Histopathological and Immunohistochemical Study of Ovine Encephalitis
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Central nervous system diseases are one of the disabilities and death causes of livestock animals in the worldwide. Our harmony of its pathobiology has increased fundamentally at recent time. The present work aimed to Detection GFAP and CD65 expression correlated with microscopically lesions in the sheep brain in Mosul city. Thirty-two samples of ovine brain were collected during the period from December 2022-February 2023, the gross lesions represented by circulatory disturbances (hemorrhage and congestion), parasitic infection, disturbance of growth and brain pigmentation. Affected samples were gathered and prepared for routine histopathology and immunohistochemistry examinations. In contrast, the inflammation finding was 31.25% represented by infiltration of mononuclear and multinucleated inflammatory cell, circulatory disturbance with 21.87% percent, disturbance of growth which accounted 12.5%, parasitic infection with a 15.62% percent and pigmentation with 6.25% percent and finally necrosis with 12.5%. Results of histological examinations revealed perivascular edema with congestion in the meningeal blood vessels, severe congestion in the cerebral and cerebellar veins, as well as infiltration of mononuclear and multinucleated inflammatory cells with condensation of inflammatory cells around the blood vessels. In addition, vacuolar degeneration and gliosis with the presence of phagocytic cells with foamy cytoplasm and suppurative encephalitis, as well as presence of larval stages of Toxoplasma gondii, Sarcocystosis parasites in the brain tissue and Hydatid cysts of Echinococcus granulosus in the cerebellum. Reproducible function of glia and neurons of ovine brain is fundamental for cellular changes study in neurodegenerative disease. Moreover, immunohistochemistry represented by CD65 as a minor E-selectin ligand and Glial fibrillary acidic protein (GFAP) as a candidate for future counting of cells and scoring as a mild, moderate and sever lesion. We concluded that ovine are exposed to different types of neurodegenerative diseases. Immunohistochemistry technique can be used as a biomarker for the severity of the disease and molecular biology techniques should be done for abnormal protein expression.

Keywords: Immunohistochemical, Histopathological lesion, Brain, Sheep.

Introduction
First and former, sheep have more comparable brain in size and structures to the brain of human than small laboratory animals, it’s considered a good example for studying of neurogenesis for multiple reasons. It’s puberty during 6-8 months and it’s age 10-11 years are longer in contrast to rodents, it’s weight between 130-140 grams while rodents’ brain weight is 1-2 grams. Neuronal maturation in adulthood is affected by lifelong differences, sheep have a gyrencephalic brain, a cortex with a laterally extended bent pial surface like human and non-human primates, and mature neurogenesis could vary from a lissencephalic brain, since both types of brain differ significantly in their developmental stages [1]. Ruminants’ disease of the central nervous system causes a worldwide economic loss. Bacterial infections are important mortality causes and neurological disorder of ruminants [2, 3]. Inflammatory neurological disorders in livestock animals can be caused by bacterial infections for example Listeria monocytogenes, cerebral and spinal cord abscess and neurotuberculosis [3-5]. Central nervous system (CNS) may be affected by microorganism infection with four pathways from direct piercing lesion; or central ascending infection, hematolog pathway and lymphatic dissemination [5 and 6]. Also, chemical poisoning may cause neurological signs [7-9]. Furthermore, parasitic infection represented by hydatid cyst and Oestrus ovis of the central nervous system continue to be a major cause of morbidity and mortality [10, 11]. The cognitive, mental health and neurological problems

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caused by parasitic infection affect health and activity of the central nervous system of small ruminants [8-10]. Brain developing needs a complex of blood vessels to deliver nutrients and oxygen. The vascular neuron unit integrates the interaction between basal lamina, pericytes, brain endothelial cells and surrounding glial [10]. Each part of this unit is intimately interconnected with other parts to establish system function and anatomical structures [12]. This system maintains homeostasis of brain, regulates flow of blood, maintains the blood brain barrier integrity and protects the CNS from any toxic exogenous or endogenous substances [13]. Any defect in this function leading to hemorrhage, edema and congestion of the CNS[14]. One of the distinctive biological markers for the diagnosis and predicting the outcome of central nervous system injuries, especially the brain ,is the using of GFAP &CD65 . This work aims to describe types of lesions, determined the ratio of Histopathological lesions and study the GFAP and CD65 expression in the brain of sheep.

