Circulatory disturbances were the most common pathological lesion encountered in all the organs. The major pathological condition encountered in liver was hepatitis and fibrosis. Interstitial pneumonia was more prevalent in the lungs than bronchopneumonia. Glomerulonephritis was more common in the kidney than tubulo-interstitial nephritis. In the heart, Sarcocyst was more prevalent than myocarditis.

Key words: Bovines, Pathology, Slaughter house.

INTRODUCTION

Cattle and buffaloes, domesticated ungulates belonging to the subfamily Bovinae and the family Bovidae, are raised as livestock for milk, meat, leather and as draught animals. India has a rapidly growing animal husbandry sector and is progressing steadily toward self-sufficiency in livestock product production (Dhama *et al.*, 2014). Livestock Sector is an important subsector of agriculture in the Indian economy. It grew at a compound annual growth rate (CAGR) of 12.99% from 2014-15 to 2022-23. The contribution of livestock in total agriculture and allied sector gross value added (GVA) has increased from 24.38% in 2014-15 to 30.23% in 2022-23 (at current prices). Livestock sector contributed 5.50% of total GVA in 2022-23 (at Current Prices). India possesses largest livestock population in world with 536 million of domesticated animals; the total bovine population is 302.79 million (Anonymous, 2019). In Mizoram, there were 45,701 cattle in 2019, out of which 53% (i.e. 24,222) were indigenous (Anonymous, 2019). The successful development of any livestock industry relies on the prevention and control of diseases among animals. Such diseases can result in significant economic losses in the milk, meat and wool industries (Behera *et al.*, 2024).

A slaughter house study helps in the assessment of the disease status of herds and also prevents distribution of the infected meat to human being (Mellau *et al.*, 2010). Abattoir data contain valuable informations about the

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incidence and epidemiology of animal diseases (Chhabra and Singla, 2009). Abattoir study also plays a significant role to know about the extent of exposure of the public to certain zoonotic diseases and helps in estimation of the financial losses due to condemnation of affected organs (Raji *et al.*, 2010; Singla and Juyal, 2014). Numerous abattoir studies have been performed to investigate both macroscopic and microscopic abnormalities of bovines in different countries (Raji *et al.*, 2010; Ahmed *et al.*, 2013). However, there is no data of proper slaughterhouse study of bovines in Mizoram even in India for determination of prevalence of pathological lesions affecting the carcasses and organs.

MATERIALS AND METHODS

The samples were collected during the period from March 2019 to March 2020 for the present study. Slaughterhouse was regularly visited and the visceral organs of slaughtered bovines were observed for the presence of any gross lesions. The representative tissue samples were randomly collected in 10% neutral buffered formalin (NBF) for further histopathological analysis. The gross findings such as cysts of visceral organs, abscesses, the appearance of congestion, necrotic patches *etc.* were recorded for each of the suspected samples collected. The histopathological analysis of all the tissue samples were carried out in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, CAU, Mizoram. The fixed tissue samples were trimmed at 4-5 mm and washed overnight in running tap water. Then the tissues were processed through paraffin embedding technique. Sections were cut at a 4-5 μ thickness from each specimen

by using semi-automatic rotary cutting machine and were stained with haematoxylin and eosin (H and E) stain. Different special staining like Masson's trichrome, Luna-Ishak, Von Kossa and Perl's Prussian blue staining were also performed for confirmation of different conditions (Bancroft and Gamble, 2008).

RESULTS AND DISCUSSION

Total of 90 samples of each organ *i.e.* liver, lungs, kidneys, heart, spleen and 35 intestine samples were collected randomly from the Government slaughterhouse, Aizawl, Mizoram during the study period. Intestine samples were less in number because of the reason that intact intestine is used for the preparation of a local delicacy in Mizoram. The incidence percentage of both gross and microscopic lesions of each organ were represented in Fig 1. Incidence of different gross lesions in all organs were given in Table 1.

Liver

Liver was found to be the most common organ in which either a gross or histopathological lesions were found during the present study. The incidence per cent of different histopathological lesions were given in Fig 2. The major gross lesion found in liver included shrunken and nodular lesions (Fig 3A), fatty liver, hepatomegaly, hydatid cyst, abscess and Amphistomiasis. Among the microscopic lesions degenerative changes were found to be the most common lesion as compared to other conditions. Degenerative changes observed include cloudy swelling, hydropic degeneration and fatty changes whose microscopic findings correlated well with the standard descriptions reported earlier. Necrosis found in liver was

Table 1: Incidence of different gross lesions in visceral organs.

