PREVALENCE OF BRAIN LESIONS IN SHEEP SLAUGHTERED AT MOSUL AREA DURING 2012-2013.

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Keywords; Sarcocystis**,** Brain**,** Edema

ABSTRACT

The present study revealed the prevalence and pathology of sheep brain(male and female). One handred brain samples were obtained randomly from Mosul animal slaughter house and butcher shops since October 2012 to August 2013 from animals with and without nervous signs. The main gross lesions were edema which accounted 53% of the cases, congestion 51% , hemorrhage 20% and 3% cyst of coenurous cerebralis. The major lesions were observed by histopathological examination of three different areas of the brain (cerebrum, cerebellum and medulla oblongata)in each case. Lesions include vascular change, neuronal degeneration, necrosis, inflammatory change, gliosis, neuronophagia, amyloid deposition. Three cases revealed the presence of the coenurous cerebralis and two cases were abscesses and one case showed the presence of Sarcocystis SPP.

INTRODUCTION

Nervous tissue is Highly specialized and structurally complex , it consist of neurons , glia,ependyma,endothelial cells and pericytes of blood vessels and surrounded by the meninges. It arranged to from two basic parts: the gray and white matter (1).

Lesions and inflammatory disease of the central nervous system (brain and spinal cord) is one of the most common causes of neurological disease in veterinary medicine (2).

Basically accumulating evidence during the last decade has shown that the central nervous system can gain a well –defence inflammatory response to avariety of insults including trauma, ischemia , transplantation viral and bacterial infections, toxins as well as neurodegenerative processes (3).

In this survey we focus on the pathological lesions of the brain of sheep due to lack of pieces of research in our country about this subject.

MATERIALS AND METHODS

Collection and examination of sheep :

A total of 100 heads of sheep (43 males & 57females) were collected from Mosul animals slaughtered house and butcher shops duration from 1/10/2012 to 1/8/2013 (96) heads of them were Suffering from nervous manifestations and staggering gait with or without blindness .

The skin was removed ,skulls were incised and the brain removed and examined visually for any pathological changes or cysts and by transverse sectioning of the cerebrum and cerebellum to detect small lesions

Histopathological studies :-

Brain tissues were fixed in 10% neutral buffer formalin followed by passages in alcohol for dehydration and then clarified in two changes of xylole and embedded in paraffin . Sections 4 micron thick then prepared and stained routinely with haematoxylin and Eosin (4).

RESULTS

Table 1. Number and Ratios of Infected Sheep with Brain Lesions Edema , Congestion and Hemorrhage , According to Sex at Different Periods of a year .

Type of lesion	sex	Months and year					Total	Ratio,%,both sexes combined
		1	2	3	4	5		
		Oct-Nov 2012	Dec-Jan 2012/2013	Feb-Mar 2013	April-May 2013	June- Jul 2013		
Edema	**M**	6	1	9	6	7	29	42.74% A
	F	6	3	1	7	7	24	
Congestion	**M**	6	2	3	5	5	21	41.13% A
	F	9	3	5	8	5	30	
Hemorrhage	**M**	1	1	2	1	2	7	16.10% B
	F	2	1	zero	6	4	13	
Total and proportions		30 (0.24)a	11 (0.08)b	20 (0.16)ab	33 (0.27)a	30 (.24)a	124	
Significancy of sex effect within a period		ns	ns	*	ns	ns	ns	

1. ns means no significant relationship between the type of brain lesions and the sex of the animals at particular month –year period
2. * indicates the presence of significant relationship between sex effect and the brain lesions at this particular period ($p<0.05$).
3. proportions of the brain lesions with different Letters are significantly different ($p< 0.1$).

The data in table 1 are referd to the number of sheep individuals which showed positive brain lesions during five 2-months successive periods , oct. 2012 through Jule.2013. Statistical analysis revealed that there is no significant relationship between the occurrence of any of the brain lesions , edema ,congestion and Hemorrhage and the sex of the animals during this different periods , except the

period lasted from Feb. to March. During the particular period , it can be noticed that female individuals were the least affected with edema and hemorrhage .

However , congestion data showed the reverse case . On the other hand, when the ratios of edema congestion and hemorrhage , expressed as a percent , were compared , regardless of the sex effect it has been found that the edema and congestion occurred at similar rates and each one of these brain lesions differed significantly ($p < 0.1$) than brain hemorrhage. Further analysis was done to detect difference in brain lesions according to the differet periods, Data were analyzed as ratios . It has been found that period 2 was significantly different than all other periods except period 3. No statistical difference were found in the occurrence of brain lesions , as a ratios, among periods 1,3,4 and 5.

Table 2 . The Numbers and Ratios of Infected and non-Infected Sheep with Coenurous cerbralis According to the Sex of Animals.

Case / Sex	infected	Non-infected
Males	Zero	43
Female	3	54
total	3	97
Proportion regardless of sex.	3% a	97% b

Percents of infected non-infected animals with different letters are significantly different (P < 0.001).

Data shown in table 2 , regarding the numbers and ratios of Coenurous cerbralis infected and non – infected individuals , clarifies that the number of infected individuals is minimal and considered , therefore , not substantial in this particular type of disease . The relationship between the infected –non –infected cases and the

sex of the animals was not significantly existed, according to Fisher exact test out come.

