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RESEARCH ARTICLE

Studies on Pathological Alterations and Diseases of Nervous System in Small Ruminants

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ABSTRACT

Neurological disorders in small ruminants present a significant challenge to livestock health and productivity, yet they remain comparatively underexplored. This study aimed to characterize the pathological alterations in the central nervous system (CNS) of goats and sheep exhibiting neurological signs. A total of 68 brain samples (44 goats; 24 sheep) were collected during post-mortem examinations. Gross lesions included meningeal congestion, cerebral and cerebellar hemorrhages, encephalomalacia and the presence of Oestrus ovis larvae. Histopathological evaluation revealed inflammatory lesions (vasculitis, meningitis, neuronophagia), reactive changes (chromatolysis, gliosis, satellitosis), vascular alterations (congestion, edema) and degenerative changes (spongiosis, axonal degeneration). Disease specific lesions consistent with listeriosis and rabies were also observed. Bacteriological culture and PCR confirmed *Listeria monocytogenes* and *Escherichia coli* as causative agents in select cases. The findings underscore the diagnostic value of integrating gross pathology, histopathology, microbiology and molecular techniques for the comprehensive identification and understanding of CNS disorders in small ruminants.

Keywords: CNS lesions, *Escherichia coli*, histopathology, *Listeria monocytogenes*, PCR, small ruminants

INTRODUCTION

Small ruminants, particularly goats and sheep, are integral to India's livestock sector, supporting the livelihoods of marginal and landless farmers due to their adaptability, low maintenance and high reproductive potential. They contribute significantly to food and nutritional security through milk, meat and wool production. As per the 2019 Livestock Census, India had 148.88 million goats and 74.26 million sheep[1], ranking first globally in goat milk production and among the top in small ruminant meat output.

Despite their importance, goats and sheep are highly susceptible to infectious and non-infectious diseases, including neurological disorders, which often go under reported. Contributing factors such as climate change, poor fodder availability and unregulated animal movement have exacerbated disease prevalence[2]. While diseases of the respiratory, digestive and reproductive systems are well studied, CNS disorders remain relatively underexplored. These include listeriosis, coenurosis, polioencephalomalacia, enterotoxaemia and congenital anomalies[3,4].

Listeriosis, a zoonotic disease caused by *Listeria monocytogenes*, is frequently associated with poor-quality silage and has been reported in northern India, including Punjab[5]. Other notable CNS conditions include coenurosis *(Taenia multiceps)* and enterotoxaemia caused by *Clostridium perfringens* type D. Definitive diagnosis relies on post-mortem examination of the brain, with characteristic lesions such as encephalomalacia, gliosis and perivascular cuffing. Ancillary tests like bacterial culture, PCR and immunohistochemistry enhance diagnostic accuracy[6].

This study aimed to investigate the pathological changes in the brains of goats and sheep showing neurological signs, document gross and microscopic lesions, identify etiological agents using conventional and molecular techniques and support improved diagnosis of CNS disorders in small ruminants.

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MATERIALS AND METHODS

Sample collection

A total of 68 brain samples were collected from post-mortem examinations of sheep (n = 24) and goats (n = 44) exhibiting neurological signs or with relevant clinical history. The animals were either brought to the Post-mortem Hall of GADVASU or were part of outbreaks on organized and unorganized farms in Punjab. The entire brain was removed aseptically and gross changes were recorded and photographed (GADVASU/2024/IAEC/75/09).

Histopathological examination

Representative brain tissue samples were fixed in 10% neutral buffered formalin (NBF), processed routinely and embedded in paraffin wax. Sections of 3-4 µm thickness were cut using a semi-automated microtome and mounted on glass slides. Haematoxylin and eosin (H&E) staining was performed for routine histopathological evaluation[7]. Selected samples were also subjected to special stains such as Silver-Golgi, Masson's Trichrome, Masson-Fontana and Triple Shorr stains to demonstrate specific tissue elements.

Bacterial isolation and identification

For bacteriological analysis, brain tissue was aseptically inoculated onto Brain Heart Infusion (BHI) agar and incubated at 37^{O}C for 24-48 hours. Pure colonies were sub cultured and identified based on colony morphology and Gram staining[8]. Selective media were used for the isolation of specific pathogens: *Listeria monocytogenes* was cultured on Listeria Selective Agar (HiCrome) and *Escherichia coli* was isolated on Eosin Methylene Blue (EMB) agar.

