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SEROLOGICAL AND HISTOPATHOLOGICAL DETECTION OF *MAEDI-VISNA* VIRUS IN MIDDLE IRAQ REGIONS

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Abstract

Maedi-Visna virus (MVv) infection is usually occurring in adult sheep and rarely in goats. It is a chronic respiratory disease characterized by progressive interstitial pneumonia and to lesser extent by neurological manifestations. This study aimed to find the seropositive rate in Awassi sheep suffered from respiratory distress, in three governorates (in middle region of Iraq), in the period from December 2018 to August 2019. Two hundred and ten (210) Awassi sheep of both sexes and different ages were used. All sera samples were tested by indirect ELISA test. Histopathological examination of lungs were done on (four sheep), gave strong seropositive reaction and showed respiratory signs. The total seropositive rate was 16.19%. Equal seropositive rates (16.19%) were found in Diwaniyah and Babylon, moreover non-significant lowered rate (14.28%) was recorded in Karbala. Non-significant effect of sex on seropositivity was noticed, moreover the seropositive rate was increased significantly with increasing ages and high seropositive rates were recorded at three-to-over six years old. The frequent signs were belonged to Maedi virus representing by emaciation and respiratory signs, whilst mastitis, arthritis and nervous disorders were rarely seen and belonged to Visna virus. Histopathological pulmonary lesions correlated to serological investigation were highly suggested for disease diagnosis. Inflammatory mononuclear cells infiltrations, emphysema with thickening of alveolar septa were most common pathological lesions.

Key words: *Maedi-Visna* virus, Iraq , Awassi sheep, ELISA.

Introduction

Maedi-Visna is the virus causing chronic infections in sheep and rarely goats. It is belonged to the genus Lentivirus and Retroviridae family, this virus is genetically related to Caprine arthritis encephalitis virus (CAEV) (Fauquet *et al*., 2005).

The disease is characterized by a long period of infection and the clinical signs don't appear until two years of age (Herrmann-Hoesing *et al*., 2010). Some infected sheep remain asymptomatic throughout their life (Radostits *et al*., 2007). The *Maedi-Visna* virus is spread via respiratory route, utero infection and by ingestion of the colostrum or milk of infected ewes, colostrum and milk contain infected mononuclear cells, these cells are capable to pass through the intestinal wall of newborn (Radostits *et al*., 2007). In addition to that the virus is shed or excreted in semen of infected rams, particularly those have leukospermia (Radostits *et al*., 2007). *Maedi-Visna* virus is responsible for respiratory and nervous clinical forms occurrence, in spite of multisystemic or organic inflammatory lesions had been recorded (Radostits *et al*., 2007; Herrmann-Hoesing *et al*., 2010). The clinical signs of Maedi infection is more prevalent than Visna (Christodoulopoulos, 2006) and mostly is manifested by emaciation, coughing and dyspnea, whereas the Visna form showed nervous disturbances: weakness of hind legs, paralysis and CNS disorders were the most frequent signs, furthermore arthritis and loss of weight were also mentioned.

As a *Maedi-Visna* virus is a fatal disease and no available vaccine is present, beside poor prognosis as well as the risk of exposure of healthy sheep to infection so that. It is are imperative to find a rapid and easy method

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to detect the infected animals. Many techniques were employed for this purpose: Agar gel immunodiffusion, Radioimmunoassay, Enzyme Linked Immunosorbent Assay. Ristocetin-induced platelet aggregation and recently the polymerase chain reaction. (Anson and Eness, 1985; Asadpour *et al*., 2014).

This study aimed to find the seropositive rate of *Maedi-Visna* virus infection in Awassi sheep in the middle region of Iraq.

Materials and Methods

Animals and samples

Two hundred and ten Awassi sheep aged from <1 -to- >6 years and of both sexes, used in this study. They were suffered from clinical respiratory signs, they reared in three governorates AL-Diwanyah, Karbala and Babylon (Middle region of Iraq). In the period from December 2018 to August 2019. History of each case was recorded in special chart and clinical examination was done.