Macroscopic lesions:

The total lesions of 100 cases of brain examined , four of them are macroscopically intact and 96 cases had lesions of congestion and edema were seen in meninges and brain tissue (figure 1), also areas of hemorrhage were noticed in cerebral leptomeninges and brain tissues (figure 2) . Cyst, were observed in the brain which located in the cerebrum and cerebellum in 3 cases diameter of cysts were ranged from (3 to 6 cm) fluid filled which are round to oval and of varied size with areas of softening in some cases (figure 3) . Edema on the dorsal surface gyri are Swollen and flattened (figure 4). Faint yellow discoloration which indicates necrosis. Cross sections of brain in some animals, revealed randomly areas of hemorrhage in the parenchyma.

Microscopic lesions:

Vascular change

- Congestion and hemorrhage: congestion and hemorrhage was observed in different areas of the brain and was very extensive and involving the whole brain tissue and meninges (figure 5)
- Edema: it appeared as spongy degeneration (spongiosis) it observed in both white and gray matter of the cerebrum and cerebellum (figure 6)
- Blood clot (thrombosis): occlusion of blood vessel with clotted blood was observed in brain tissue of meningies , also capillary damage was seen in the cortical and medullary regions of the brain. (figure 7)
- Neurophagia: which appeared as aggregation of glial cells around the damaged or necrotic neurons. (figure 8)

- Neuronal degeneration the degenerative change varied from multifocal to diffuse in brain tissue , cell body appeared shrunken and the cytoplasm stained highly esinophilic , and in some cases the cell body was swelled and peripheral displacement of the nucleus and disappearance of nucleus and Nissl (figure 9)
- Amyloid : wich appeared as a pale eosinophilic amorphous material consistent with amyloid deposition (figure 10)
- Necrosis: necrosis of neurons was observed in many cases in brain tissue.
- Purkinje cell degeneration and necrosis:

  Degeneration and necrosis observed in many cases by disappear of nuclear details and the cytoplasm appeared granular and eosinophilic , also necrosis of these cells observed in many cases. (figure 11)

Inflammation:

- Meningitis: abnormal large number of glial cells and mono nuclear cells were present in the space around the meningeal vessels walls in the leptomeningies. (figure 12)
- Meningio–encephalitis:- vasculitis and perivascular cuffing in both meningies and brain parenchyma by gemistocytic astrocytes , plasma cells and mono nuclear cells. (figure 13)
- Abscessation: which appeared as an area of necrotic tissue surrounded by thick capsule of connective tissue infiltered by mono nuclear cells in one cases (figure 14) and multiple abscess in medulla oblongata. (figure 15)
- Spongiform changes: spongiform changes in the brain tissue (cerebrum and cerebellum). Most cells reaveled one or more vacuoles and the nucleus

displaced spongy appearance in the white matter of the brain referred to encephalomalasia. (figure 16)

- Demyelination: It was evident in cerebrum and cerebellum in some cases. (figure 17)
- Gliosis: Abnormally large number of glial cells could be detected as a diffuse gliosis and focal gliosis with neuronal degeneration particularly in the cerebral cortex. (figure 18)
- Cyst: are observed in 3 cases seen in the cerebral ,cerebellum and medulla in the gray and white matter which lead to edema and atrophy due to compression on the parenchyma. (figure 19)
- sarcocystis had been observed in one` case seen in the medulla oblongata.(figure 20)

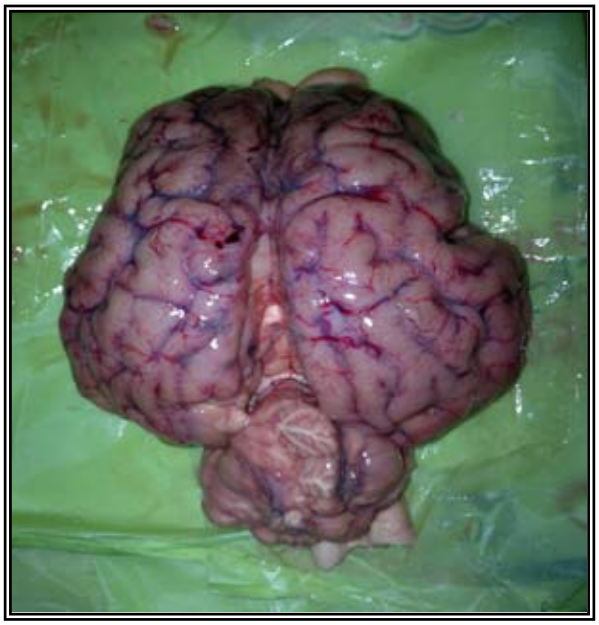

Fig. (1): sever congestion and edema in all part of brain.congestion and edema were seen in meninges and brain tissue.