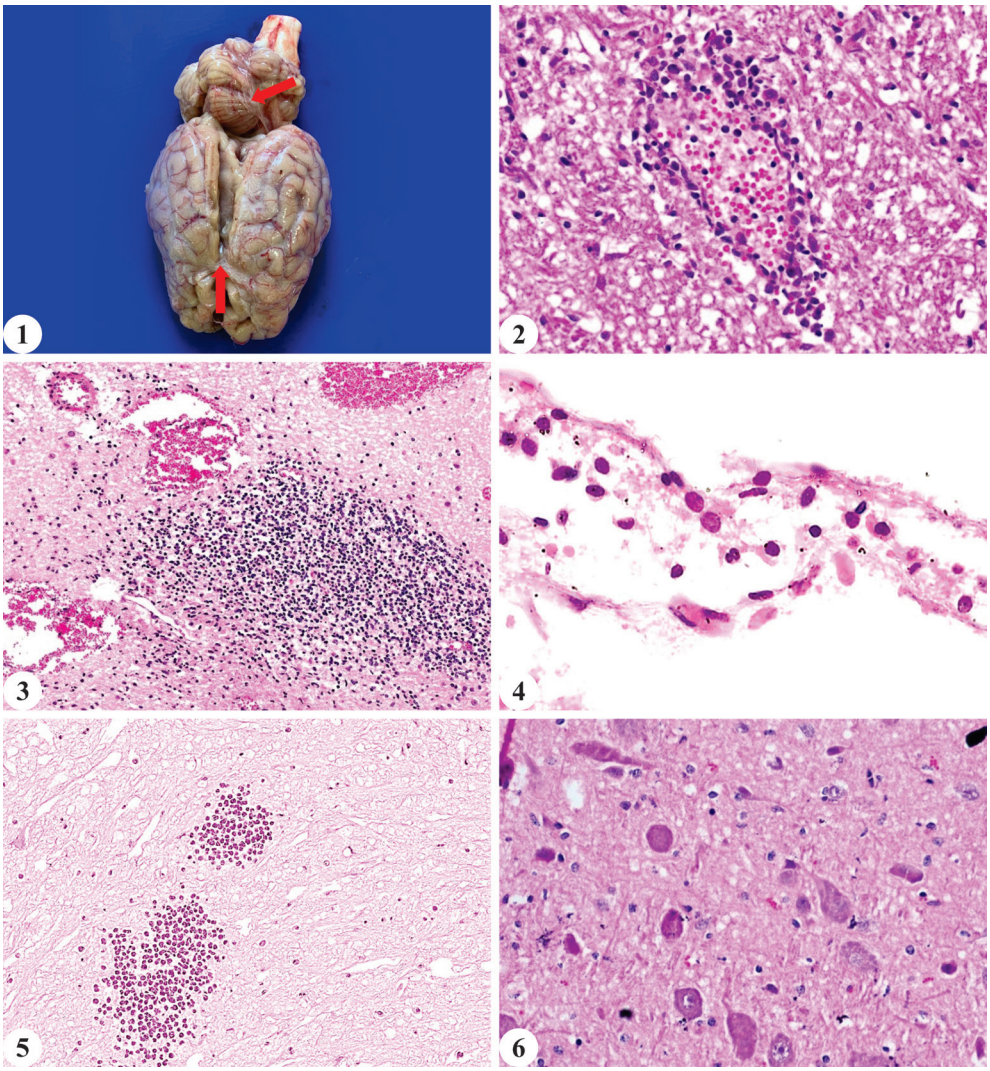

Fig. 1. Fibrinous meningitis with adhesions on the brain surface of goat (arrow); **Fig. 2.** Perivascular cuffing characterized by accumulation of mononuclear inflammatory cells around blood vessels of goat (H&E x400); **Fig. 3.** Non suppurative encephalitis: Cerebellar haemorrhage with lymphocytic infiltration characterized by extravasated red blood cells (haemorrhage) and a dense accumulation of lymphocytes in sheep brain (H&E x200); **Fig. 4.** Meningitis infiltration of mixed cell in goat (H&E x1000); **Fig. 5.** Suppurative encephalitis showing microabscess in brain of sheep (H&E x200); **Fig. 6.** Chromatolysis characterized by a swollen cell body and dispersion of Nissl substance in goat (H&E x400).