Blood samples were collected via jugular vein puncture by vacutainer tubes system, free of anticoagulant compounds. Sera were separated by centrifugation at 3000 rpm and stored in -20°C until analysis.

Indirect Enzyme linked Immunosorbent Assay (ELISA) were used for detection of specific *Maedi-Visna* glycoprotein (gp) 135 and protein 25 antibodies. ID screen (CAEV/MVV Indirect screening) test was used for this purpose. The test was performed on available commercial plate (96 wells), supplied by (ID Vet Innovative Diagnostics/France).

Calculation of *Maedi-Visna* antibodies values were done according to equation: Cut-off value (Khalaf, and Aldoori, 2018). ELISA reader was adjusted at 450 nm wave length. The results were read according to kit protocol: The S/P ratio over 60 percent was considered positive, 50-60% suspect and less than 50% negative.

Histopathological sections: Lung specimens were taken from 4 sever affected sheep 10 cm in size, they were cut into small pieces (0.5 cm), they were washed by immersing in distilled water for an overnight, then placed in several gradual ascending alcohol concentrations for dehydration of tissues. The dehydrated tissues washed with xylene and then soaked in paraffin, for preparation of blocks. The blocks were dissected in to 4-5 micrometers, and stained by Hemetoxylene and Eosine (Bancroft and Layton, 2012). Examination of all section were done by ordinary light microscope (Olympus, Japan) under (10X).

Statistical analysis: Data were analysed by using SPSS program, version 23.0 (Chicago. USA) and the significant variation were evaluated by employing Chi-square test (Al-Ukaelii and Al-Shaeb, 1996).

Results

The total seropositive rate for *Maedi-Visna* virus antibodies was 16.19% in Awassi sheep suffered from respiratory signs. Although in Karbala governorate shower lower positive rate (14.28%) but non-significant variation between the three governorates were observed table 1.

In the fact the positive sera differ in their reactions: thirty sera samples were given highly strong positive reactions >60% whilst the four remaining sera gave weak reaction between 50-60%, in spite of these were considered positive.

There was no significant effect of sex variation on the positive rate.

The significant ($P \leq 0.05$) high positive rate was recorded in advance aged sheep, particularly those 3 -to- >6 years old (14.87-25%) and the low positive rate were recorded in young sheep less than 1 to >1-3 years table 3.

The seropositive sheep showed normal vital clinical

Table 1: Seropositive rates for *Maedi-Visna* virus Antibodies in Awassi sheep in three governorates.

Governorate	Positive (%)	Total No.
Babylon	12 (17.14%)	70
Al-Diwaniyah	12 (17.14%)	70
Karbala	10 (14.28%)	70
Total	34 (16.19%)	210

$X^2 = 0.281$, P value= 0.869 (NS)

Table 2: The ELISA positive samples with variable reaction.

Reaction stat	Strong +	Weak +	Total
N. of samples	30	4	34
X2	39.76 (HS)		
P value	0		

HS : Highly significant difference (P <0.01)

Table 3: Infection rates with *Maedi-Visna* virus in sheep according to different ages.

Age/year	<1	>1-3	>3-6	>6	Total
No. of animals	10	35	121	44	210
Positive (%)	1 (10)	4 (11.42)	18 (14.87)	11 (25)	34 (16.19)

$X^2 = 3.53$, P value= 0.016 (S).

Table 4: Clinical parameters of seropositive sheep to MV-virus antibodies.

Parameter	Temperature (°C)	Pulse rate/minute	Respiratory cycle /minute
Average	38.5-42.0	75-95	25-55
Mean±SE	(40.0 ±0.17)	(84.11±1.002)	(40.02±1.36)

Table 5: Clinical signs in seropositive Awassi sheep to MV-virus antibodies.