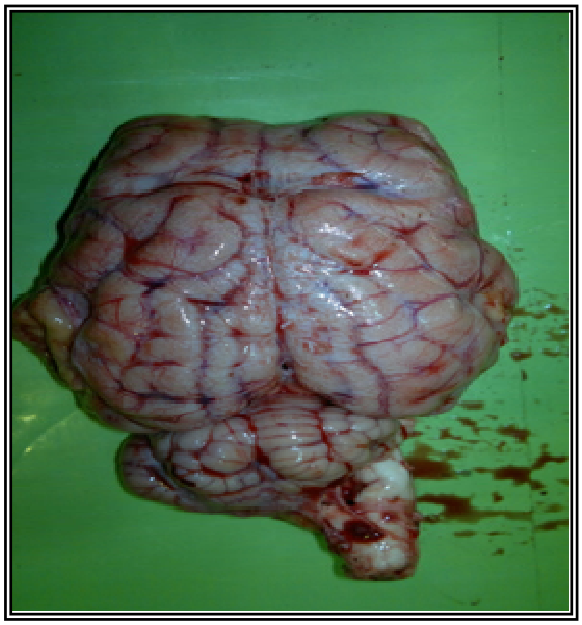

Fig. (2): sever hemorrhage in cerebrum ,cerebellum and medulla oblengata

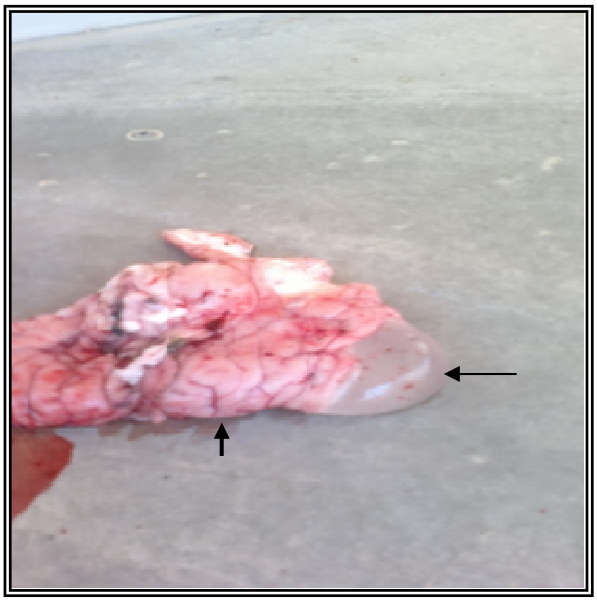

Fig.(3): cyst in the cerebrum which is oval in shape(⟵) with area of softening in brain tissue (↑).

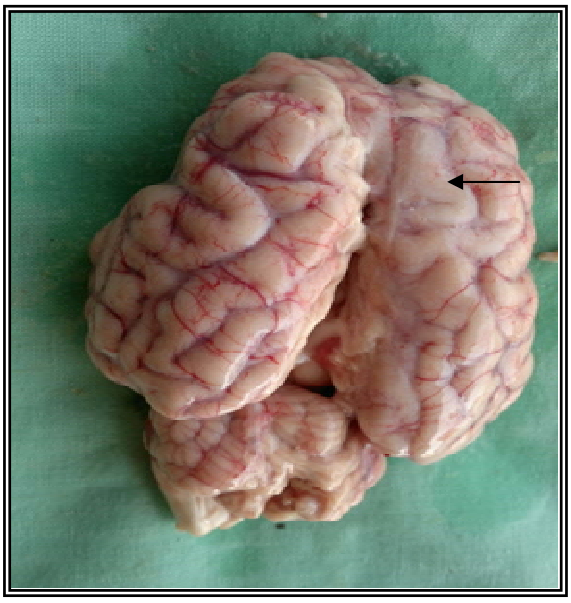

Fig.(4): edema in dorsal surface. gyri are swollen and flattend (⟵).

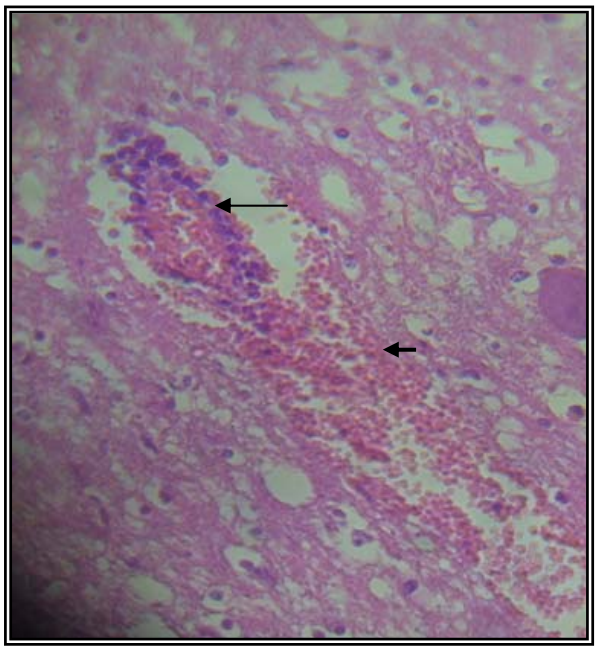

Fig. (5): section of cerebrum showed congestion (⟵) and hemorrhage (←) in the white matter-cerebrum. H & E stain. 420x.

