**Egyptian Journal of Veterinary Sciences**
**https://ejvs.journals.ekb.eg/**

# 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 lingad 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**: Immunhistochemical, 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, hematology 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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(Received 23/09/2023, accepted 09/11/2023)
DOI: 10.21608/EJVS.2023.238191.1625

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 injures, 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 IJG. 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.

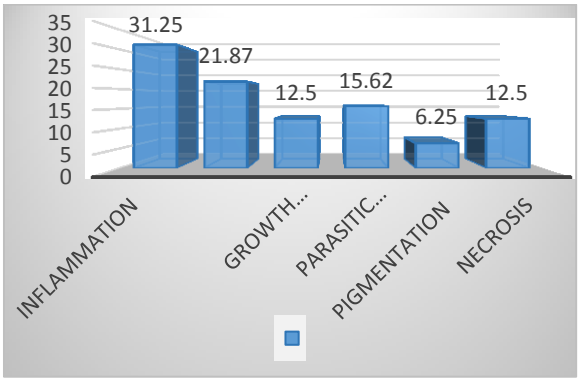


**Fig. 1. Diagram showed types of brain lesion and finding of it.**

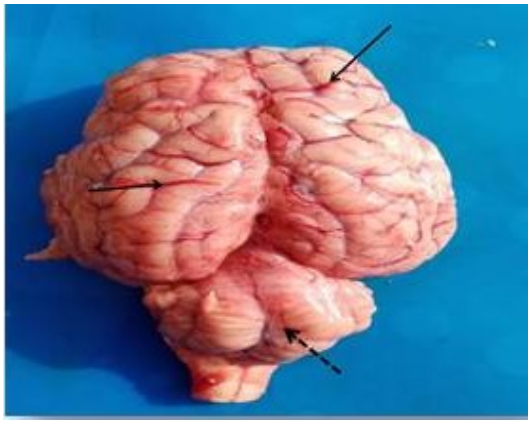

**Fig. 2. Gross section of sheep brain shows hemorrhage and congestion in the brain surface (arrows) and cerebellum surface (dashed arrow)**

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

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

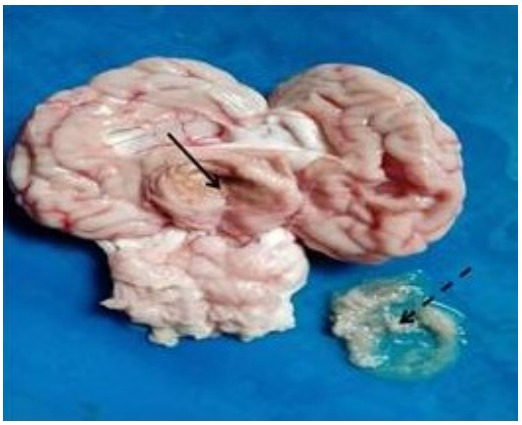

**Fig. 5. Gross section of sheep brain shows the presence of *Echinococcus granulosus* inside the cerebellum (arrow) with the presence of ruptured cyst (dashed arrow)**