Material and Methods

-Collection of Brain tissue

This work was constructed on tissue sample from sheep brain that delivered to the Veterinary Teaching Hospital of the College of Veterinary Medicine, University of Mosul. 32 sheep brains of both sexes were collected immediately after slaughter, these sample collected during December 2022 -February 2023.

-Gross examination

All brain samples were examined macroscopically to determine any abnormal changes in the appearances measurement and texture of the brain. The suspected part of the organs was excised and placed in a clean and labeled container and sent to the laboratory for downstream analysis.

-Histopathological examination

Sheep brain samples were treated and fixed in 10% formalin, then these tissue samples were embedded in blocks of paraffin wax, 3-4 µm section of tissue sample from each animal stained routinely by (H&E). A 4-µm section of brain tissue from each animal was stained and mounted on Poly-L-lysine slides for Hematoxylin and Eosin (H&E) for microscopic evaluation. The stained slides were assessed according to the references [15,16].

-Immunohistochemistry analysis

Samples were de-waxed in Xylene, rehydrated in a series of alcohol (ethanol) followed by washing in PBS in order to suppress the activity of endogenous peroxidase. 3% methanol solution was added for 30 minutes. Then, these sections were blocked and incubated for overnight with primary antibodies at 4 Celsius, CD65 and GFAP (1:2000 Z0334 Dako cyto matin) were used as a primary antibody at the current work. These sections were washed 3 times and incubated for 60 minutes in poly-HRP Goat Anti-Mice IGG. After that, these sections were washed 3 times and detected with a complex of avidin-biotin and stained for 1 hour with Hematoxyline, followed by washing in distilled water, dehydrated. Finally, these slides were covered by cover slipped and photographed by a digital camera [17].

-Grade of immunohistochemistry

Brain section were exam at low-power magnification and high –power magnification in order to detection antibody reaction places .CD65&GFAP showed positive expression of CD65&GFAP were estimated as the positive average of brain lesion and staining density was evaluated in three grade . CD65& GFAP and immune staining estimated was done according to a weight grade system [18].Positive lesion proportion was recorded as mild, moderate and sever lesion.

Analytical Statistics

One-way ANOVA was used to analyze all data, and the post-hoc Duncan’s test was used [19].

Results

Brain lesion

Gross examination of infected sheep brain showed disturbances of circulatory system represented by (hemorrhage and congestion on the surface of cerebellum, parasitic infection including larvae of oster ovies and Echinococcus granulosus tapeworm ,which appeared as a rupture cysts, as well the gross section shows disturbances of growth (atrophy )and pigmentation .Result in figures (1 and 2) revealed types of brain gross lesions , lesion description, number of affected animals and percentage of it.

![Diagram showed types of brain lesion and finding of it.](image-url)
Fig. 2. Gross section of sheep brain shows hemorrhage and congestion in the brain surface (arrows) and cerebellum surface (dashed arrow)

Fig. 3. Gross section of sheep brain shows severe hemorrhage inside brain (arrow) and inside cerebellum (dashed arrow)

Fig. 4. Gross section of sheep brain shows the presence of *Oestrus ovis* (arrow)

Fig. 5. Gross section of sheep brain shows the presence of *Echinococcus granulosus* inside the cerebellum (arrow) with the presence of ruptured cyst (dashed arrow)

Fig. 6. Gross section of sheep brain shows severe atrophy of the cerebral lobes and cerebellum

Fig. 7. Gross section of sheep brain shows pigmenting of brain with complete disappearance of the cavities (arrow)
Photograph of brain showed hemorrhage and congestion in figures (2 & 3), parasitic infection with cyst rupture (4 & 5) and atrophy of lobes and pigmentation (6 & 7)

**Histopathological lesions**

Histopathological grades for the brain lesions explained in table (1), as a mild lesion, moderate lesion & sever lesion. Microscopic examination of brain tissue using Hematoxylin and eosin stain (H and E) revealed presences of inflammation with dense infiltration of inflammatory cell in addition to necrosis of the cerebellum figures (8 & 9). Also, there was vacuolar degeneration and thickening of blood vessels wall figures (10,11), increased number of satelliosis cells and swollen of gitter cell represented as a growth disturbance. Parasitic infection was clear with egg and larval phase of *Sarcozystosis ovis* and *Toxoplasma gondii*, figures (12,13, 14 and 15).