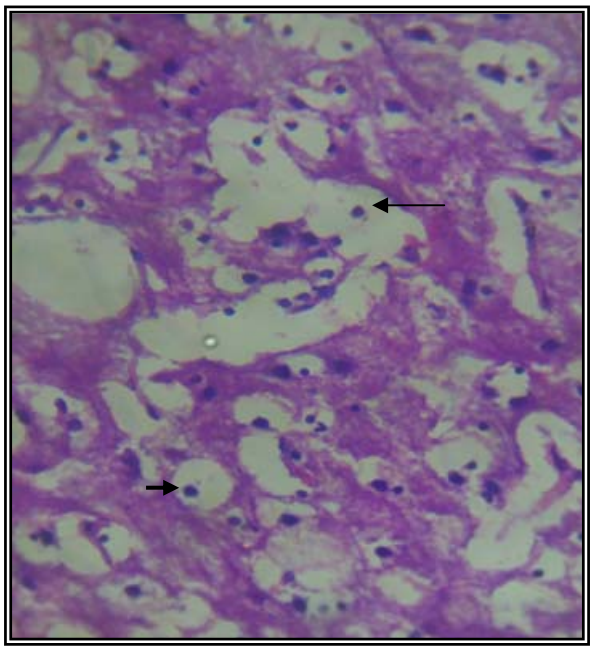

Fig. (6): vasogenic Edema (⟵) with spongiosis (→) in gray matter of cerebrum. H & E stain. 420x.

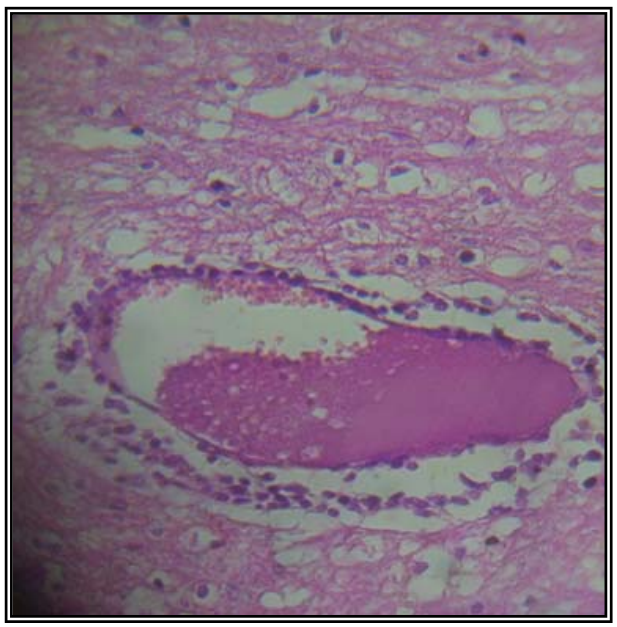

Fig. (7): thrombosis with vasculitis in the white matter of medulla oblongata. H & E stain.

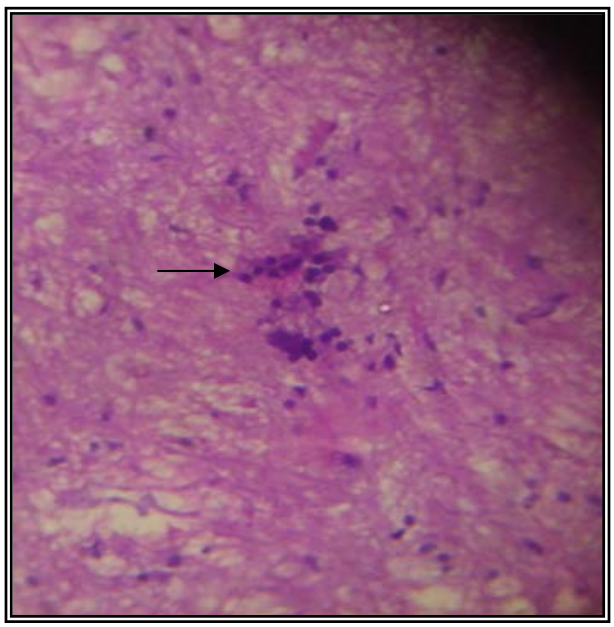

Fig. (8): Neurophagia (⟶) in cerebellum - white matter. H & E stain. 420x.

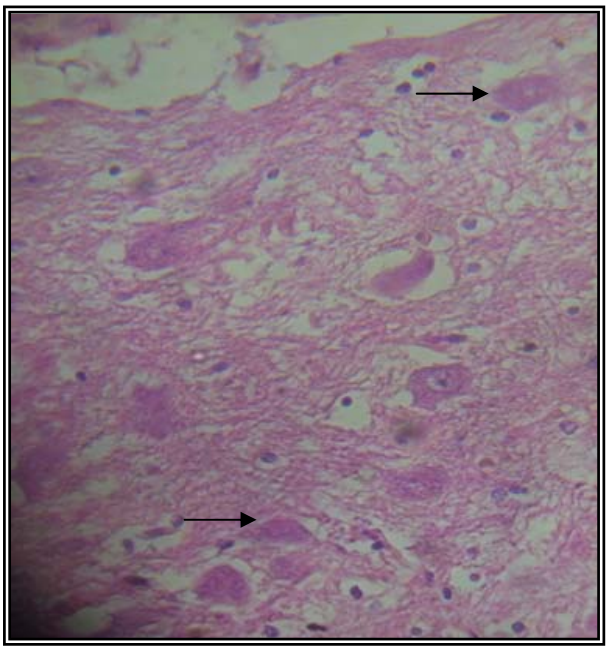

Fig. (9): Neuronal degeneration and necrosis (⟶) in the medulla oblongata. H & E stain. 420x.