Bacteria	Forward Primer	Reverse Primer	Gene	Product Size (bp)
E. coli	CTTACCGGGCAATACACTCACTA	CTTACCGGGCAATACACTCACTA	*phoA*	622
Listeria Monocytogens	ATGAATATGAAAAAAGCAACGATC	TAGCACTTGCACTTGAATTGCTG	*iap*	1120

Molecular diagnosis

DNA was extracted from bacterial isolates using the crude-boiling TENT buffer method[9,10,11] and the DNeasy Blood & Tissue Kit (Qiagen, Germany). Species-specific primers targeting the *phoA* gene for *E. coli* and the *iap* gene for *L. monocytogenes* were used[12]. PCR amplification was performed in a final volume of 25 µl and products were visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

RESULTS

A total of 68 brain samples, including 44 from goats and 24 from sheep were examined to investigate central nervous system (CNS) disorders through gross pathological, histopathological, bacteriological and molecular analyses.

Gross pathological findings

Gross lesions were observed in 18 of 44 goat brains (40.90%) and 13 of 24 sheep brains (54.17%). Key findings included meningeal congestion (6 goats, 33.3%; 3 sheep, 23.07%), fibrinous adhesions (Fig. 1) (4 goats, 22.2%; 2 sheep, 15.38%), cerebral hemorrhages (2 goats, 11.1%; 3 sheep, 16.6%) and cerebellar hemorrhages (4 goats, 30.76%; 2 sheep, 15.38%). Encephalomalacia was recorded in 2 goats (11.1%) and 1 sheep (7.69%). Oestrus ovis larvae were found in the cranial cavities of one goat (5.55%) and one sheep (7.69%) highlighting incidental parasitic infestation.

Histopathological findings

Neuropathological lesions were detected in 35 goat brains and 17 sheep brains. Lesions were classified as inflammatory, reactive, vascular, degenerative and disease specific.

Inflammatory lesions

Inflammatory changes were present in 20 goats (45.45%) and 9 sheep (37.5%). Vasculitis was observed in 4 goat brains (20%) and 2 sheep brains (22.2%). Perivascular cuffing (Fig. 2) occurred in 6 goats (30%) and 3 sheep (33.3%). Neuronophagia (6 goats, 30%; 2 sheep, 22.2%) and microgliosis (4 goats, 20%; 3 sheep, 33.3%) were also noted. Non-suppurative encephalitis (Fig. 3) was seen in 3 goats (15%) and 2 sheep (22.2%), while suppurative encephalitis was detected in 2 cases each of both species. Meningitis was noted in 3 goats and 2 sheep (Fig. 4) and ependymitis in 2 goats and 1 sheep.

Reactive changes

Reactive alterations were observed in 15 goats (34.09%) and 7 sheep (29.17%). Chromatolysis was the most frequent reactive change in goats (6 cases, 40%) but was infrequent in sheep (1 case, 14.2%). Satellitosis was more prominent in sheep (3 cases, 42.8%) than in goats (5 cases, 33.3%) (Fig. 7). Gliosis was equally frequent in both species (4 goats, 26.6%; 3 sheep, 42.8%).

Vascular lesions

Vascular lesions were recorded in 20 goats (45.45%)

