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Prevalence of Respiratory Syncytial Virus Infection in Sheep at Babylon Governorate, Iraq

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ABSTRACT

The current study aimed to determine the occurrence of the ovine respiratory syncytial virus (ORSV) infection in sheep employing sandwich enzyme-linked immunosorbent assay (sandwich-ELISA) and look into some disease risk factors. We collected blood samples (n=460) from sheep of various ages and sexes in Babylon Governorate/Iraq. The epidemiological data obtained during the interview with the owners of the animals. The prevalence of ORSV infection at Babylon Governorate was 58.91% based on sandwich-ELISA. The risk factors related to ORSV infection included: sheep with 7months-2years old, imported animals, outdoor feeding, large herd size, in northern parts of the Governorate and winter and spring seasons. There was a significant elevation in RBC, Hb, PCV and a significant reduction in WBC and lymphocytes. There was a significant elevation in AST, CK, LDH, TP, globulin, and total bilirubin. At the same time, there was a significant reduction in albumin, creatinine, and glucose in the infected animals with ORSV. In conclusion, ORSV is prevalent in sheep at Babylon Governorate, with a higher prevalence of the disease in the northern parts of the Governorate. The present report is the first for detecting ORSV antigens in sheep at Babylon Governorate/Iraq.

Key words: Respiratory syncytial virus, Prevalence, Sheep.

INTRODUCTION

The respiratory diseases are very important in sheep because they cause high economic losses in sheep farms (Franco et al. 2020). There are many predisposing factors related to the respiratory infections including intensive management, failure of passive immunity transfer, weaning, transportation, dehorning and cold climate (Mahdi et al. 2015; Navarro et al. 2019). The ovine respiratory syncytial virus (ORSV) is an important respiratory pathogen which is responsible for various types of lower and upper respiratory tract infections including bronchopneumonia, bronchiolitis, bronchitis, tracheitis and rhinitis in sheep (Valarcher and Taylor 2007; Bell 2008; Al-Sadrani and Abedelsalam 2010). The ORSV is an RNA virus belonging to the genus Pneumovirus and the family Paramyxoviridae (Lamb and Parks 2007; Hussain et al. 2019). ORSV infection leads to sudden fever, dry and non-productive cough, polypnea and severe dyspnea, bilateral nasal discharge, abdominal breathing, reduction in feed consumption, inappetence, nasal and lacrimal discharges, lethargy and death within 2-5 days after onset of the disease (Contreras-Luna et al. 2017; Constable et al. 2017). Aerosols and direct contact between animals are the main transmission routes of various pathogens (Ferella et al. 2017). According to earlier epidemiological data, the incidence of ORSV in sheep ranged from 49.3 to 60.86% (Cabello and Rivera 2006; Calderon 2011) depending on animal age and predisposing factors as weather condition, inappropriate handling, stress (Socha and Rolla 2013; Sacco et al. 2014). Many laboratory procedures, including viral isolation and serological procedures, immuno-peroxidase method, immunofluorescent method and ELISA can confirm the diagnosis of ORSV infection (Ceribasi et al. 2013). The reverse transcription polymerase chain reaction (RT-PCR) can also be used to properly diagnose ORSV infection (Contreras-Luna et al. 2017; Constable et al. 2017; Timurkan et al. 2019).

In the Babylon Governorate, Iraq, no ORSV prevalence survey in sheep has ever been conducted. As a result, the goal of this study was to estimate the prevalence of ORSV infection in this area and to look into the disease risk factors.

MATERIALS AND METHODS

Study Area and Collection of Samples

This research was performed in the eastern, northern, western and southern parts of Babylon Governorate, Iraq.

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In this investigation, sheep of various ages, sexes and breeds without a history of ORSV immunization were employed at various seasons. A total of 460 blood samples were taken. Twenty-five animals were considered as control group. Animal ages, sex, breed, origin, type of breeding, season, number of animals in the herd and geographical region were all documented. The serum was obtained and stored at–20°C till final testing.

Sandwich Enzyme-linked Immunosorbent Assay (Sandwich- ELISA)

The serum samples were tested by the sandwich enzyme-linked immunosorbent assay (Sandwich-ELISA) kit (MyBioSource, Biotechnology company: San Diego, California, United States) to qualitative analyze the existence or absence of respiratory syncytial virus (RSV) in sheep serum, the procedures were applied according to the manufacturer's instructions.

Biochemical Tests

The serum samples of the animals were used for biochemical tests comprising aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), total protein (TP), albumin, globulin, creatinine, glucose and total bilirubin (TB) through using special cassettes for each test in a chemistry analyzer (IDEXX- Vet Test, Arachem, USA). The 40µL of serum samples was used and the procedure was done according to the chemistry guidebook.