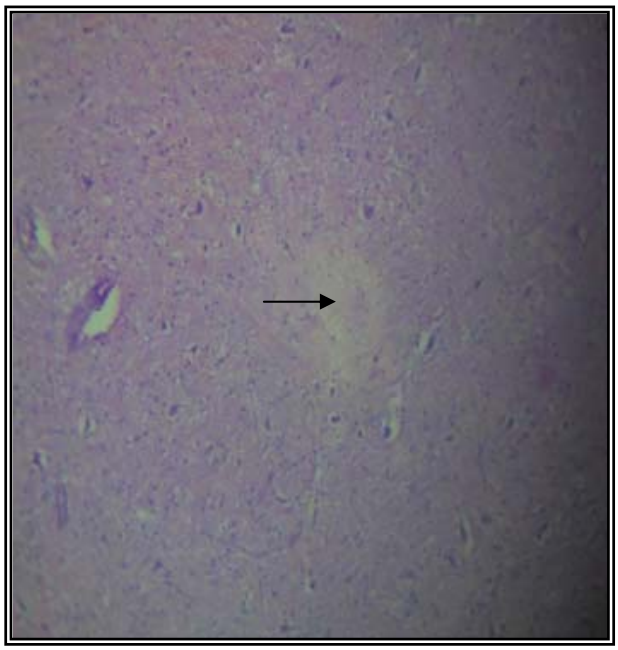

Fig. (10): cerebral amyloid pale esinophilic amorphous material (⟶) . H & E stain. 420x.

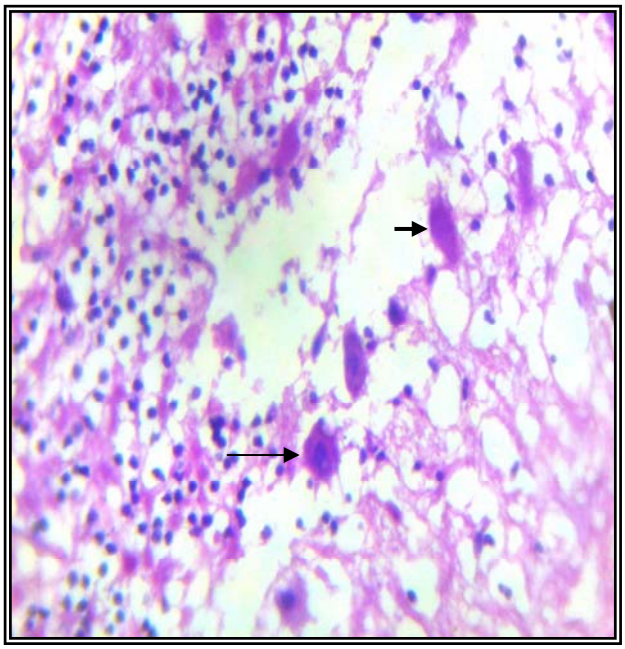

Fig. (11): cerebellum gray matter. Degeneration (⟶) and necrosis of purkinji cells (➞). H & E stain. 420x.

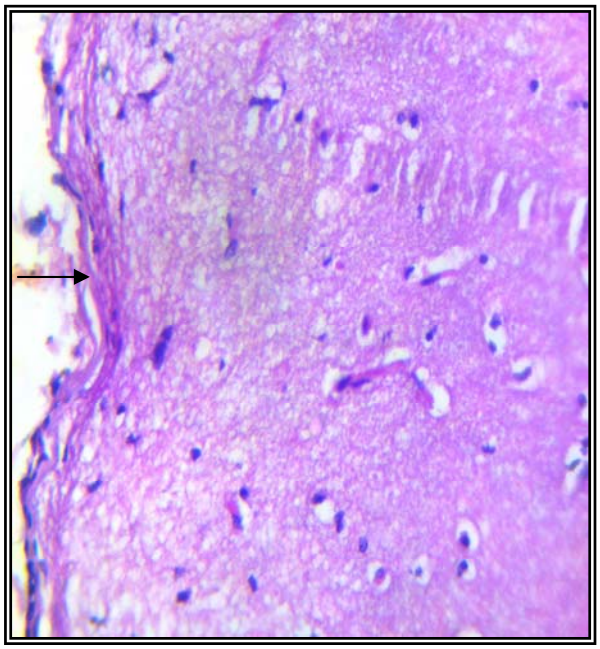

Fig. (12): cerebral meningies, leptomeningitis , congestion and infiltration of glial cells. (⟶)H & E stain. 420x.