Organ	Total number of cases with gross lesions	Different gross lesions incidence %						
Liver	25	Circulatory disturbances (36)	Shrink and Nodular lesion (24)	Fatty liver (12)	Hepatomegaly (12)	Hydatid cyst (8)	Amphistomiasis (4)	Abscess (4)
Lungs	16	Circulatory disturbances (43.75)	Emphysema (25)	Pneumonia (31.25)	-	-	-	-
Kidney	10	Circulatory disturbances (70)	Cystic kidney (10)	Renal necrosis (20)	-	-	-	-
Heart	5	Circulatory disturbances (100)	-	-	-	-	-	-
Spleen	7	Circulatory disturbances (57.14)	Splenomegaly (28.57)	Abscess (14.29)	-	-	-	-
Intestine	7	Circulatory disturbances (71.42)	Ulcer and erosion (28.57)	-	-	-	-	-

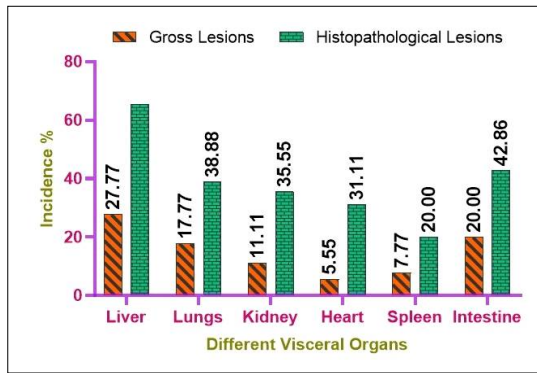


Fig 1: Incidence % of gross and microscopic lesions in different visceral organs.

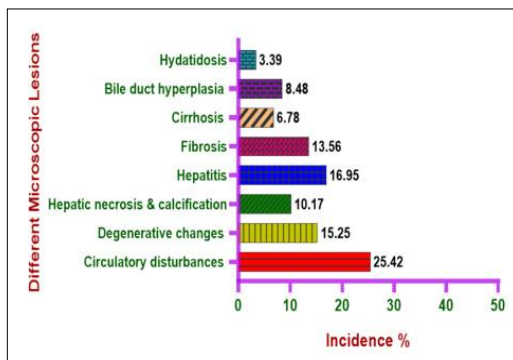


Fig 2: Incidence % of different microscopic lesions in liver.

of either coagulative or caseous type with calcifications (Vegad and Swamy, 2010). Bile duct hyperplasia was indicated by proliferation of new biliary duct around the portal area with infiltration of mononuclear cell (Fig 3B) and the bile canaliculi cell was indicated by Luna-Ishak stain (Fig 3C) which was also reported previously (Laven *et al.*, 2004). Liver with fibrosis showed proliferation of fibrous connective tissues with infiltration of inflammatory cells (Fig 3D) and hepatitis was indicated by presence of congestion and haemorrhages with inflammatory cells (Fig 3E). Microscopically, cirrhotic liver was indicated by presence of island of degenerating and necrotic hepatocytes with infiltration by mononuclear cells in between the excessively proliferating fibrous connective tissues (Fig 3F). El-Dakhly *et al.* (2007) and Raji *et al.* (2010) also reported similar findings in cirrhosis cases in their studies. One case showed presence of multiple abscess formations with thick purulent masses (Fig 3G), which was also observed in previous study (Ahmed *et al.*, 2013). In Amphistomiasis, liver became hard with thickened bile duct (Fig 3H) and microscopically, infiltration of reactive cells with moderate fibrosis was observed. The same type of lesions was observed in previous study (Ahmedullah *et al.*, 2007). In one case, shrunk and constipated hydatid cyst was observed and histopathological changes included presence of laminated wall and hepatic degeneration with infiltration by mononuclear cells in between cyst wall and liver parenchyma (Fig 3I) which was in accordance with previous report (Mekuriaw *et al.*, 2016).

