ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Histopathological and ultrastructural alterations of neurons and glial cells of infected sheep brain with *Coenurus cerebralis*

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Manuscript Info

Manuscript History:

Received: 15 November 2014
Final Accepted: 22 December 2014
Published Online: January 2015

Key words:

Coenurus cerebralis, sheep, pathology, oligodendroglia, nissl granules

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Abstract

One of the serious nervous diseases of livestock animals is coenurosis which cause high economic losses in the sheep herds. In the present study, the pathological effects of *Coenurus cerebralis* on neurons and glial cells were studied in a trial to elucidate the pathway of the associated serious nervous manifestations. Brain specimens from 20 coenurosis infected sheep were examined parasitologically, histologically and ultrastructurally. Degeneration and necrosis of the ependymal cells were the most obvious lesions in the examined histological sections, beside the granulomatous encephalitis. In addition, the cresyl violet stained sections demonstrated the depletion and loss of Nissl granules responsible for the neurotransmission. Ultrastructurally, loss of the myelin sheath, edema and degeneration of the interfasicular oligodendroglia were evident. On conclusion, *Coenurus cerebralis* causing progressive and fatal nervous manifestations not only as a space occupying lesion as previously mentioned but mainly due to their pathological effect on the ependymal and oligodendroglia cells leading to progression of the neurological signs.

INTRODUCTION

Coenurosis (gid or sturdy) is an economically important disease caused by *Coenurus cerebralis* (the metacestode of *Taenia multiceps*). This parasite usually inhabits the central nervous system especially the left and right cerebral hemisphere (Desouky et al., 2011). Domestic and wild canids constitute the definitive hosts, while a wide range of herbivores including sheep, goats, cattle, buffaloes, camels, yak and equines are the intermediate hosts. Coenurosis is quite common in sheep (Sharma and Chauhan, 2006).

Infected animals showed sever nervous manifestations including circling towards the side of the cyst location, blindness, convulsions, cerebral atrophy, thinning and morphologic changes in the cranium (Yoshino and Momotani, 1988). Various reported human cases were infected with *C. cerebralis* in the central nervous system (Benifla et al., 2007) and the intraocular cavities resulting in endophthalmitis and retinal detachement (Inechukwu and Onwukeme, 1991).

Different pathological studies were developed to elucidate the causes of the nervous manifestations associated with coenurosis infection. Kheirandish et al. (2012) found that *C. cerebralis* considered as a space occupying lesion which lead to marked pressure atrophy and liquifactive necrosis in the brain tissues, hyperemia, neural degeneration, satellitosis and diffuse microgliosis. While, Bussell et al. (1997) noted severe deleterious granulomatous encephalitis.

Moreover, Mohi El-Din (2010) stated that this metacestode utilize the CSF for its differentiation and growth. Avcioglu (2012) noted a neural necrosis with sheath demylination beside astrocytosis and microgliosis.

Herein, we focused on the pathological effects of *Coenurus cerebralis* on the neurons and glial cells of infected sheep brain, which may explain the progresssion of the serious nervous manifestations.

Material and methods

1. Specimens' collection:

A total number of 20 sheep from 8 small flocks and showed nervous symptoms were included in this study. Sheep were necropsied and brains were inspected for the presence of *C. cerebralis* cysts.

2. Parasitological examination:

The diameter of the collected coenuri were measured using a caliber, and the harvested protoscolices were permanently mounted and stained with acid carmine stain (Richard and Kruse, 1982).

3. Histopathological examination:

The coenuri and the surrounding tissues were fixed in 10% neutral buffered formalin for histopathological examination (Bancroft and Stevens, 1996). Special staining was carried out for some sections using the cresyl violet stain for demonstrating the Nissl substances (Carson, 1990).

4. Electron microscopic examination:

Pin head sized brain samples were fixed in buffered 5 % glutaraldehyde for 24 hours. Then, they washed in cacodylate buffer (0.1 M, pH 7.2) 3-4 times for 20 minutes for each and post fixed in 1% osmium tetroxide for 2 hours. Samples dehydration was done by immersing them in ascending grades of ethyl alcohol up to 100% (30, 50, 70, 80, 90 and 100% /2 hours). Using the gelatine capsule, samples were embedded in Epon 812. For polymerization, the embedded samples were kept in incubator at 35 C˚ for one day, at 45 C˚ for another day and three days at 60 C˚. Semithin sections (0.5-1µ thick) were prepared, then stained with toluidine blue and examined by light microscope. Regions for preparation of ultrathin sections were oriented. The ultrathin sections at a thickness of 500-800 Å were made and fixed on copper grids (200µ meshes), they were contrasted in uranyl acetate for 15 minutes and lead citrate for 5 minutes and examined by JEOL, CX11 transmission electron microscope (Bozzola and Russell, 1991).