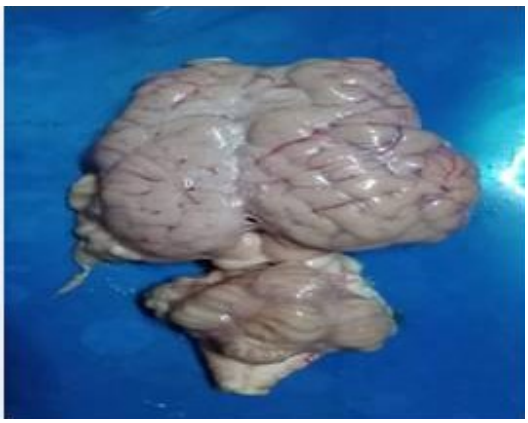

**Fig. 6. Gross section of sheep brain shows sever atrophy of the cerebral lobes and cerebellum**

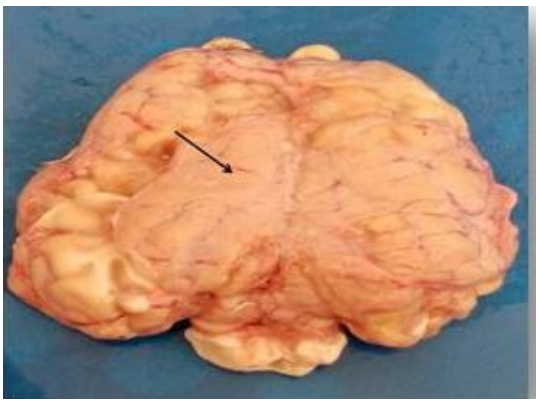

**Fig. 7. Gross section of sheep brain shows pigmenting of brain wit complete disappearance of the cavities (arrow)**

Photograph of brain showed hemorrhage and congestion in figures (2 & 3), parastic infection with cyst ruputerd (4 & 5) and atrophy of lobes and pigmention (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 *Sarcosystosis ovis* and *Toxoplasma gondii*, figures (12,13 , 14 and 15).

**Table 1. Histopathological grading criteria for brain lesion**

| Grade | Mild | Moderate | Sever |
|---|---|---|---|
| **Brain** | Infiltration of lymphocytes& and macrophage .figs 8,9 | Inflammation of cerebellum (figs 8,9) | Multiple cysts of Echinococcus granulosus .figs12,13. |
| | Condensation of inflammatory (figs 8,9). | Congestion &hemorrhage(fig. 14). | Sarcosystosis and toxoplasma gondii larvae stage(fig 14,15). |
| | | Thickening of blood vessels , abscess and Vasogenic Edema(Fig. 11) | Gliosis and vaculor degenration of glial cell .figs 10,11. |

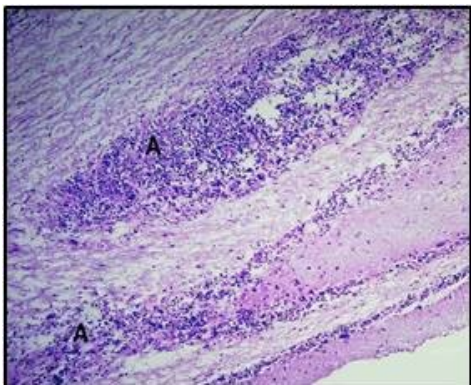


**Fig. 8. Histopathological section of sheep brain shows sense infiltration of inflammatory cells (A) H&E stain, 150X**

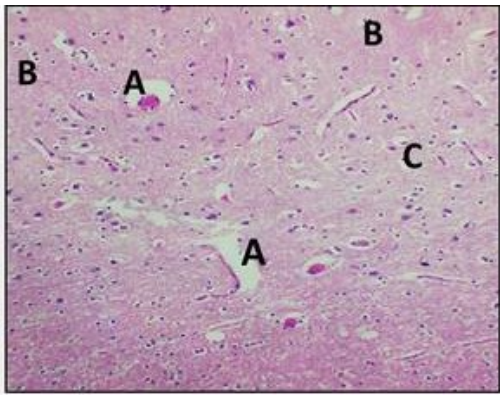


**Fig. 10. Histopathological section of sheep brain shows blood clot (A) and gliosis of glia cell and vacuoles (B) H&E, 150XB**

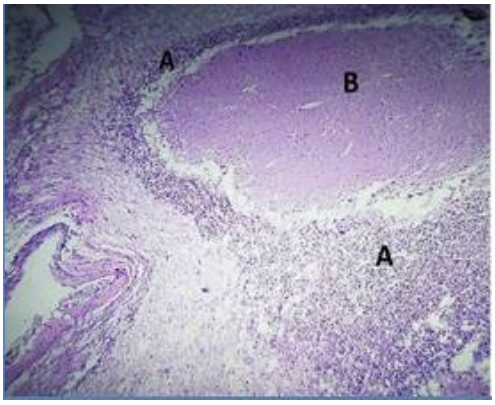


**Fig. 9. Histopathological section of sheep brain shows chronic abscess with necrosis of cerebellum (A) with dense infiltration of inflammatory cells (B) H&E, 150X**

