PATHOLOGICAL STUDIES ON SOME PARASITIC INFESTATIONS OF BRAIN IN SHEEP

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SUMMARY

The present study was carried out on 500 brain of sheep slaughtered in Cairo abattoirs and reared in different sheep's farms. The parasitological results revealed that, the incidence of the parasitic affection of the brain was 21% and including Coenurus cerebralis 9.4 %, Oestrus ovis infestation 10.2% and sarcocystosis 1.4%. Clinically, in coenuriosis, the infested sheep hold their heads on one side and suffered from uni- or bilateral blindness, recumbancy and complete paralysis. The gross examination of the brain infested with C. cerebralis, revealed that, the live cysts appeared as bladder like cysts with translucent membrane and contained clear fluid and they were found mostly in the cerebellum. While the dead cysts appeared as old calcified grayish white small nodules. Microscopically, the walls of live cysts of C. cerebralis were formed from thick outer connective tissue layer and inner germinating layer carried many scolices. These cysts induced inflammatory reaction in the form of mononuclear and eosinophilic cellular aggregations around their walls. Meanwhile, the dead parasitic cysts were represented by focal areas of caseous necrosis and dystrophic calcification which enclosed by thick connective tissue capsule infiltrated with chronic inflammatory cells. Cerebral congestion, perivascular hemorrhages and cerebral encephalomalacia with gliosis, neural degeneration and necrosis of Purkinje cells were also observed. While in O. ovis infestation, the infested sheep were clinically emaciated and rub their noses to the ground or the adjacent animals. Grossly, the brain was congested and showed petechiae on the cerebellum, thickening in the meninges with presence of few larvae attached to the brain surface. Histopathologically,
Congestion, perivascular hemorrhages, cerebral edema, focal encephalomalacia, mononuclear cellular infiltration with neural degeneration and thickening of the meninges were recorded. On other hand, no characteristic clinical signs or gross lesions were observed in sarcocystosis. Microscopically, oval parasitic cysts of sarcocyst filled with PAS positive bradyzoites without any inflammatory reaction were noticed the examined brain.

Finally, it concluded that, *C. cerebralis* and *O. ovis* infestations of the brain of sheep were accompanied with irritation of the brain tissue resulting in focal encephalomalacia with congestion, hemorrhages and mononuclear inflammatory cellular reaction. While in sarcocystis no tissue host reaction was recorded in the brain of infested sheep.

**INTRODUCTION**

Sheep constitute the main income of Desert habitat and farmers in Egypt. They were raised mainly on naturally growing herbage that allows spreading of the parasitic infestations among them. *C. cerebralis* is the most common dangerous parasite infesting the central nervous system of sheep and goats causing primarily focal encephalopathy (23). Contamination of sheep feeds with dog feces increasing the incidence of larval invasions (18). The majority of *C. cerebralis* cysts was located in the cerebrum and cerebellum, and not found caudally beyond the cerebellum (9, 10, 25). *O. ovis*, nasal bot fly, is world wide mayiasis-producing fly in all sheep farming countries. It usually deposits its first stage larvae around the nose of sheep causing rhinitis (12). Migration of these larvae through the ethmoid bone, penetrating the cranial cavity resulting in damage and inflammatory lesions in the brain and with death of infested sheep. (13, 17). Moreover, sheep are the intermediate host of different types of sarcocysts. Most of these sarcocysts were found in the brain of sheep without any inflammatory reaction (19, 21), however, Caldow et al. (5) recorded non suppurative encephalomyelitis in sheep suffered from sarcocystosis.
The present study was carried out to record the incidence of parasitic infestations in the brain of sheep and describe the associated gross and microscopic changes.

MATERIAL AND METHODS

The present study was carried out on 500 brain of sheep slaughtered in Cairo abattoirs and reared in different farms at Kafr El Sheikh, Bourg ElArab, Ismailia and Cairo University. The age of the animals were ranged from 4 months to 2 years in these farms and were 1.3 to 2.6 years in Cairo abattoirs.

For parasitological examination, the suspected cysts of *C. cerebralis* were separated from the brain and preserved in 10% neutral buffered formalin. Parts of these cysts were compressed between two glass slides stained in acetic acid alum carmine and examined microscopically to show up the structure of scolices according to Wasser (28).

In suspected cases of *O. ovis* infestation, the heads of the sheep were cut from the nose up to the base of skull and the recovered larvae were carefully picked up, preserved in ethyl alcohol and then examined macroscopically after Soulsby (24).

For bacteriological investigations, samples from brain tissues were collected in sterile plastic bottles surrounded with ice and brought to lab under aseptic condition.