Results

Parasitological examination:

Brain inspection of the 20 coenurosis suspected slaughtered sheep revealed transparent wall vesicles (0.7 – 8 cm in diameter) with whitish clusters of protoscolices attached to their walls. Each protoscolex consisted of 4 cup shaped muscular suckers and an about 30 hooked rostellum. The coenuri were distributed in the right and left cerebral hemisphere and the cerebellum, figure (1).

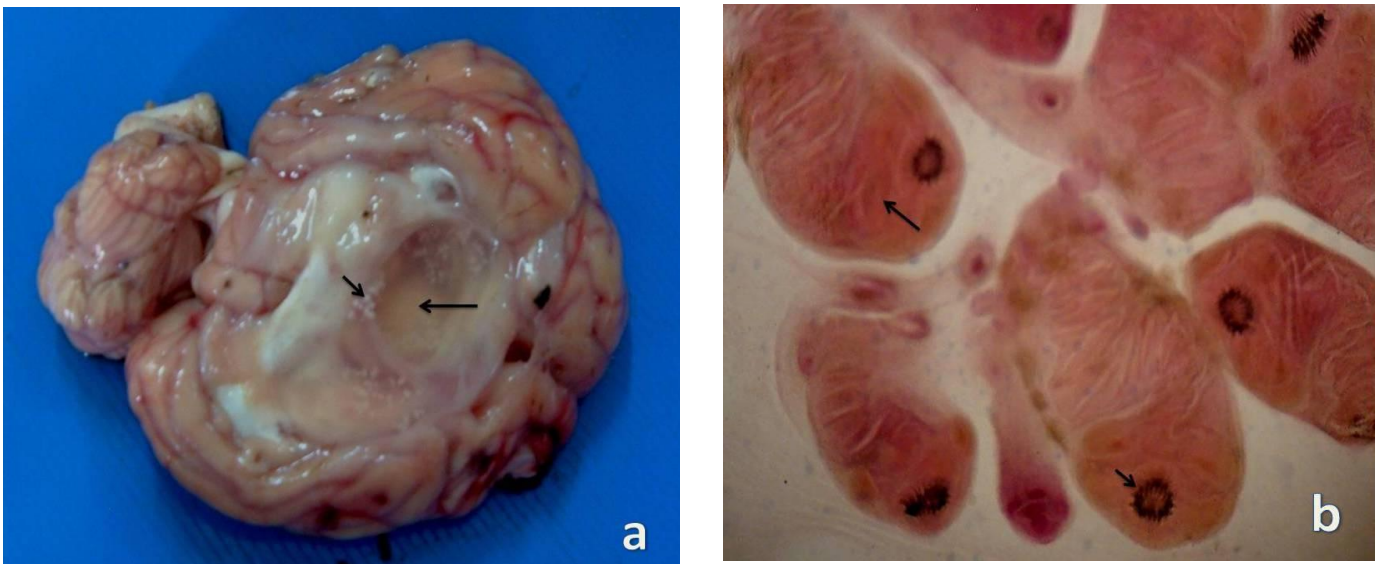
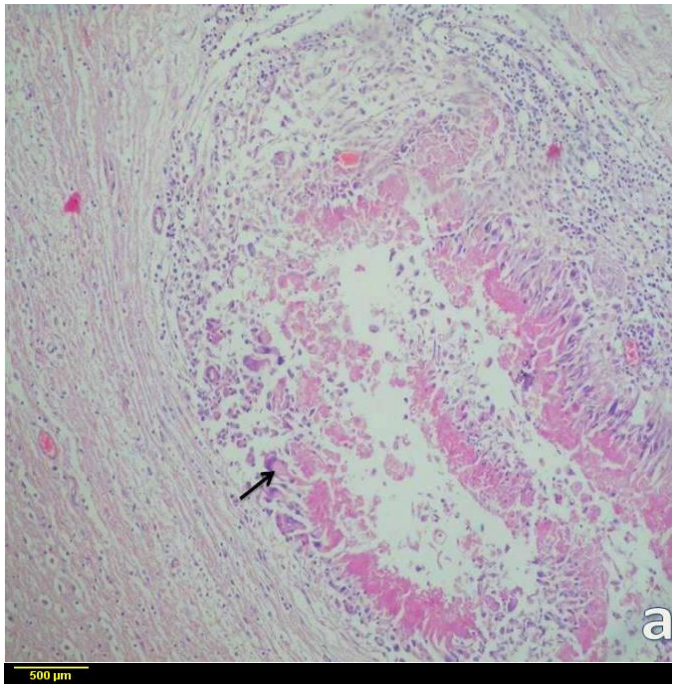
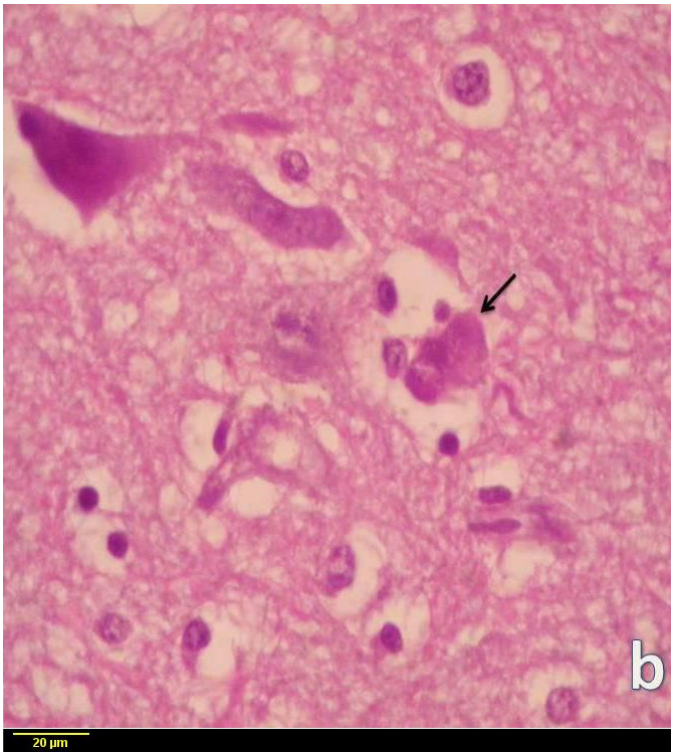