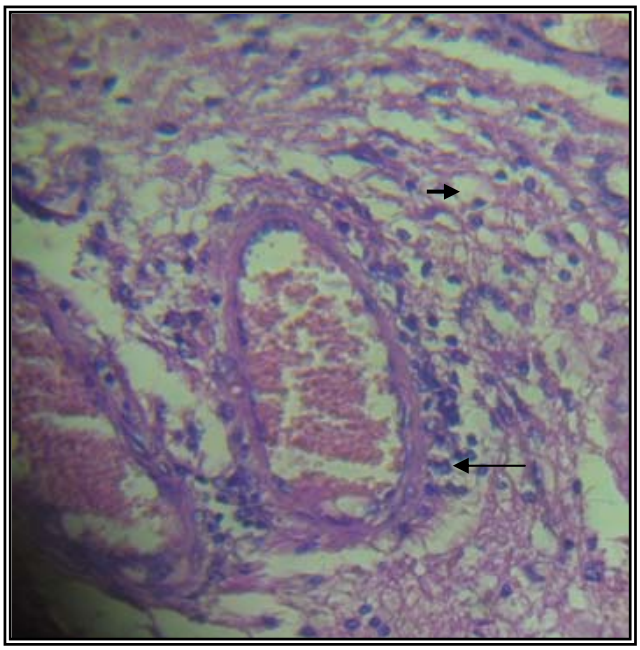

Fig. (13): Meningio encephalitis, vasculitis (⟵) with infiltration of glial cells in the white matter (→) H & E stain. 420x.

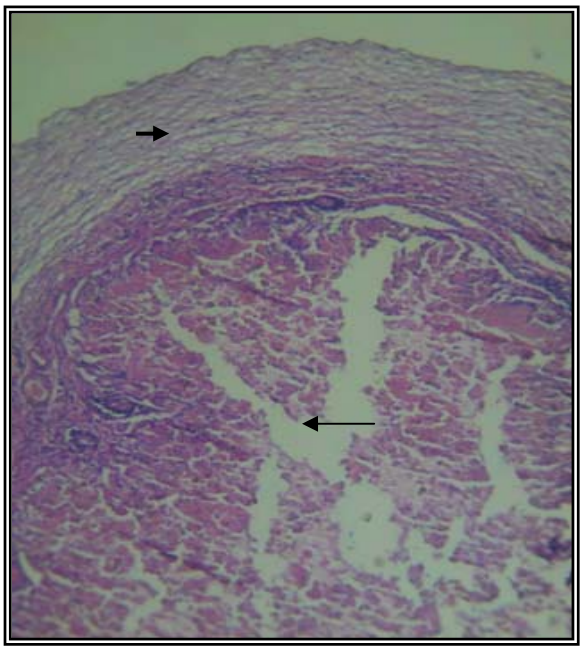

Fig. (14): cerebral abscess. The necrotic tissue (→), surrounded by thick fibrous tissue (⟵) H & E stain. 420x.

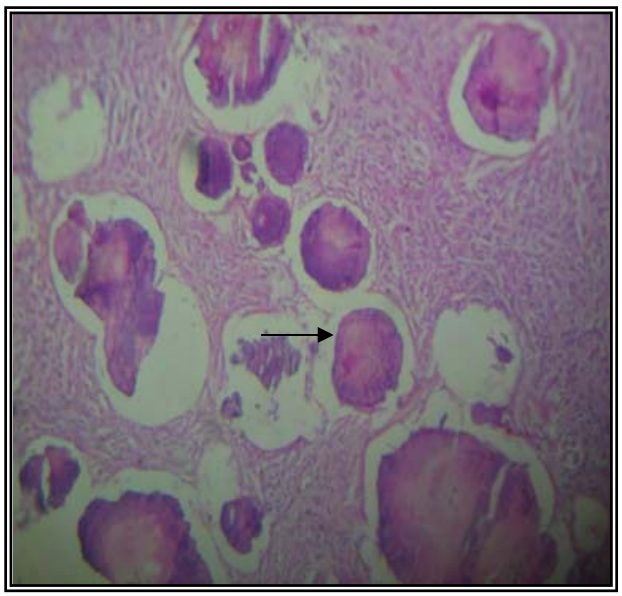

Fig. (15): Multiple abscesses in medulla oblongata (⟶) H & E stain. 105x.

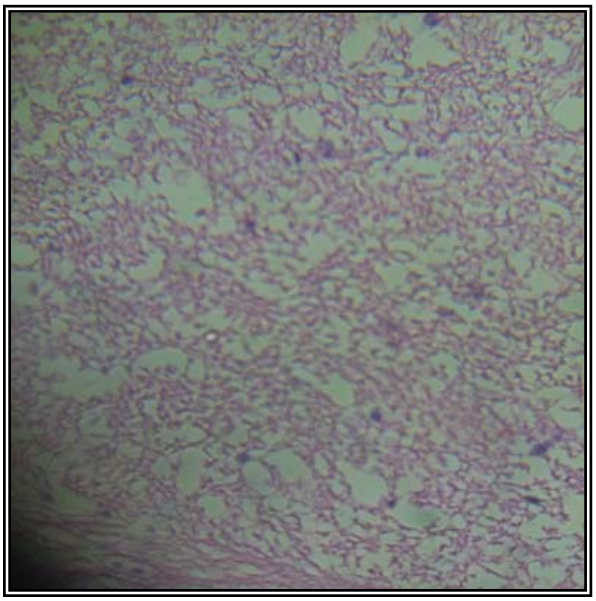

Fig. (16): Encephalomalasia. Area of malacia in medulla oblongata. H & E stain. 420x.