Signs	Emac-iation	coug-hing	dys-pnea	mas-titis	nervous signs	arth-ritis
No. of sheep	28	28	18	2	1	1
Percentage (%)	82.35	82.35	52.94	5.88	2.94	2.94

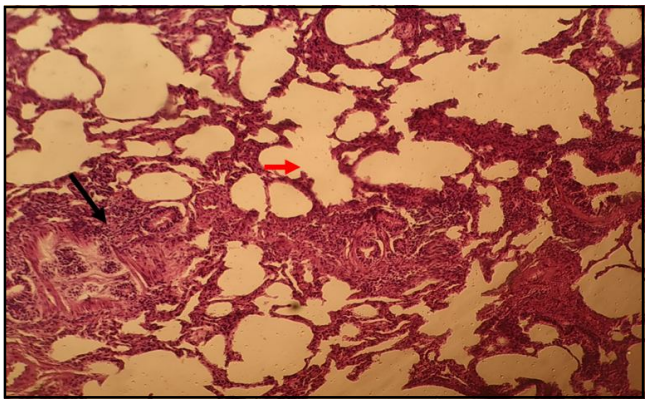

Fig. 1: Histopathological section in the lung of sheep infected with *Maedi-Visna* virus shows inflammatory cell infiltrate and alveolar emphysem a with thickness alveolar septa (H & E stain 10X).

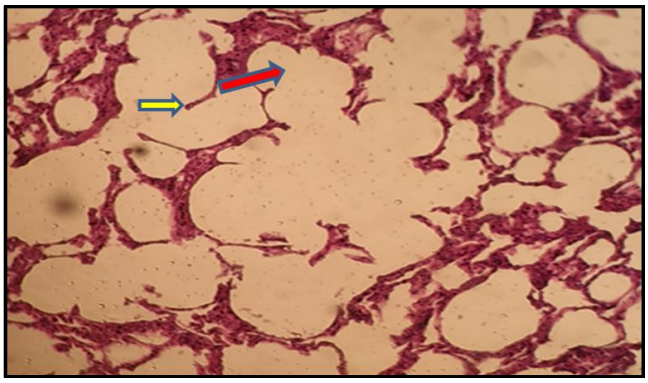

Fig. 2: Microscopic examination of the lung of the sheep infected with *Maedi-Visna* virus shows alveolar emphysema with thickness alveolar septa (H&E stain 10X).

parameters: temperature (ºC) and pulse rate (pulse/minute) excepted the respiratory rate (Resp/cycle) was increased (40.02±1.36) at rest table 4.

The most frequent clinical manifestations were emaciation and coughing, whereas in contrary the nervous signs, arthritis and mastitis were the less frequent signs table 5.

The histopathological section Fig. 1 and 2 showed chronic interstitial inflammation of the lung manifested by inflammation mononuclear and lymphocytic cells aggregations and highly infiltrated between alveoli, diffuse thickening of interalveolar septa accompanied with alveolar emphysema.

The smooth muscles of interalveoli showed hyperplasia and vacuolation in the tissues Fig. 2.

Discussion

The total seropositive rate was 16.19% in Awassi sheep suffered from respiratory signs, this rate was lowered that found in by (Azkur *et al*., 2011) registered a rate of 19.4 % in Kirikkale city located in Central Anatolia region of Turkey. Whereas (Norouzib *et al*., 2015) reportd 34.5% of the samples were seropositive in sheep population of Khorasan-e-Razavi province in Iran. A large number of similar studies has been performed in the other parts of the world. The difference in the prevalence of an infectious disease in different regions is evident. For example, in the study by Tabet *et al.,* (2017) in Lebanon, the seroprevalence of the disease was determined 71%. In a survey done by Giangaspero *et al.,* (2011) in Japan using three methods AGID, ELISA and polymerase chain reaction (PCR) on serum samples from 267 sheep reported the prevalence 1.1%, 0% and 0% for each method, respectively.

The different in the prevalence of an infectious disease in different regions of a country is unavoidable. Some factors such as different susceptibility of different breeds in studied regions, management practices and the biosecurity affect on the prevalence of the disease. Two later factors are also related to weather conditions and experience and economic statues of farmers (Shuaib *et al*., 2010).

Moreover, we did not notice significant differences between infection rates between governorates table 1 due to the lack of environmental differences and similarity of breeding methods in the three governorates.

With ELISA, the high percentage 30 samples gave positive reaction ≥ 60% and the remained 4 samples considered doubtful between 50% and 60% with the test was observed, this result may probably be due to variable specificity of the serum antibodies or the presence of existing high levels of antibodies resulting from a chronic natural infection in these animals.