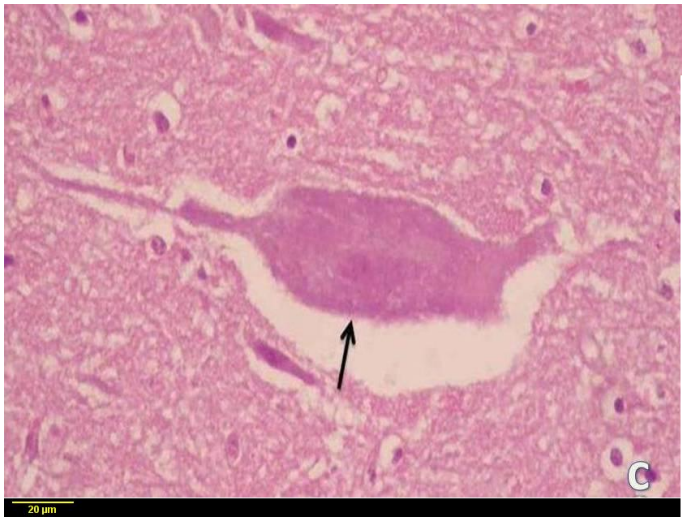
Figure (1): *Coenurus cerebralis*. a: Sheep brain with 5 cm in diameter transparent wall cyst (long arrow) with clusters of white dots (short arrow). b: Permanent mount section of protoscolices showed 4 cup shaped suckers (long arrow) and an armed rostellum (short arrow), X40.

Histopathological examination:

Examination of the H&E stained sections showed granulomatous encephalitis with caseation, encephalomalacia and langhanz giant cells (Fig. 2a). Neuronal necrosis was predominant with satellitosis (Fig. 2b), neuronophagia, perivascular cuffing, diffuse gliosis and choroid plexitis, beside endothelial necrosis and hemorrhage. Necrosis and dissociation of ependymal cells (Fig. 2d) with subventricular edama was consistent. Degeneration and necrosis of oligodendroglial cells was noticed, in addition to the axonal swelling (Fig. 2c) and demyelination. Furthermore, the histopathological sections stained with Cresyl violet stain elucidate severe depletion of Nissl granules from the cytoplasm of the neurons (Fig. 3).