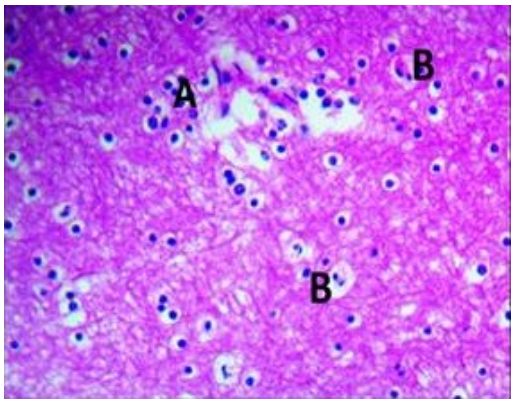


**Fig. 11. Histopathological section of sheep brain shows gliosis (A) with vacuolar degeneration (B) H&E, 150X**

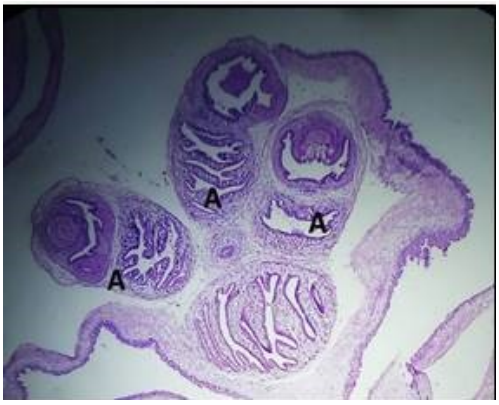


**Fig. 12. Histopathological section of sheep brain shows eggs (A) and larval stage (B) of *cysticercus ovis* H&E, 150X**

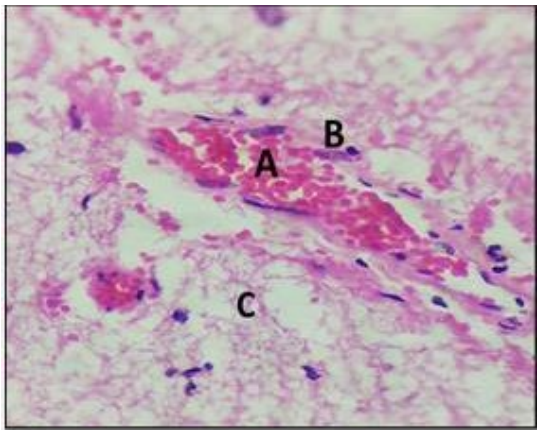


**Fig. 14. Histopathological section of sheep brain shows sever hemorrhages (A) and *Toxoplasma gondi* stages (B) and edema (C) H&E, 450X**

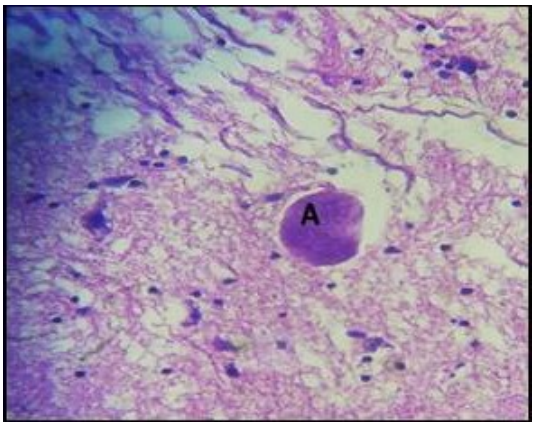


**Fig. 13. Histopathological section of sheep brain shows head of (A) and larval stage (B) of *Echinococcus granulosus* H&E, 150X**

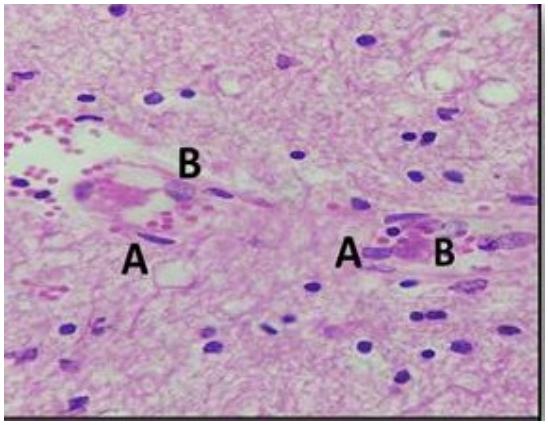


**Fig. 15. Histopathological section of sheep brain shows different stages of *Sarcocystis* different stages (A), *Toxoplasma gondi* stages (B) H&E, 450X**