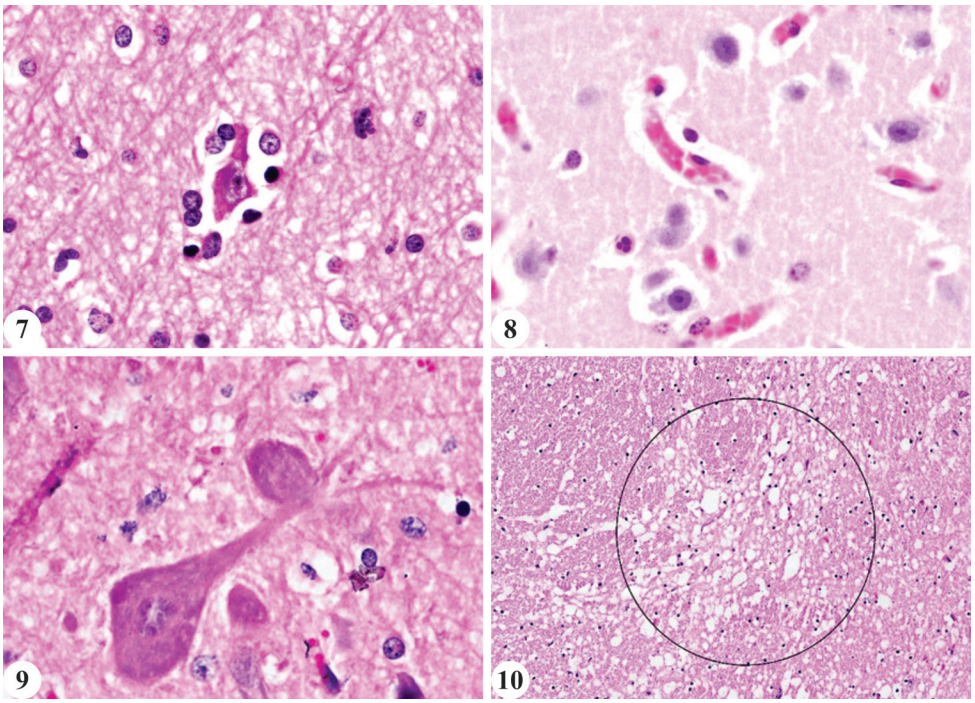

Fig. 7. Satellitosis: Several smaller cells with darkly stained, condensed nuclei are clustering around the neuron in sheep (H&E x1000); **Fig. 8.** Perivascular edema characterized by clear halos surrounding neuronal cell bodies and blood vessels of goat (H&E x1000); **Fig. 9.** Axonal spheroid: Rounded, eosinophilic swellings within the neuropil of sheep (H&E x1000); **Fig. 10.** Spongiosis: Multiple clear vacuoles or empty spaces in the neuropil of goat (H&E x200).

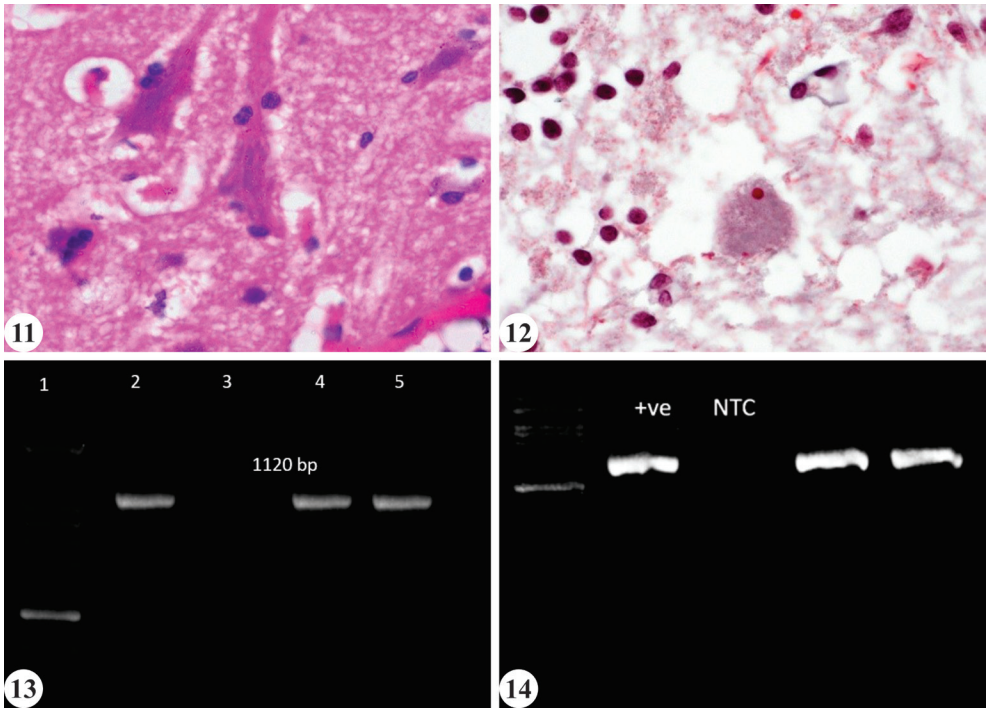

Fig. 11. Neurofibrillary tangle dense, brightly eosinophilic (pink-red) fibrillary mass within the cytoplasm of the neuron in sheep (H&E x1000); **Fig. 12.** Rabies: Large intracytoplasmic Negri body within a neuron of goat (Triple Shorr stain x1000); **Fig. 13.** Agarose gel electrophoresis. PCR analysis for sample suspected for *L. monocytogenes*, Lane 1: 100 bp DNA ladder, Lane 2: Positive control (1120 bp), Lane 3: Negative control, Lane 4 & 5: Samples; **Fig. 14.** Agarose gel electrophoresis. PCR analysis for sample suspected for *E. coli*, Lane 1: 100 bp DNA ladder, Lane 2: Positive control (622 bp), Lane 3: Negative control, Lane 4 & 5: Samples.

and 9 sheep (37.5%). Haemorrhages were found in 14 goat brains (70%) and 8 sheep brains (88.8%), while congestion was present in 13 goats (65%) and all 9 sheep (100%). Vasogenic oedema was identified in 8 goats (40%) and 7 sheep (77.7%). Perineuronal, perivascular and perixonal oedema was noted in 5 goats (25%) and 4 sheep (44.4%) (Fig. 8).