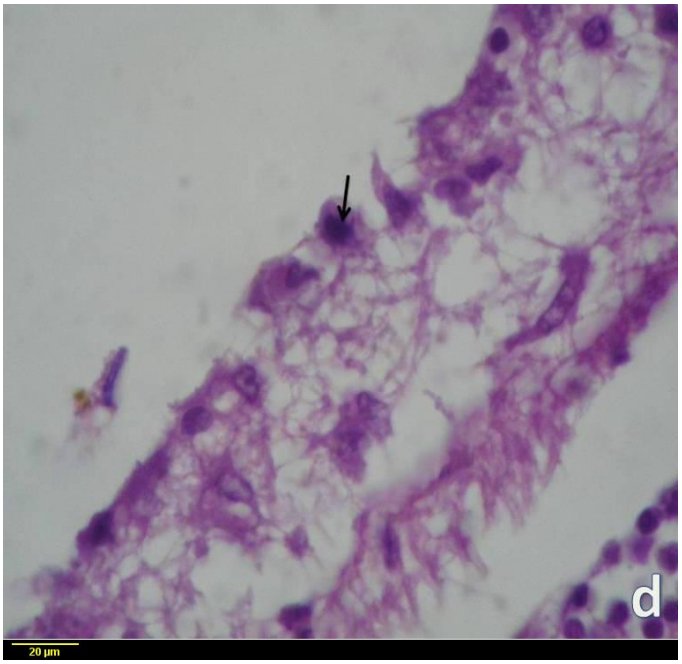


Figure (2): Histopathological sections stained with H&E stain showing a. Brain granulomatous encephalitis with caseation and langhanz giant cells (arrow), X4. b. Neuronal necrosis (arrow) with satellitosis, X40. c. Axonal swelling (arrow) with esinophilic degenerated oligodendroglia, X40. d. Necrosis (arrow) and dissociation of ependymal cells, X100.

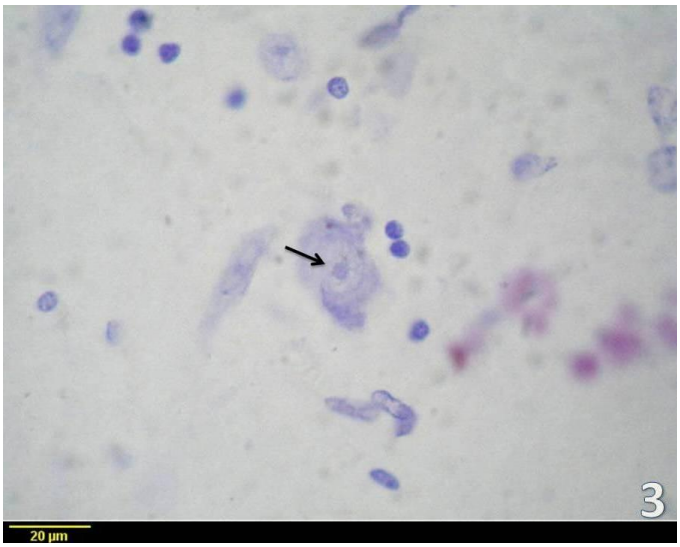


Figure (3): Cresyl violet stained section showing violet stained nissl granules forming cap like around nucleus (arrow), X100.

Examination of the semithin sections revealed severe edema, degenerated interfasicular oligodendroglia and loss of axons, with marked astrocytosis (Fig. 4). Moreover, electron microscopic examination estimates loss of myelin sheath with edema in white matter, in addition to marked degeneration and loss of oligodendroglial cells (Fig. 5).

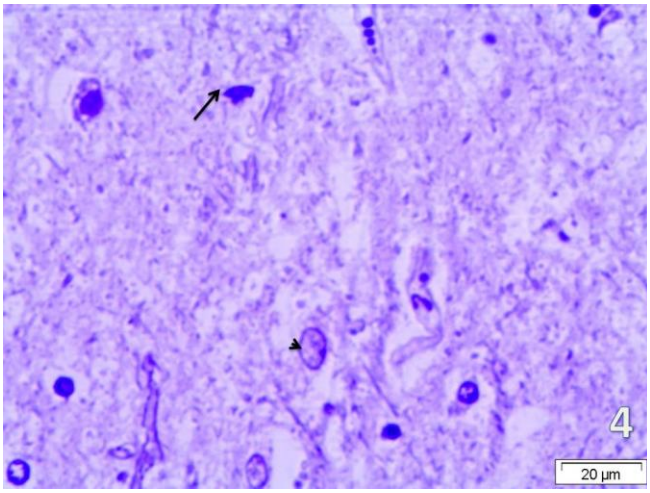


Figure (4): Toludine blue stained semithin section showing degenerated oligodendroglia (arrow), edema and marked astrocytosis (arrow head).