For histopathological examination, tissue specimens from the brain of infested sheep were collected and immediately fixed in 10% neutral formalin. After proper fixation, thin paraffin sections were routinely prepared and stained with hematoxylin and eosin stain after Drury and Wallington (7). Moreover, special staining techniques as Masson's trichrome stain, Von kossa's stain, and PAS stain were also performed for demonstration of connective tissues, calcium salts and parasitic cysts respectively according to Clayden (6).
RESULTS

The parasitological examination of the brain of sheep under investigation revealed that, the total numbers of different parasitic infestations of the brain were 105 out of 500 examined brain with an incidence of 21%. These parasitic infestations were classified into 47 cases of *C. cerebralis* infestations (9.4%), 51 cases of *O. ovis* infestations (10.2%) and 7 cases of sarcocyst infestations (1.4%).

The bacteriological examination of the brain revealed negative result except isolation of *Staphylococcus aurous* in two cases associated with *O. ovis* infestations.

*Coenurus cerebralis* infestation:

The parasitological diagnosis of the *C. cerebralis* was depend on the morphological characters and microscopic examination of the parasitic cysts after staining with acetic acid alum carmine stain. These cysts were 1-5 cm in diameter, enclosed in a translucent membrane and contained many scolices. These scolices were varied in size according to the stage of their development from 0.12 X 0.15 to 1.2 X0.8mm. Moreover, in well developed scolex, the rostellum was markedly armed with 30 hooks arranged in two rows (Fig. 1).

Clinically, the affected sheep hold their heads to one side and some of them suffered from uni- and bilateral blindness. Complete paralysis and sternal recumbancy were observed in few examined sheep.

The gross examination of the brain in cases of coenurirosis, revealed presence of one or more cysts embedded mostly in the cerebral hemisphere (Fig.2). In one case, the recorded parasitic cyst was found between the cerebrum and cerebellum. The recorded cysts were mostly live, bladder like in appearance and of variable sizes. The cut sections of these cysts revealed grayish white translucent outer walls and clear fluid containing, over one hundred scolices in their lumens. Moreover, two old calcified dead cysts appeared as grayish white small foci we detected.

Microscopically, the walls of the live cysts were formed from thick outer connective tissue layer and inner germinating layer (Fig. 3). Many scolecies were observed attached to the germinating layer and free
inside the lumens of these cysts (Fig.4). Congestion of the cerebral blood vessels, focal aggregation of mononuclear cells mostly lymphocytes, macrophages and plasma cells with few eosinophils were observed mainly near to these cysts. Meanwhile, the dead parasitic cysts were evidenced by focal areas of caseous necrosis surrounded by thick connective tissue capsule infiltrated with mononuclear inflammatory cells and few multinucleated giant cells (Fig. 5A&B and 6). Dystrophic calcification of the dead cyst which takes blackish coloration with Von Kossa's stain was noticed in one case (Fig. 7). Cerebral congestion and multiple areas of hemorrhages particularly around the blood vessels were detected in the cerebral hemisphere. Necrosis of Purkinje cells and focal cerebral encephalomalacia were observed. Perivascular inflammatory cellular aggregation and proliferation of glial cells were noticed. Neural degeneration, satellitosis and neurophagia were seen (Fig. 8). Moreover, in the meninges, congestion of the blood vessels, edema and fibrous connective tissue proliferation were also detected.

**Oestrus ovis infestation:**

The parasitological detection of *O. ovis* larvae was depending on their morphologic characters. The recovered larvae were yellowish white in color and their length ranged from 1 to 3 cm. Moreover, these larvae were segmented and each segment showed transverse bands on its dorsal surface and small spines on its ventral aspect.

Clinically, the affected sheep were emaciated and rub their nostrils to the ground and the adjacent animals.

Macroscopically, the brain of these infested sheep were congested and showed patechieae on the surface of cerebellum and thickness of the meninges. Moreover, one or more larvae were found attached to the surface of the brain.

The histopathological examination of this brain showed severe congestion and perivascular hemorrhages (Fig. 9). Focal areas of encephalomalacia were prevalent (Fig. 10). Multiple areas of hemorrhages and edema scattered throughout the cerebral hemisphere were found. In addition, neural degeneration and thickening of the meninges were detected.
Moreover, focal gliosis, neurophagia with mononuclear inflammatory cellular infiltration of the cerebellum mainly lymphocytes were observed in cases of *O. ovis* complicated with *Staphylococcus aureus* infection (Fig. 11).

**Sarcozyst infestation:**

Seven cases of sarcocystosis were accidentally recorded during the histopathological examination of the brain of sheep. Neither characteristic clinical signs nor gross lesions were recorded.