The higher and lowest prevalence of *Maedi-Visna* virus were observed among age group of >6 and <1 years old, respectively. The high seropositive ELISA seropositive results rate in >6 years age group recorded, the results of the current study is in agreement with Gurcay and Parmaksiz, (2013) and Norouzi *et al.,* (2015). The reason of increasing ELISA positive result with age is due to the chronic progress of the disease and long replication period of the virus in host cells: monocyte and macrophages (Pepin *et al*., 1998).

Clinical findings of Visna disease occur less commonly than Maedi (Herrmann-Hoesing *et al*., 2010). Maedi showed clinical signs include dyspnea, coughing, emaciation, and mastitis (Straub, 2004). The clinical signs of visna are arthritis, weakness in the hind legs, mastitis and weight loss. These findings might be continued until complete paralysis occurs and some time central nervous system disorders arise (Muz *et al*., 2006). In terminal stage, the body temperature in both Maedi and Visna rises due to secondary infection (Straub, 2004). Clinical signs observed in several *Maedi-Visna* related studies (Fournier *et al*., 2006; Muz *et al*., 2006) are mostly computable consistent with the signs observed in this study.

In this study, there were several clinical signs recorded that including: emaciation (28 cases), coughing (28 cases), dyspnea (18 cases), mastitis (2 cases), nervous signs (1 cases), arthritis (1 cases), in addition to the vital signs temperature 38.5-42.0^{o}C, pulse rate 75-95 beat/min and respiratory rate 25-55 cycle/min. The severity of clinical signs may depend on the severity of the infection, infective dose and willingness of sheep to infection (Radostits *et al.,* 2007).

In present study, higher percentage of some clinical signs such as emaciation, dyspnea and coughing observed in infected sheep than the research done by (Lamontagne *et al*., 1983) was reported. However, in another study (Benavides *et al.,* 2007) no clinical findings like dyspnea and emaciation were found, and no findings related to the respiratory system were found. In the present study, we presume the reason of this condition as all the blood samples were taken from infected sheep with chronic pneumonia.

In present study, lower percentage of some clinical signs seen in some adult sheep had been reported neurological symptoms. This results agrees with (Pritchard *et al.,* 1995; Benavides *et al.,* 2007) that Neurological signs have been observed in some of the animals. Besides, in other studies (Fournier *et al.,* 2006; Asadpour *et al*., 2014) researchers reported mastitis, which is similar to results in our study, and in present study, the ELISA tests showed (2) of the infected sheep with mastitis and one animal suffering from arthritis in this study were seropositive. The percentage of some clinical signs such as mastitis and arthritis identified in seropositive sheep in the present study was lower than the results recorded by (Christodoulopoulos, 2006). We assume the cause of this condition more cases of the samples were collected from adult sheep with chronic pneumonia.

The main histological change in the *Maedi-Visna* virus is the chronic interstitial inflammation with thickening of the interalveolar septa which sometimes leads to the completely alveoli obliterated. The thickening of alveolar septa and alveolar emphysema Fig. 1 is primarily duo to infiltration with large mononuclear cells and to a lesser extent to lymphocyte.

In our research, *Maedi-Visna* virus infected sheep show pulmonary lesions were consistent with previous report observations (Villagra-Blanco *et al.,* 2015; Singh *et al.,* 2017). The dominant feature was chronic interstitial pneumonia characterization by diffuse thickening of the interalveolar septa, primarily due to the involvement of macrophages, lymphocytes and plasma cells.

Another conspicuous alteration is smooth muscles hyperplasia in the interalveolar walls with vacuolation in the tissue and inflammatory cell aggregate and infiltration. Fig. 2. The muscular hyperplasia in Maedi and associated pulmonary diseases may be a secondary phenomenon, reflecting a compensatory response to decreased elastic lung recoil caused by thickening of interalveolar septa and fracturing and breakdown of elastic fibers that occur in Maedi an in zwoegerziekte (Ressang *et al.,* 1968).

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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