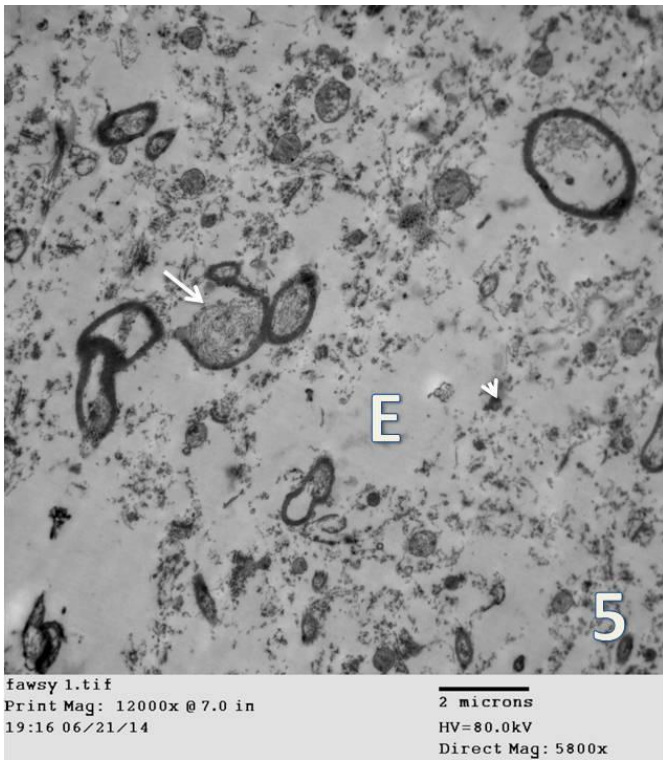


Figure (5): Transmission electron microscopic micrographes showing marked edema (E) and loss of myelin sheath (arrow) with degenerated and few oligodendroglia (arrow head).

Discussion

Coenurosis is an important parasitic disease of sheep causing fatal nervous manifestations hence it has various pathological alterations on the brain. We aimed at studying the pathological alterations caused by *C. cerebralis* on brain glial cells as a trial to explain the pathway of the associated nervous manifestations. In the present work, *Coenurus cerebralis* caused serious pathological effect on the ependymal and oligodendroglia cells mainly with subsequent neuronal injury.

The revealed pathological findings are consistent with what have been reported by (Gogoi et al., 1992; Tafty et al., 1997; Sharma et al., 1998; Achenef et al., 1999).

Examinations of the histopathological sections demonstrated sever neuronal necrosis, satellitosis and neuronophagia and loss of nissl granules, which are responsible for the neurotransmission (Cragg, 1970). This explains the chromatolysis in response to axonal injury, which lead to loss of the neurotransmission and the subsequent clinically reflected sever irritability.

Moreover, the ependyma is a simple ciliated epithelium that lines the ventricular surface of the central nervous system, extending from the lateral ventricles to the filum terminale (Marc and Bigio, 2010). Degeneration and necrosis of the ependymal cells lead to leakage of the cerebrospinal fluid into the brain tissue, this may explain the severe interstitial edema, demyelination and axon loss (Mc gavin, 2007) accompanied cyst which worseness the case and progression of the neurological signs.

Furthermore, oligodendrocytes are the myelinating cells of the central nervous system (CNS), they are the end product of a cell lineage which has to undergo a complex and precisely timed program of proliferation, migration, differentiation, and myelination to finally produce the insulating sheath of axons. They are not only ensheath the axons to electrically insulate these structures, but also induce a clustering of sodium channels along the axon, at the node of Ranvier, which is one important prerequisite for saltatory nerve conduction (Kaplan et al., 2001). In our results, the degeneration and necrosis of oligodendroglia as a bystander reaction of the inflammatory process lead to demyelination in the brain white matter (Bradl and Lassmann, 2010) resulting in a series of progressive neurological manifestations.

Conclusion

It can be concluded as a first report that the progressive nervous manifestations associated with *Coenurus cerebralis* infection are mainly due to its pathological effects on the interfasicular oligodendroglia and ependymal cells leading to fatal brain edema and axonal injury with subsequent neuronal injury, rather than the space occupied by the cyst.