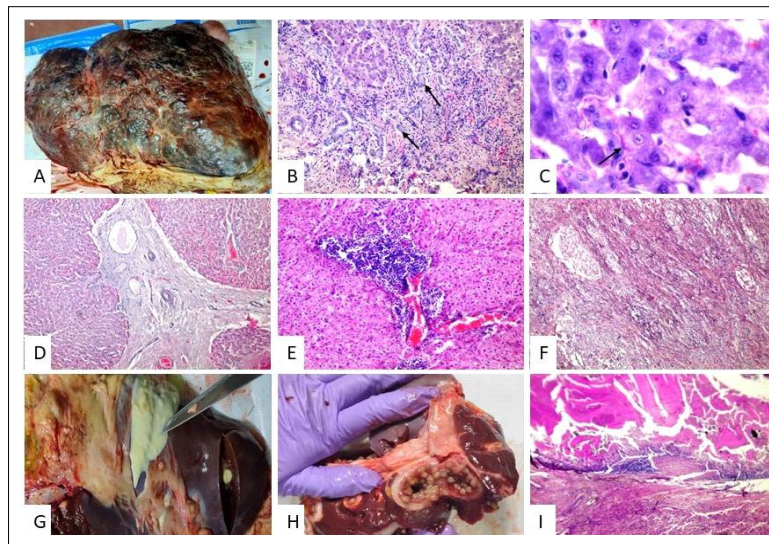


Fig 3: A: Hard, shrunken and nodular liver indicating cirrhosis. B: Liver showing bile duct hyperplasia with fibrosis and infiltration of mononuclear cells (H and E, 200x). C: Liver showing hyperplasia of bile canaliculi (Luna Ishak, 1000x). D: Section of liver showing fibrous tissue proliferation and congested blood vessel in portal areas (H and E, 100x). E: Liver showing hepatitis with severe congestion and mononuclear cells infiltration (H and E, 200x). F: Cirrhotic liver with islands of degenerating necrotic hepatocytes and infiltration by mononuclear cells in between proliferating fibrous tissues (H and E, 100x). G: Liver showing multiple abscess formation of varying size. H: Liver showing thickened bile duct with parasites (*Gigantocotyle explanatum*). I: Liver showing hydatid cyst with laminated wall and hepatic degeneration with infiltration by mononuclear cells and fibrosis (H and E, 100x).

Lungs

The incidence percent of different histopathological lesions were presented in Fig 4. Interstitial pneumonia was the most common as compared to other types. Other than pneumonia hemosiderosis, emphysema, anthracosis and hydatid cyst formation were the other microscopic lesions encountered. In a study by Ahmed *et al.* (2013), the author reported congestion (6.5%), lung abscesses (9.4%) and pneumonia (28.7%) as the common findings. Mekuriaw *et al.* (2016) reported emphysematous lungs were non collapsible with pale or white patches and microscopically, there was diffuse, distended and broken alveoli (Fig 5A) which was also found in emphysema cases during this study. Improper stunning, delayed slaughter after stunning may also contributed to emphysema in lungs (Kusiluka and Kamarage, 1996). Cases with bronchopneumonia, anterior and ventral portion of lungs were severely affected

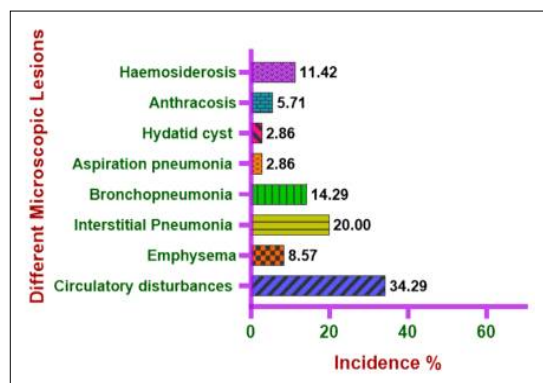


Fig 4: Incidence % of different microscopic lesions in lungs.

as compared to other parts. There was also congestion and hemorrhages with areas of consolidation and hepatisation (Fig 5B). In interstitial pneumonia, mottling of lungs with thickened interalveolar septa was observed. Bronchopneumonia was indicated by presence of congestion along with serous exudates and inflammatory reactions surrounding the bronchus (Fig 5C). In interstitial pneumonia, thickening of interalveolar septa with infiltration of inflammatory cells predominantly mononuclear types with varying degree of serous or fibrinous exudates could be observed (Fig 5D). In aspiration pneumonia, there was presence of severe congestion, hemorrhages and serofibrinous exudates in the lungs parenchyma (Fig 5E). Verminous pneumonia with hydatid cyst was observed in one case. Microscopically, there was presence of laminated cyst wall surrounded by proliferation of connective tissues (Fig 5F). The gross and histological lesions associated with each of these types of pneumonia correlated well with the lesions previously reported by other research (Caswell and Williams, 2007; Dar, 2011). Bronchopneumonia was most often associated with *P. multocida* infection, although other respiratory bacteria may also produce the same lesions (Caswell and Williams, 2007). Pneumonia is important in animals because of extreme weather conditions during dry season and verminous pneumonia in rainy season. One case of hydatid cyst was observed in the present study which was also reported by earlier workers like Islam *et al.* (2014) and Belkhiri *et al.* (2009). Anthracosis was indicated by presence of black colour deposits in the lungs parenchyma (Fig 5G) which was also recorded by Rahman *et al.* (2006). Hemosiderin pigments were observed as brownish yellow granules inside