Microscopically, oval parasitic cysts filled basophilic rounded bradyzoites and enclosed with cystic capsule were observed in the cerebral parenchyma without any inflammatory reaction (Fig. 12 -A). These bradyzoites gave positive reaction with PAS stain (Fig. 12-B).

Finally, it concluded that, *C. cerebralis* and *O. ovis* infestations of sheep were accompanied with irritation of the brain tissue resulting in cerebral encephalomalacia and vascular changes in the form of congestion and hemorrhages with mononuclear inflammatory cellular reaction. Meanwhile, no tissue host reaction was observed in the brain of sheep in sarcocystosis.

**DISCUSSION**

Nothing is known of what motivates and directs of the migration of the larval parasite. Aberrant pathways include the nervous system with such frequency suggest that parasitic larvae have a special propensity for wandering in the central nervous system (15). Therefore the present study was carried out as a trial to record the incidence of some parasites infested the brain of sheep and in the same time to describe the associated gross and microscopic changes.

In the present study, the parasitological examination of 500 brain of sheep slaughtered in Cairo abattoirs and reared in different sheep's farms, recovered 105 cases of different parasitic infestations. According to the causative parasite, these infestations were classified into 47 cases of *C. cerebralis* infestations (9.4 %), 51 cases of *O. ovis* infestations (10.2 %) and 7 cases of sarcocyst infestations (1.4%). The incidence of the recorded coenurusosis was nearly equal to those recorded by previous
studies (20,18) who mentioned that, the incidence of C. cerebralis infestations were 3.7% and 5.8% respectively and was lower than those mentioned by Saleh and Idris (22) who found that, the incidence of coenuriasis in sheep was 21.8%. This difference could be attributed to the recent improved hygienic measurements which reduced the number of dogs that responsible for contamination of sheep feeds and so lowering the incidence of larval infestation of sheep in present study. This opinion was supported by those mentioned in previous work (18).

In the present work, the sheep infested with C. cerebralis, holds their heads on one side and most of them suffered from uni- or bilateral blindness, recumbancy and complete paralysis. These neurological disorders could be attributed to the pressure exerted by the parasitic cysts on the surrounding cerebral tissues. Moreover, nearly similar clinical signs were observed in previous investigations (4,11,16).

The gross examination of the brain of sheep infested with C. cerebralis in the present work showed that, the live cysts were found mostly in the cerebellum and appeared as bladder like cysts contained clear fluid with translucent membrane. These macroscopic lesions were in a complete agreement with that recorded by previous workers (14,11,10,16). Moreover, in present study two dead C. cerebralis cysts were recorded and grossly appeared as old calcified grayish white small foci in the cerebellum.

Regarding to the microscopic findings of the brain in coenurosis, the walls of the live cysts were formed from outer connective tissue layer and inner germinating layer carried many scolices. These cysts induced inflammatory reaction in the form of mononuclear and eosinophilic cellular aggregations around their walls. This microscopic picture was in a harmony with those described in previous studies (30,20,2). Meanwhile, the dead parasitic cysts recorded in the present work were represented microscopically by focal areas of caseous necrosis and dystrophic calcification enclosed with thick connective tissue capsule and chronic inflammatory cells. These microscopic results were in complete accordance with that recorded by (18) and partially in agreement with Gogoi et al. (9) who found liquefactive necrosis Summers et al. (25) who found focal coagulative necrosis.
In the present study, presence of dead cysts of *C. cerebralis* was accompanied with microscopic brain lesions represented by congestion, perivascular hemorrhages, encephalomalacia with focal gliosis, neural degeneration and necrosis of Purkinje cells. These results were supported by Gogoi *et al.* (9).

Concerning *O. ovis* infestations of sheep, the recorded incidence of infestations in the present investigation was 8.2 %. This incidence was similar to those reported by Abd el-Rahman *et al.* (1) and lower than those mentioned by Yilma and Dorchica (29) and Gabaj *et al.* (8) who found that, the incidences of *O. ovis* infestations among sheep were 24.8 and 22.6 % respectively. These differences in the prevalence of infestations could be attributed to the variation in seasons during which the specimens were collected. Our opinion was supported by the previous investigation (3).

Clinically in the present work, the sheep suffered from *O. ovis* infestation, were emaciated and rub their noses to the ground or the adjacent animals. Grossly, the brain of these sheep was congested and showed petechiae on the cerebellum, thickening in the meninges together with the presence of few larvae attached to the brain surface. These results were in accordance with those mentioned in previous works (24,27).