References

Achenef, M., Markos, T., Feseha, G., Hibret, A. and Tembley, S. (1999): *Coenurus cerebralis* infection in Ethiopian Highland Sheep: incidence and observations on pathogenesis and clinical signs. Trop. Anim. Health Prod., 31: 15-24.

Avcioglu, H., Kapakin, T. and Yildirim, A. (2012): Clinical, morphological and histopathological features of bovine coenurosis: case reports. Revue Vet. Med., 163 (6): 295-298.

Bancroft, J. D. and Stevens, A. (1996): Theory and practice of histological technique. 4th Edn., Churchill, Livingston, Edinburgh, London, Melbourne, New York.

Benifla, M., Barrelly, R., Shelef, I., El-On, J., Cohen, A. and Cagnano, E. (2007): Huge hemispheric intraparenchymal cyst caused by *Taenia multiceps* in a child. Case report. J. Neurosurg., 107 (6): 511-514.

Bozzola, J. and Russell, L. (1991): Electron microscopy principles and techniques for biologists. Jones and Bartlitt publishers, Boston, USA., 2116.

Bradl, M. and Lassmann, H. (2010): Oligodendrocytes: biology and pathology. Acta Neuropathol., 119:37–53.

Bussell, K. M., Kinder A. E. and Scot, P. R. (1997): Posterior paralysis in a lamb caused by *Coenurus cerebralis* cyst in the lumber spinal cord. Veterinary Record, 140: 560.

Carson, F. (1990): Histotechnology: A self instructional text. Ascp., I11., 170-171.

Cragg, B. G. (1970): What is the signal for chromatolysis? Brain Research, 23 (1): 21.

Desouky, E. A., Badawy, A. I. and Refaat, R. A. (2011): Survey on coenurosis in sheep and goats in Egypt. Veterinaria Italiana, 47 (3): 333-340.

Dinev, I., Stoykov, D. and Bozadjiev, I. (1999): Clinical and morphological studies in spontaneous coenurosis in calves. Bulgarian J. Vet. Med., 2: 131-136.

Inechukwu, B. I. and Onwukeme, K. E. (1991): Intraocular coenurosis: a case report. Br. J. Opthalmol., 75 (7): 430-431.

Kaplan M. R., Cho, M. H., Ullian, E. M., Isom, L. L., Levinson, S. R. and Barres, B. A. (2001): Differential control of clustering of the sodium channels Na(v)1.2 and Na(v)1.6 at developing CNS nodes of Ranvier. Neuron, 30:105–119

Kheirandish, R., Sami, M., Azizi, S. and Mirzaei, M. (2012): Prevalence, predilecton sites and pathological fndings of *Taenia multceps* coenuri in slaughtered goats from south-east Iran. Onderstepoort Journal of Veterinary Research, 79(1): 436-441.

Marc, R. and Bigio, D. (2010): Ependymal cells: biology and pathology. Acta Neuropathol., 119: 55–73.

Mohi El-Din, M. (2010): The Significance of Subarachnoid Cerebrospinal Fluids (CSF) in the Development of metacestode of *Coenurus Cerebralis* in Sheep with reference to its Pathological Effect. Global Veterinaria, 4 (4): 343-348.

Mc gavin, M. D. and Zachary, J. F. (2007): Pathological basis of veterinary disease. 4th Ed., Mosbi, Philadelphia, USA, 858.

Oruç, E. and Uslu, U. (2006): Comparative cytopathological and histopathological studies of sheep with suspected *Coenurus cerebralis* infection. Türkiye Parazitoloji Dergisi, 30: 285-288

Richard, M. H. and Kruse, G. O. W. (1982): The collection and preservation of animal parasites. Boston, London.

Sharma, D.K., Singh, N. and Tiwari, H. A. (1998): Prevalence and pathology of coenurosis in organized goat farms. J. Vet. Parasitol., 12: 30-32.

Sharma, D. K. and Chauhan, P. P. S. (2006): Coenurosis status in Afro-Asian region: A review. Small ruminant research, 64: 197-202.

Tafty, A.K., Oryan, A. and Maleki, M. (1997): Pathological changes due to coenurosis in a wild ewe in Iran. J. Vet. Parasitol., 11: 65-68.

Yoshino, T. and Momotani, E. (1988): A case of bovine coenurosis (*Coenurus cerebralis*) in Japan. Japanese J. Veterinary Sci., 50: 433-438.