**Immunohistochemistry Grading**

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

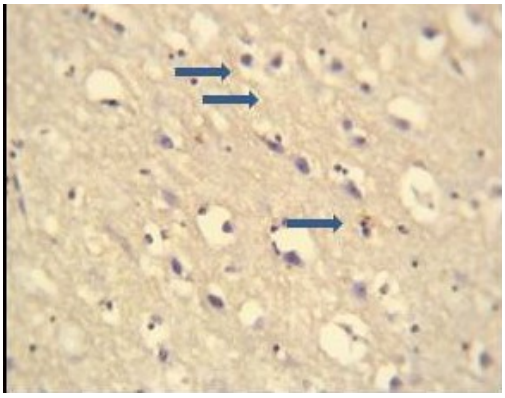

**Fig. 16. Immunohistochemistry scoring with $CD^{65}$ antigen shows mild inside microglia (score 1) X400**

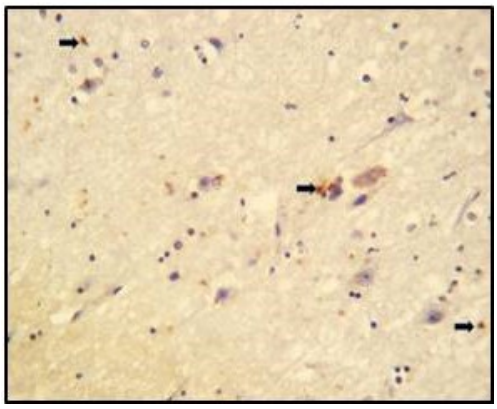

**Fig. 17. Immunohistochemistry scoring with $CD^{65}$ antigen shows moderate grade inside microglia (score 2) X400**

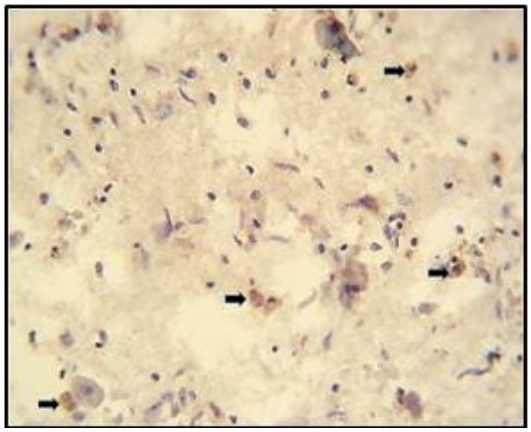

**Fig. 18. Immunohistochemistry scoring with CD$^{65}$ antigen shows sever expression inside microglia (score 3) X400**

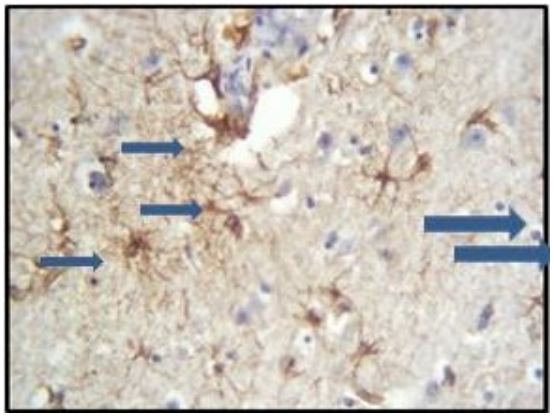

**Fig.20. Immunohistochemistry scoring with GFAP antibody in spinal cord shows moderate grade of astrogliosis antigen (score 2) X400**

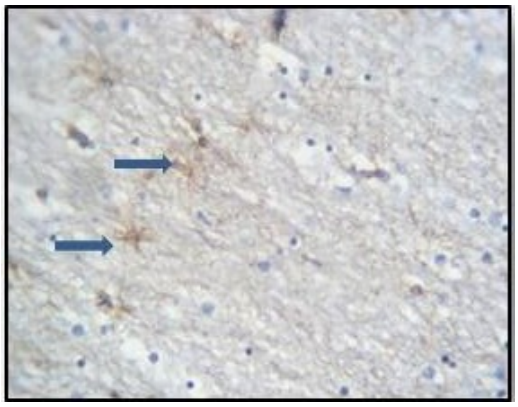

**Fig. 19. Immunohistochemistry scoring with GFAP antibody in spinal cord shows mild grade of astrogliosis antigen (score 1) X400**