In the present study the histopathologic examination of the brain of the sheep infested with *O. ovis* revealed that, the larvae of *O. ovis* induced some vascular changes in the form of congestion perivascular hemorrhages and cerebral edema in addition to brain damages manifested by focal encephalomalacia, mononuclear cellular infiltration of the cerebellum together with neural degeneration and thickening of the meninges. These microscopic findings were partially in agreement with the results of Parihar (21) who found giant cell granuloma and Linklater and Smith (17) who recorded necrosis in the brain of sheep in *O. ovis* infestation.

In the present work, seven cases of sarcocystosis were accidentally recorded during the microscopic examination of the brain of sheep. Neither clinical signs on the sheep nor characteristic gross brain lesions were observed in sarcocystosis. This result was partial in
agreement with *Caldow et al.* (5) and *Ugglā* (26) who recorded some nervous manifestation without any gross brain lesions of sheep infested with sarcocyst. Microscopically, oval cysts filled with PAS positive bradyzoites without any inflammatory reaction in the brain of examined sheep were noticed. These results were nearly similar to the previous studies (19,21) and in the same time disagree with *Ugglā* (26) who recorded nonsuppurative encephalitis in ovine sarcocystosis. These differences could be attributed to the species of the infested sarcocyst. This opinion was supported by the previous work (26).

Finally, it concluded that, *C. cerebralis* and *O. ovis* infestations of the brain of sheep were accompanied with irritation of the brain tissue resulting in focal encephalomalacia and mononuclear inflammatory cellular infiltration with vascular changes represented by congestion, edema and hemorrhages. On other had, no tissue host reaction was recorded in the brain of sheep infested with sarcocyst.

**REFERENCES**


Legends:

Fig.(1) Brain of sheep infested with *C. cerebralis* showing presence of bladder like cyst in the cerebellum (arrow).

Fig.(2) Cyst of *C. cerebralis* showing rostellar hooks. Acetic acid alum carmine stain X 400.

Fig.(3) Brain of sheep infested with *C. cerebralis* showing the inner germinating layer of the parasitic cyst. H& E stain X 100

Fig.(4) Brain of sheep infested with *C. cerebralis* showing inverted scolex. H& E stain X 400

Fig.(5) A-Brain of sheep infested with *C. cerebralis* showing dead cyst surrounded by fibrous CT capsule infiltrated with mononuclear inflammatory cells. H& E stain X100  
B- Showing positive reaction of connective tissue capsule with Crossman's trichrome stain X100

Fig.(6) Brain of sheep infested with *C. cerebralis* showing many giant cells at the periphery of dead cyst (Arrows) H& E stain X 400.

Fig.(7) Brain of sheep infested with *C. cerebralis* showing dystrophic calcification of dead cyst with thick connective tissue capsule. H& E stain X100.

Fig.(8) Brain of sheep infested with *C. cerebralis* showing neural degeneration and satellitosis H& E stain X400

Fig.(9) Brain of sheep infested with *O. ovis* showing focal and perivascular hemorrhages. H& E stain X400

Fig.(10) Brain of sheep infested with *O. ovis* showing cerebral encephalomalacia. H& E stain X200

Fig.(11) Brain of sheep infested with *O. ovis* showing lymphocytic cellular infiltration of the brain tissues H& E stain X 200

Fig.(12) A- Brain of sheep infested with sarcocyst showing oval shaped cyst filled with basophilic small bradyzoites within the brain tissues H& E stain X 200  
B- Brain of sheep infested with sarcocyst showing PAS positive reaction of bradyzoites within the parasitic cyst. PAS stain X 200.
دراسات بالاتولوجية على بعض الإصابات الطفيلية للدماغ في الخراف

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في مزارع خراف مختلفة أظهرت نتائج الفحوص الطفيلية أن نسبة حدوث الإصابات الطفيلية بين دماغ الخراف المفحوحة كانت 21% وتضمنت إصابات بالسينيروس سيتيرلايس 9%، وعندو أورسترس أوفرز، 20% و إصابات بالساركورستس 4%.

وأظهر النحص الكنكيكي أن الخراف المصابة بالسبيرس سيتيرلايس تلوى رأسها على أخرى جواربها كما تعلت من عمي في خرابة البطنين أو كلاهما، مع ظهورها الدائمين.

وأظهر النحص المخبرى للمتحورات أن هناك قشور طفلاً ناجية تمثل حوالي 50% من نسلها، وعند ما تدخل من الفوهة، يظهر احتقان الدماغ، ووفرة نسيج الورم، وجدت عادة في الجوانب.

وأظهر النحص المخبرى للمتحورات وجود خلال الحيوانات الحية لداء الطفيلي المتراكم، وعندما تدخل نسيج الورم في جوانب الدماغ، تتبعها نسيج الورم، وجدت عادة في الجوانب.

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