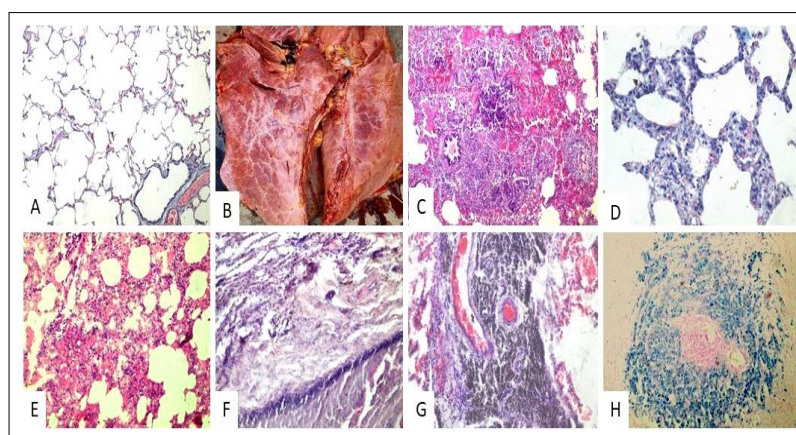


Fig 5: A: Lungs showing emphysema with diffuse distended and broken alveoli with congestion (H and E, 100x). B: Lungs showing areas of consolidation and red hepatisation. C: Lungs showing bronchopneumonia with congestion, hemorrhages and infiltration of both mononuclear and polymorphonuclear cells around the bronchiole (H and E, 100x). D: Lungs showing interstitial pneumonia with thickened interalveolar septa and infiltration of mononuclear cells (H and E, 400x). E: Aspiration pneumonia: Lungs showing hemorrhages, congestion with hemosiderosis and serofibrinous exudates in alveoli (H and E, 200x). F: Lungs showing verminous pneumonia with presence of laminated walls of hydatid cyst and fibrous encapsulation (H and E, 100x). G: Lungs showing deposition of dark black deposit (anthracosis) with hemorrhagic areas (H and E, 200x). H: Hemosiderosis in lung demonstrated by Perl's Prussian blue staining (H and E, 100x).

macrophages which was confirmed by Perl's Prussian blue staining (Fig 5H). This correlated with the findings of Jongsam *et al.* (2002).

Kidney

The major gross changes included circulatory disturbances followed by a case of necrosis, cystic kidney. The incidence % of different histopathological lesions were presented in Fig 6. In one case, there was presence of solitary cyst of 4 cm diameter containing clear fluids (Fig 7A). On histopathological examination, indistinct cyst wall separated by a zone of proliferating connective tissues surrounded by a zone of infiltrating mononuclear cells separating the kidney parenchyma from the cyst was observed (Fig 7B). Tavassoly (2003) also reported the presence of cystic kidney conditions in his study. Among the histopathological lesions tubulo-interstitial nephritis was the most common finding other than the circulatory disturbances. The other important lesions included glomerulonephritis, degenerative changes in the tubular epithelial cells and necrosis of renal