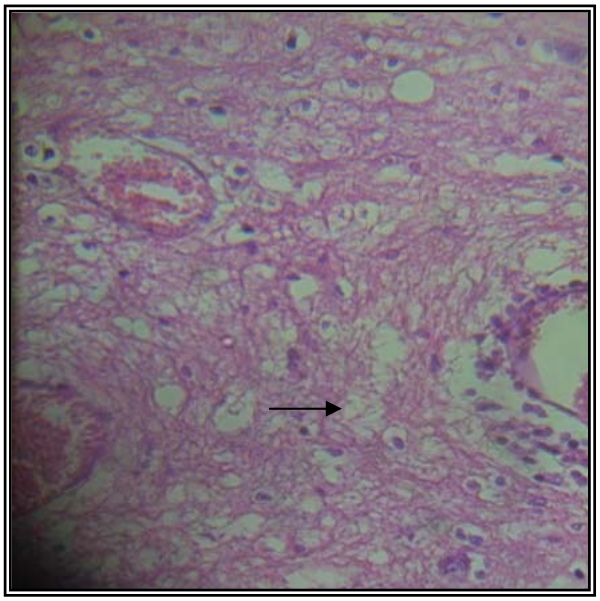

Fig. (17): Demyelination in medulla oblongata (⟶) .H & E stain. 350x.

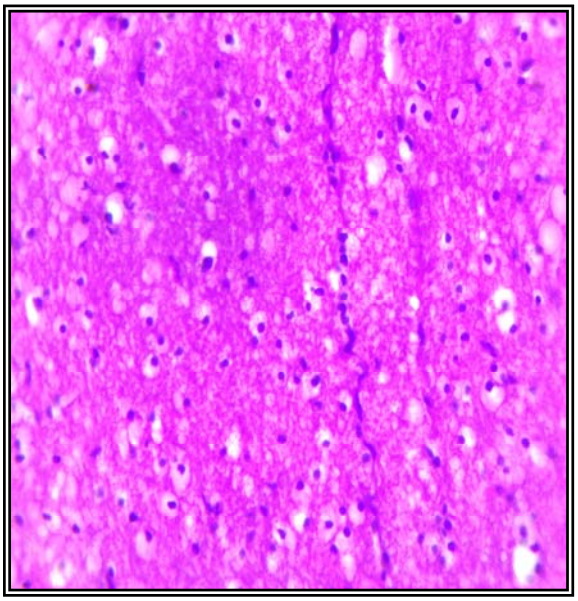

Fig. (18): diffuse gliosis with neuronal degeneration – cerebrum in white matter . H & E stain. 105x.

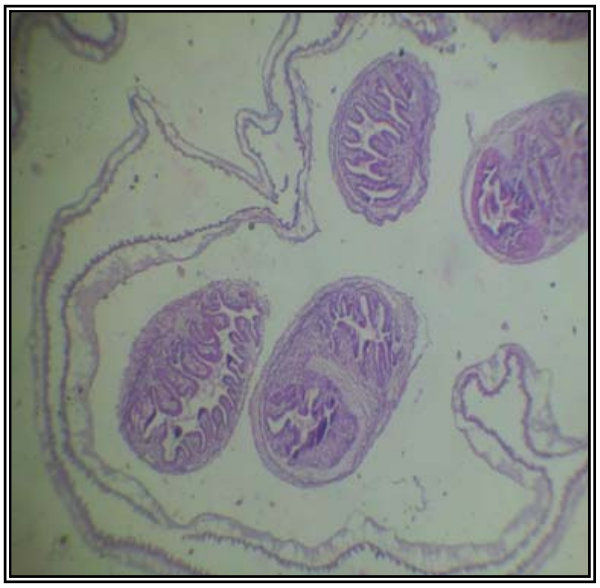

Fig. (19): Cyst of coenurus cerebralis in brain parenchyma H & E stain. 43x.

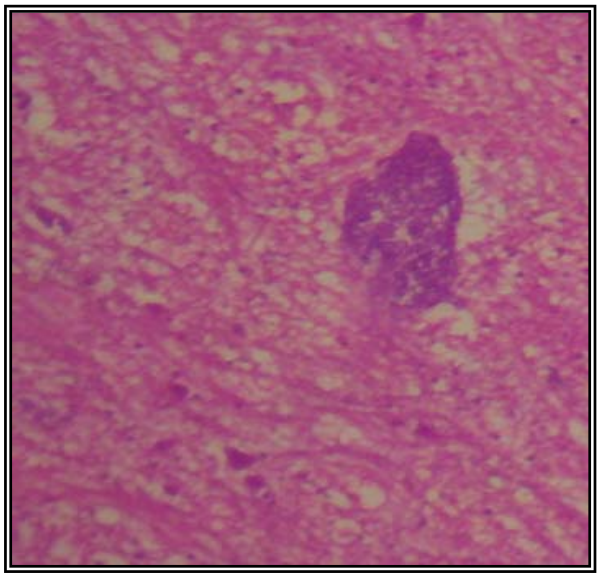

Fig. (20): sarcocystis arranged in medulla oblongata H & E stain. 350x.