Table 1. Histopathological grading criteria for brain lesion

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mild</th>
<th>Moderate</th>
<th>Sever</th>
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<tr>
<td>Brain</td>
<td>Infiltration of lymphocytes &amp; and macrophage .figs 8,9</td>
<td>Inflammation of cerebellum (figs 8,9)</td>
<td>Multiple cysts of Echinococcus granulosus .figs 12, 13.</td>
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<td>Condensation of inflammatory (figs 8,9).</td>
<td>Congestion &amp;hemorrhage(fig. 14).</td>
<td>Sarcozystosis and toxoplasma gondii larvae stage(fig 14,15).</td>
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<td></td>
<td>Thickenning of blood vessels , abscess and Vasogenic Edema(Fig. 11)</td>
<td>Gliosis and vacuolar degeneration of glial cell .figs 10,11.</td>
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Fig. 8. Histopathological section of sheep brain shows sense infiltration of inflammatory cells (A) H&E stain, 150X

Fig. 9. Histopathological section of sheep brain shows chronic abscess with necrosis of cerebellum (A) with dense infiltration of inflammatory cells (B) H&E, 150X

Fig. 10. Histopathological section of sheep brain shows blood clot (A) and gliosis of glia cell and vacuoles (B) H&E, 150XB

Fig. 11. Histopathological section of sheep brain shows gliosis (A) with vacuolar degeneration (B) H&E, 150X
Immunohistochemistry Grading

The analysis of immunohistochemistry scoring (CD65 and GFAP) in brain sections showed diffused and severe positive staining (score 3) figs (16, 17 and 18). Both CD65 and GFAP showed mild, moderate and severe expressions (19, 20 and 21)

![Image](image1)

**Fig. 16.** Immunohistochemistry scoring with CD<sup>65</sup> antigen shows mild inside microglia (score 1) X400

![Image](image2)

**Fig. 17.** Immunohistochemistry scoring with CD<sup>65</sup> antigen shows moderate grade inside microglia (score 2) X400

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**Fig. 12.** Histopathological section of sheep brain shows eggs (A) and larval stage (B) of *cysticercus ovis* H&E, 150X

**Fig. 13.** Histopathological section of sheep brain shows head of (A) and larval stage (B) of *Echinococcus granulosus* H&E, 150X

**Fig. 14.** Histopathological section of sheep brain shows sever hemorrhages (A) and *Toxoplasma gondii* stages (B) and edema (C) H&E, 450X

**Fig. 15.** Histopathological section of sheep brain shows different stages of *Sarcocystis* different stages (A), *Toxoplasma gondii* stages (B) H&E, 450X
Fig. 18. Immunohistochemistry scoring with CD68 antigen shows severe expression inside microglia (score 3) X400

Fig. 19. Immunohistochemistry scoring with GFAP antibody in spinal cord shows mild grade of astrogliosis antigen (score 1) X400

Fig. 20. Immunohistochemistry scoring with GFAP antibody in spinal cord shows moderate grade of astrogliosis antigen (score 2) X400

Fig. 21. Immunohistochemistry scoring with GFAP antibody in spinal cord shows severe grade of astrogliosis antigen (score 3) X400