parenchyma along with calcification and fibrosis. A study done by Vikas and Deepika (2016) revealed that congestion (57.14%), haemorrhages (29.42%) and necrotic foci (14.29%) as the common findings in kidney. Raji *et al.* (2010) reported that there was prevalence of abscesses (4.55%) and hydronephrosis (0.08%). Ahmed *et al.* (2013) investigated kidneys of cattle in Ismailia abattoir, Egypt and revealed that there were kidney cysts (6.9%), hydronephrosis (1.6%), white spots (6.7%), fibrosis (2.6%) and pyelonephritis (3.6%) led to the condemned kidneys. Tubulo-interstitial nephritis was more common as compared to glomerulonephritis. In tubulo-interstitial nephritis, infiltration of inflammatory cells was observed surrounding the tubule and in the interstitial spaces of the kidney parenchyma along with congestion and haemorrhages (Fig 7C) whereas in glomerulonephritis, infiltration of inflammatory cells (Fig 7D) and mesangial cell hyperplasia was found in the glomerulus. Similar lesions associated with tubule-interstitial nephritis and glomerulonephritis were also reported by different other studies (Vegad and Swamy, 2010; Ahmed *et al.*, 2013). Glomerulonephritis may be seen in the infection of *Coliforms*, *Leptospira* (Sharma *et al.*, 2022), *Streptococci*, *Staphylococci*, *Pasteurella* and *Salmonella* (Jubb *et al.*, 1993). Interstitial nephritis may occur due to toxicities of ochratoxins, atrinin and pesticides or due to leptospirosis, Herpes virus etc. (McGavin *et al.*, 2001). Degenerative changes were also observed with sloughing of tubular epithelium in the present study which were also reported by previous report (McGavin *et al.*, 2001). Calcification was confirmed by Von Kossa stain (Fig 7E) whereas proliferated collagen fibres in fibrosis was stained with Masson's trichrome stain (Fig 7F). The same reports were recorded by Tavassoly (2003).

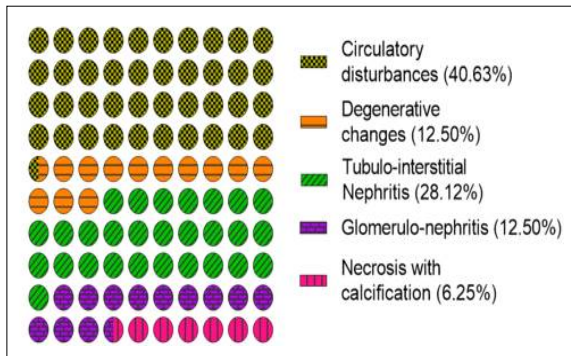


Fig 6: Incidence % of histopathological lesions in kidneys.

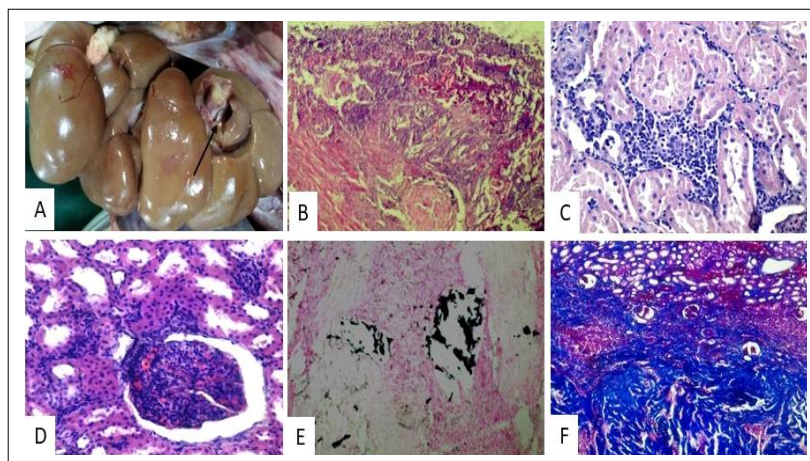


Fig 7: A: Kidney showing a cyst formation. B: Kidney section showing indistinct cyst wall separated by a zone of proliferating connective tissues with infiltrating mononuclear cells (H and E, 100x). C: Kidney section showing interstitial nephritis with severe mononuclear cell infiltration in the interstitium (H and E, 400x). D: Kidney showing glomerulonephritis indicated by congestion and hyper cellularity (H and E, 400x). E: Kidney Section stained with Von Kossa stain showing areas of calcification (H and E, 100x). F: Kidney showing severe fibrosis demonstrated by the presence excess collagen deposition (MTC, 100x).