DISCUSSION

Our findings observed that there is no significant relationship between the occurrence of the macroscopic brain lesions, edema, congestion, and hemorrhage and the sex of animals during the different periods of months, except the period lasted from Feb. to March which showed that female individuals were the least affected with edema and hemorrhage while congestion showed the reverse case this result may be due to the number of samples that collected in that period. There was no significant sex difference in the incidence of infestation of Coenurous cerbralis that's agree with (5 ,6) who found that sex of sheep does not have correlation with the prevalence of this parasite. This study indicate that the lesions identified in the brain with or without nervous singnes. Because many of the infectious disease pathogenesis spread through the body by circulatory system, endothelial cells which lining blood vessels especially capillaries are exposed to injuries which lead to congestion, vasculitis and thrombosis as well as hemorrhage as found in our study similar findings were reported by (7,8,9). Also the traumatic injury and metabolic-toxic diseases to the central nervous system resulting to hemorrhage and malasia (10)

This endothelial injury can be reversible or non reversible resulting in necrosis and dysfunction by activation and release of vasoactive mediators such as histamine which lead to local or systemic changes in vascular flow and permeability (11).

However in these cases vasogenic edema in central nervous system was reported which may be as a result of bacterial products and inflammatory cytokines that cause vascular injury and break down of the blood-brain barrier that results in movement of plasma constituents into perivascular extracellular space, particularly that of the white matter (12).

Our finding observed a vacuolar degeneration which characterized by a small clear vacuoles of varied sizes in the cytoplasm of neuron cell bodies and necrotic changes in neurons which appeared shrunken and the cytoplasm was highly eosinophilic with pyknotic nuclei these changes occurs in ischemic injury and viral disease (13,14). It is seen also in deficiencies and toxins which cause a spongy vacuolar change (10).

The neurons have large requirements for energy to maintain normal metabolism , but it lack adequate intracellular glucose , so they depend for survival on adequate blood supply to provide glucose. As well as neurons are vulnerable to free radical oxidative stresses, so for this reasons neurons are very sensitive to injuries such as cerebral ischemia , inflammatory mediators bacterial toxins, thermal injury, heavy metals, nutritional deficiencies and truma, these are very common in sheep (3,15).

Gliosis was seen in many cases which was more evident in the white matter due to injury of the brain, these changes could be due to bacterial infection by clostridium and viral infection as it seen by (16).

Also the perivascuar cuffing within the brain was observed in many cases by the astrocytes, plasma cells and mononuclears (17).

Demyelination which is the destruction of myelin sheath were reported in few cases. Demyelination was found in case of intoxication as it was reported by (18,19).

Meningitis was reported in this study which caused by bacteria (20, 21). That reach the leptomeninges and subarachnoid space hematogenously or direct extension also it may be secondary to viral infection (22).

The hallmark of the inflammatory diseases is the paranchymal invasion of blood-derived leucocytes around blood vessels (perivascular cuffing) and infiltrating into parenchyma as well as proliferation of endogenous microglia resulting in encephalitis, meningitis, ependymitis. (23).

Cerebral abscess was reported in few cases in cerebrum and cerebellum and in medulla oblongata , in one case the abscess surrounded by thick fibrous tissue it seen by (24).

Protozoa sarcocysts had been observed in the brain parenchyma it may be associated with encephalomyelitis in sheep and cattle (25).

Coenurus cerebralis which are the most commonly infests sheep was observed in few cases. The larva reach the central nervous system hematogenously and cause damage during migration and encystations and forming lesions (26).

One case of Amyliodiosis have been reported by the presence of homogenous esinophilic area in the white matter similar lesions was reported by (27)in case of scrapie in sheep in UK.

حدوثية آفات الدماغ في الأغنام المجزورة في منطقة الموصل للفترة ٢٠١٢-٢٠١٣.

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الخلاصة

كشفت هذه الدراسة عن مدى اصابة الدماغ في الاغنام (ذكور واناث). حيث تم جمع ١٠٠ عينه دماغ عشوائيا من مجزرة الموصل ومحلات القصابة للفترة من تشرين الثاني ٢٠١٢ ولغاية اب ٢٠١٣ من الاغنام السليمة والاغنام التي كانت تعاني من العلامات العصبية ، تمثلت الافات العيانية بوجود الوذمة وبنسبة ٥٣% من اجمالي الحالات المفحوصة يليه الاحتقان والنزف وبنسبة ٢٠% وبلغت نسبة الاصابة بطفيلي الدودة المثانية 3%. اما اهم التغيرات النسيجية في اقسام الدماغ المختلفة (المخ والمخيخ والنخاع المستطيل) لكل حالة ، تمثلت بوجود التغيرات الوعائية، تنكس ونخر الخلايا العصبية، التهاب السحايا والدماغ، ظاهرة الدباق

والبلعمة العصبية وترسب المادة النشوانية. كما اظهرت ٣ حالات وجود اكياس طفيلي الدودة المثانية وحالتين من الخراجات وحالة واحدة اوضحت وجود الحويصلات الصنوبرية في نسيج الدماغ.

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