**Discussion**

The central nervous system is apart from the nervous system, The nervous systems involve two parts peripheral and central nervous system which is consist from brain and spinal cord. its responsible for senses, cognition, emotion and movement. CNS exposure to different microorganism and disease like traumatic injury, and neurodegenerative diseases that effects on activity,function or even morphology of it[20]. Our results showed that these cell was predominantly inflammatory cell with a moderate number of other inflammatory cell, the occurrence of inflammation in the brain tissue is comparatively low in comparison to inflammation of others organs; nonetheless, the etiological agent are quite changeable with bacterial agent infection commonplace in domestic animals While parasitic and viral infection agent are more common in cats and dog[21], and these results agreement with[22,23 and 24].Neuron necrosis can results from vascular thrombosis ,cerebral ischemia, inflammatory mediators,bacterial toxin, thermal injury, cardiac failure, nutritional deficiency , heavy metal toxicity and trauma [25].Degenerative changes represented by cavitation and vacoulolation give deeply affected brains tissue a squishy appearance .The cavitation and vacoulolation are betimes as results of the empty area left after death of cell and resorption, neuropil degeneration and vacoulolation [26].Expression of GFAP has been related to the utmost damage of human and animal cell with infection[27]. This is comparable to the present study observation of notably significant GFAP immunostaining in the medulla. CD65 protein was observed in the CNS infection. CD65, a minor E-selectin ligand acts as an independent hazard agent for extravascular dissemination of acute myeloid leukemia, adhesion molecule presence in acute myeloid leukemia explains their tendency to cross the vascular wall and establish infiltration extravascular leukemic[28].CD65 can be considered as a diagnostic marker in acute leukemia. Moreover, Histopathological and immunohistochemical examination showed the presence of parasitic infection (egg& larvae) and these contributed to several stage of livestock animal infection with these parasite , healthy condition , immunity statuses [29], as well as random distribution of parasite may be laboror [30 and 31]. Further our results highlights on disturbance of growth especially (atrophy )in different part of the brain and these contributed to some neurodegenerative clinical disease such as multiple sclerosis and Alzheimer’s disease[32]. The blood stream is the more common entry portal of the central nervous system in neonates animals ,microorganism enter the blood stream through venous system and umbilical vein[33,34], in adults animals chronic inflammation site like bacterial skin disease, ear infection and abscess, can also acts as a bacteria sustained sources, which enter and disrupted in different distant site of the CNS through the hematogenously of the blood, choroid plexuses, neuropil and meninges capillary bed[35]. are consider the most common location for microorganism localization and these contributed to mediated receptor or patterns of the vascular flow related to infectious pathogen size .As well blood stream acts as portal CNS entry for tumors (36).

**Conclusion**

Inflammation and circulatory disturbances are the more common lesion at the current work, CD65 and GFAP is a significant marker in neurodegenerative disease shows the most ample immunopositisiv reaction. Moreover, we suggest that molecular biology techniques like PCR and RT-PCR should be done for the purpose of abnormal proteins associated expression.

**High lights:**

1- CD65 and GFAP are considered significant markers of expression in neurodegenerative disease.

2- The incidence of parasitic infection in sheep was high.

3- The more significant pathological lesions were vascular changes, necrosis, edema and inflammation.

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**Conflict of interest**

The authors announced that there are no conflicts of interest with this research.

**Ethical approve**

All sample were accepted by ethical commission for extermination of animal based on meet of 24-10-2022.brain sample were surgically eradicate at the pathology lab, faculty of veterinary medicine.

**References**


HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF OVINE …


دراسة نسيجية مرضية وكيماوي مناعية لالتهاب الدماغ في الأغنام

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تعد امراض الجهاز العصبي المركزي واحدة من اسباب العجز والوفاة بحولوات الماشية في العالم. في الاونة الأخيرة ازداد انسجامنا في علم الاحياء المرضي وتهدف الدراسة الحالية إلى الكشف عن التعبير البروتيني للGFAP,CD65 في اللافات المجهرية في دماغ الاغنام في مدينة الموصل. تم جمع عينة دماغ لمجموعة من الإصابات المرضية للفترة من شهر ديسمبر 2022 إلى شهر فبراير 2023، وتمت تحليل النتائج باستخدام تقنيات الكيميا مناعية. اكتشفنا وجود ارتفاع في مستوى التعبير البروتيني للGFAP,CD65 في الإصابات المرضية، حيث تم التحقق من وجود تعبير للGFAP,CD65 بمستويات عالية في الإصابات المرضية. فشل الرطبة المريحة، وحكة، نزف، اختلال الوعي، وفعالة الجلد هي بعض التعبيرات المرضية المميزة. النتائج تظهر أن الاغنام عرضة للإصابة بتغيرات عصبية، ويمكن استخدام تقنيات الكيميا مناعية للتنبؤ بالحالة المرضية في هذا النوع من الامراض.