Heart

The incidence % of different histopathological lesions were presented in Fig 8. Mainly congestion and hemorrhages in epicardium (Fig 9A) and endocardium (Fig 9B) were observed in heart. Myocarditis characterised by infiltration of mononuclear cells in between muscle fibres (Fig 9C) was observed in few cases in the present study. Myocarditis may occur in diseases like Pasteurellosis, FMD, Blackleg, Listeriosis, Tuberculosis *etc.* (Kumar *et al.*, 2007). Pericarditis was observed in few cases in which congestion and infiltration of the pericardium with inflammatory cells was observed. Sastry and Rao (2013) reported that pericarditis may be observed in diseases like Pasteurellosis, Coliform infections and Tuberculosis. Presence of Sarcocyst in the

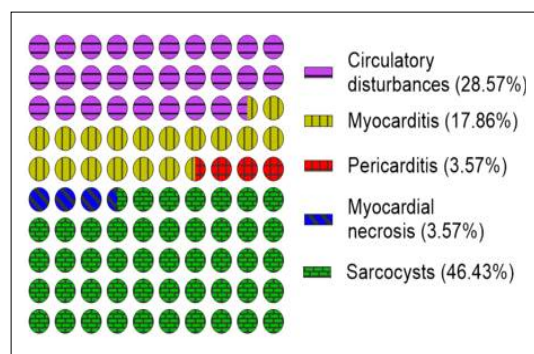


Fig 8: Incidence % of histopathological lesions in heart.

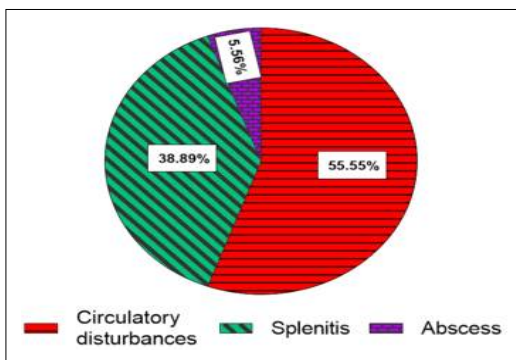


Fig 8A: Incidence % of histopathological lesions in spleen.

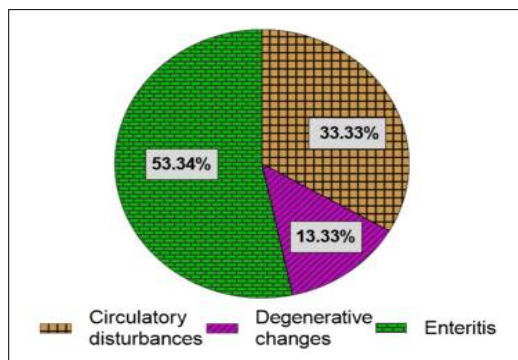


Fig 8B: Incidence % of histopathological lesions in intestine.

myocardium (Fig 9D) was most commonly observed in my study period, which could be attributed to the wet and moist agro-climatic conditions prevailing in this region and myocardial necrosis was observed in one case. Similar observations were also reported in the previous studies (Seema *et al.*, 2007).

Spleen

Like heart, except for congestion and abscess formation no other gross lesion was observed in spleen. The incidence % of different histopathological lesions were presented in Fig 8A. Among the microscopic lesions, spleen showed lesions associated with circulatory disturbances, splenitis and suppurative necrosis and fibrous encapsulation. Splenomegaly was indicated by rounding of edges with congestion and the spleen was soft in consistency. Gracey *et al.* (1999) described the same lesions in spleen was due to blood parasite infestations. Splenitis and circulatory disturbances were found more commonly in the present study. Microscopically, splenitis was indicated by presence of necrosis in red pulp areas with congested blood vessels and neutrophilic infiltrations. In some cases, there was proliferation of reticuloendothelial cells with severe macrophages infiltrations in red pulp areas (Fig 9E). Grossly, one large abscess with thick pus was observed in one case (Fig 9F). Abscess formation may be of bacterial infections which is aggravated due to migrating intestinal parasite (Rosa *et al.*, 1989). In the case of abscess, severe fibrous tissue proliferation with neutrophilic infiltrations separating the splenic parenchyma from the abscess cavity could be observed (Fig 9G). Same results were reported earlier (Jubb *et al.*, 1993). Splenitis may occurs due to hemoparasitic infections (Anaplasmosis, Babesiosis and Theileriosis) and also in Salmonellosis, Anthrax, Tuberculosis, Pseudotuberculosis and Actinomycosis *etc.* (Maxie *et al.*, 2007).