Degenerative lesions

Degenerative changes were seen in 9 goat brains (20.45%) and 6 sheep brains (25%). Axonal spheroids were seen in 1 case each (11.1% goats; 16.6% sheep) (Fig. 9). Spongiosis was observed in 3 goats and 2 sheep (33.3% each) (Fig. 10). Neuronal degeneration (2 goats, 22.2%; 1 sheep, 16.6%) and vacuolation (2 goats, 22.2%; 1 sheep, 16.6%) were also recorded (Fig. 3). Neurofibrillary tangles were found in one goat and one sheep (Fig. 11).

Disease specific lesions

Listeriosis was diagnosed in one goat and one sheep, exhibiting non-suppurative meningoencephalitis with microgliosis and perivascular cuffing, especially in the brainstem. Rabies was confirmed in one goat and one sheep, characterized by neuronal degeneration, perivascular lymphocytic cuffing, babe's nodule and Negri bodies (Fig. 12).

Other lesions

Rare findings included meningeal melanosis in one goat and one sheep and neoplastic growths in another goat and sheep. These cases suggest potential congenital pigmentary anomalies or rare intracranial neoplasms.

Bacteriological and molecular findings

Bacterial culture identified *Listeria monocytogenes* and *Escherichia coli* in 2% of goat brains and 5.55% of sheep brains, respectively. PCR confirmed their identity using the *iap* gene for *L. monocytogenes* (Fig. 13) and *phoA* gene for *E. coli* (Fig. 14).

DISCUSSION

This study highlights the diverse spectrum of CNS pathologies in small ruminants. Gross lesions, including meningeal congestion, hemorrhages and encephalomalacia were more frequently observed in sheep, suggesting a potentially higher susceptibility or advanced disease progression at the time of death. Histopathologically, inflammatory changes predominated, with perivascular cuffing, microgliosis and neuronophagia indicating active encephalitic processes. These lesions, especially prominent in the brainstem and cortex are consistent with bacterial infections such as listeriosis or coliform septicemia aligning with previous reports[13]. Reactive gliosis, chromatolysis and satellitosis were common and reflect the CNS's attempt to counteract injury. Chromatolysis in goats

may indicate sustained neuronal stress, possibly from chronic infection or metabolic dysfunction[14]. Vascular disturbances such as congestion and haemorrhages, observed in nearly all sheep and many goats, suggest significant cerebrovascular compromise. Vasogenic edema and associated perivascular/perineuronal swelling underscore disruptions to the blood-brain barrier frequently seen in septic or toxic encephalopathies.

Degenerative lesions, while less frequent were noteworthy. Spongiosis and neuronal vacuolation suggest metabolic or toxic causes like polioencephalomalacia. The detection of neurofibrillary tangles in both species, though rare is notable and suggests possible age-related brain degeneration in ruminants[15]. Disease specific diagnoses were confirmed in a few cases through molecular techniques, including PCR. *Listeria monocytogenes* was detected in samples exhibiting classical signs of non-suppurative meningoencephalitis, including micro abscess formation and extensive perivascular cuffing. These findings were in line with the neuropathological hallmarks described in earlier studies. Similarly, rabies was confirmed in two animals one goat and one sheep based on the presence of Negri bodies, gliosis, babe's nodule and neuronophagia. These lesions support existing descriptions in the literature[16,17]. Rare lesions such as meningeal melanosis and CNS neoplasia although incidental, underscore the importance of comprehensive neuropathological assessments. These findings also hint at the broader spectrum of neuropathological entities in small ruminants some of which may go unnoticed in routine clinical diagnosis[18].

In conclusion, the high prevalence and variety of lesions observed in both goats and sheep highlight the diagnostic value of postmortem CNS evaluation. Integration of histopathology with bacteriological and molecular tools is critical for accurate diagnosis, epidemiological tracking and understanding disease pathogenesis in small ruminant neurology.

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