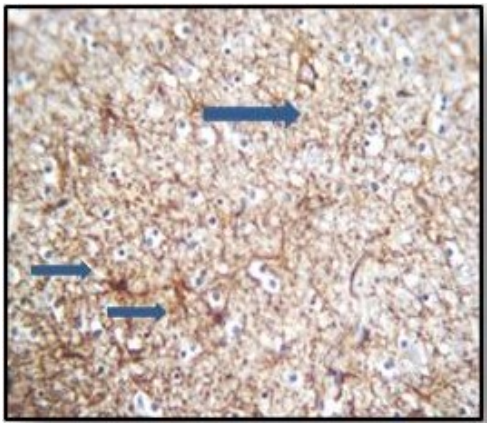

**Fig.21. Immunohistochemistry scoring with GFAP antibody in spinal cord shows sever grade of astrogliosis antigen (score 3) X400**

## 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 vacoulation give deeply affected brains tissue a squishy appearance .The cavitation and vacoulation are betimes as a results of the empty area left after death of cell and resorption, neuropil degeneration and vacoulation [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 presences 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 laborer [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 immunopositivit 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.

*Acknowledgments*

The authors are very grateful for the support received from the department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul.

*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 pathology lab, faculty of veterinary medicine.

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## دراسة نسيجية مرضية وكيمياء مناعية لالتهاب الدماغ في الاغنام

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تعتبر امراض الجهاز العصبي المركزي واحدة من اسباب العجز والوفاة لحيوانات الماشية في العالم.في الاونة الاخيرة ازداد انسجامنا في علم الاحياء المرضي وتهدف الدراسة الحالية الى الكشف عن التعبير البروتيني للGFAP,CD65 للافات المجهرية في دماغ الاغنام في مدينة الموصل،تم جمع 32 عينة دماغ للفترة من شهر ديسمبر لسنة 2022 الى شهر فبراير لسنة 2023 ،شملت الافات العيانية على اضطرابات جهاز الدوران متمثلة بالنزف والاحتقان ،اصابات طفيلية ،اضطرابات نمو مع وجود التصبغات ،تم جمع الافات المصابة وتهيئتها لاجراء الفحص النسجي المرضي والكيماء المناعية كانت نسبة الالتهاب 31.25 ٪ متمثلة بالخلايا الالتهابية الوحيدة والمتعددة النواة،فيما كانت نسبة اضطرابات جهاز الدوران21.87 ٪ ،واضطراب النمو بنسبة 12.5 ٪ فيما مثلت نسبة الاصابة الطفيلية 15.62٪ والتصبغات بنسبة 25. 6 ٪وكانت نسبة التنخر بالافات المرضية هيا 12.5 ٪.اظهر الفحص النسجي المرضي وجود الوذمة حول الاوعية الدموية،مع احتقان شديد في الاوعية الدموية السحائية والدماغية والمخيخة مع تكثف شديد للخلايا الالتهابية حول الوعاء الدموي بالاضافة الى وجود التنكس الفجوي وظاهرة الدباق والخلايا البلعمية في السايتوبلازم الرغوي والتهاب الدماغ القيحي ، كما لوحظ وجود مراحل طفيلي التوكسوبلازما وطفيلي المكيسات الصنوبرية في أنسجة المخ بالإضافة إلى وجود الأكياس العدارية من المشوكة الحبيبية في المخيخ تعدالوظيفة القابلة للتكاثر للخلايا الدبقية والخلايا العصبية في دماغ الاغنام امرا اساسيا لدراسة التغيرات الخلوية ، ان الكيماء المناعية النسيجية المتمثلة بالCD65 والGFA تستخدم لعد التفاعل الموجب والتي تم تصنيفها كافة خفيفة ومتوسطة وشديدة .نستنتج ان الاغنام عرضة للاصابة بانواع مختلفة من التغيرات العصبية ،تقنية الكيماء المناعية يمكن استخدامها لتقيم شدة المرض ويجب استخدام التقنية البايولوجية الجزئيية لتحديد التعبير البروتيني الغير طبيعي

**الكلمات الدالة**: الكيمياء المناعية ،التغيرات المرضية ،الدماغ ،الاغنام .