Intestine

The major gross changes found in intestine was congestion of the mucosa and some cases showed haemorrhages. The incidence % of different histopathological lesions were presented in Fig. 8B. Erosion and ulcer formation was observed in one case (Fig 9H). Enteritis was the most common microscopic findings during the study period. There was sloughing of villous epithelial cells with inflammatory cells in the lumen of intestine and in some cases mild to severe infiltration of mononuclear cells in the mucosa and submucosa (Fig. 9I). Degeneration and necrosis of mucosa and crypt epithelial cells were also observed. Similar findings were reported by Cho and Yoon (2014). Several aetiologies are responsible for enteritis in bovines which include chemicals, bacteria, viruses, protozoa, rickettsia, helminths, fungi, metabolic disorders *etc.* (Kashyap *et al.*, 2017).

Study about abattoir slaughter is crucial for understanding the public's exposure to zoonotic diseases and estimating financial losses caused by the condemnation of affected

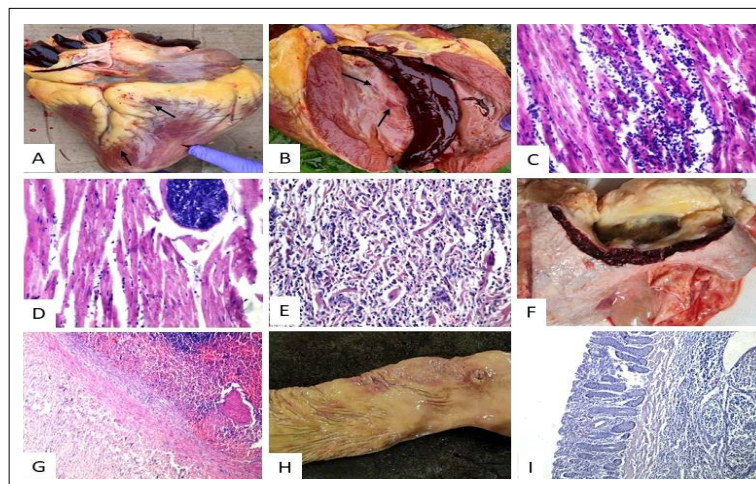


Fig 9: A: Heart showing petechial hemorrhages in the epicardium. B: Heart showing hemorrhages in the endocardium. C: Heart showing severe infiltration of mononuclear cells in between muscle fibres (H and E, 400x). D: Heart showing presence of Sarcocyst with mild mononuclear cells infiltration (H and E, 400x). E: Spleen showing splenitis indicated by proliferation of reticular endothelial cells (H and E, 400x). F: Spleen showing single large abscess of 8 cm diameter with thick inspissated pus. G: Spleen showing abscess wall with fibrosis (H and E, 100x). H: Intestine showing areas of necrosis and ulcer. I: Intestine showing enteritis with severe infiltration of inflammatory cells in the mucosa and submucosa (H and E, 100x).

organs (Chhabra and Singla, 2009). Different affected organs were condemned during the slaughter which causes huge financial losses during this study periods. Some zoonotic diseases may be fatal in human beings if they will eat the affected meat. Therefore, a slaughter house study helps in the assessment of the disease status of herds and also prevents distribution of the infected meat to human being (Mellau *et al.*, 2010).

CONCLUSION

Abattoir data provide valuable insights into the incidence and epidemiology of animal diseases. They play a crucial role in assessing public exposure to zoonotic diseases and help estimate financial losses resulting from the condemnation of affected organs. Among all the organs studied, liver (65.55%) showed the highest prevalence of pathological lesions followed by intestine (42.86%), lungs (38.88), kidneys (35.55%), heart (31.11%) and spleen (20%). Circulatory disturbances were the most common pathological lesion encountered in all the organs. The major pathological condition encountered in liver was hepatitis. In lungs, bronchopneumonia was more common followed by interstitial pneumonia. Tubulo-interstitial nephritis was more in kidney followed by glomerulonephritis. Sarcocyst was more common in heart followed by myocarditis. Different veterinary inspection protocols and policies in Mizoram includes Strengthening slaughterhouse regulations, Enhanced meat inspection procedures like standardized ante-mortem and post-mortem inspection protocols, conduct of regular training programs for veterinary inspectors, butchers and abattoir workers on disease identification, safe handling and proper disposal

of condemned meat, better Surveillance and Reporting System (SRS), Strict zoonotic disease monitoring, Upgradation of abattoir facilities, Public awareness and Consumer Education, Coordination Between Departments like Strengthen collaboration between veterinary authorities, public health officials and municipal bodies to ensure effective enforcement of meat safety regulations. Further research can be performed to explore the potential aetiologies (which may be environmental, nutritional or infectious factors) of different pathological lesions observed in different organs.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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