Hematological Examinations

Heparinized blood samples were used to calculate the hematological parameters including red blood cell count (RBC), hemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC) by the use of Hematology analyzer (Mythic 18VET/France). Two mL of blood samples was used for applying the test and the procedure was performed according to the hematology guidebook, while the lymphocyte count was carried out by staining the blood smears with Giemsa stain and the parameters were calculated properly (Weiss and Wardrop 2010).

Statistical Analysis

IBM SPSS Statistical for Windows, version 19 was used for statistical analysis (IBM Corp, Armonk, NY, USA). The ORSV prevalence and numerous risk variables in the sheep groups were assessed using the two-sided Chi-square and Fishers exact test. Using Epi-Info TM 7, version 7 (CDC, Atlanta, GA, USA), the rate of relative ratio (RR) between ORSV risk variables was estimated at 95% significance, while T-test was used for differences in the clinical symptoms, hematological and biochemical parameters at ($P \leq 0.05$).

RESULTS

The results of the sandwich enzyme-linked immunosorbent assay (Sandwich-ELISA) revealed that in the Babylon Governorate, the total prevalence of ORSV infection was 58.91% (271 out of 460). There was a significant elevation in the heart rate, respiratory rate and body temperature of the infected animals with ORSV in comparison with the control group, while there was a significant reduction in the ruminal movements of the infected animals with the virus in comparison with the control group (Table 1). Depending on Sandwich-ELISA, the prevalence of ORSV infection was significantly higher among the sheep that were aged 7months-2years old 88.27% [Relative risk (RR): 3.215 times, Confidence Interval (CI): 2.470-4.185] than old aged sheep (less than 2 years) and young lambs (1-6 months) which were 59.31 and 27.45%, respectively.

There were no significant differences in the prevalence of infection between male and female animals. The prevalence of ORSV infection revealed a significant difference between sheep origin ($P \leq 0.05$). The results revealed that the imported animals had a significantly higher prevalence of 73.82% (RR: 1.836 times, CI: 1.530-2.204) than the native animals at 40.19% (Table 2).

The prevalence of ORSV infection was significantly higher in the outdoor feeding sheep with 77.82% (RR: 2.115 times, CI: 1.751-2.554) than indoor feeding sheep with 36.79%. The prevalence of ORSV infection was significantly higher in the large size herds 93.53% (RR: 3.949 times, CI: 3.120-4.997) compared to the small size herds 23.68% (Table 3). The prevalence of ORSV infection was considerably affected with the geographical regions in

Table 1: Comparison of clinical symptoms of the infected sheep with ORSV and the control group

Clinical symptoms	Control group (n=25)	Clinical cases (n=271)
Heart rate/min	74.42±3.2a	96.8±4.6b
Respiratory rate/min	28.73±2.6a	64.52±4.2b
Ruminal movements/5min	3.21±0.2b	1.28±0.3a
Body temperature °C	38.61±0.09a	40.14±0.12b

Values (Mean±SE) bearing different alphabets in a row differ significantly($P<0.05$).

Table 2: The relative risk of animal variables linked to ovine respiratory syncytial virus prevalence in sheep based on sandwich-ELISA

Factors	Animals tested	Positive number (%)	Relative Risk (RR)	Confidence Interval 95% (CI)	P value
Age					
1-6 months	153	42 (27.45)a	1		
>2 years	145	86 (59.31)b	2.160	1.615-2.889	0.000
7months-2years	162	143 (88.27)c	3.215	2.470-4.185	0.001
Gender					
Males	218	126 (57.79)a	1		
Females	242	145 (59.91)a	1.036	0.889-1.208	0.644
Origin					
Native	204	82 (40.19)a	1		
Imported	256	189 (73.82)b	1.836	1.530-2.204	0.001

Values bearing different letters within a factor and in a column are statistically ($P<0.05$) different.

Table 3: The relative risk of management variables linked to ovine respiratory syncytial virus prevalence in sheep based on sandwich-ELISA

Factors	Animals tested	Positive number (%)	Relative Risk (RR)	Confidence Interval 95% (CI)	P value
Feeding status					
Indoor feeding	212	78 (36.79)a	1		
Outdoor feeding	248	193 (77.82)b	2.115	1.751-2.554	0.001
Herd size					
Small size <20	228	54 (23.68)a	1		
Large size >60	232	217 (93.53)b	3.949	3.120-4.997	0.001

Values bearing different letters within a factor and in a column are statistically (P<0.05) different

Table 4: The relative risk of regional variables linked to ovine respiratory syncytial virus prevalence in sheep based on sandwich-ELISA

Region	Animals tested	Positive number (%)	Relative Risk (RR)	Confidence Interval 95% (CI)	P value
Western	112	18 (16.07)a	1		
Southern	108	68 (62.96)b	3.917	2.504-6.127	0.000
Eastern	118	86 (72.88)c	4.534	2.928-7.022	0.001
Northern	122	99 (81.14)d	5.049	3.278-7.775	0.001

Values bearing different letters within a column are statistically (P<0.05) different.

Table 5: The relative risk of seasonal variables linked to ovine respiratory syncytial virus prevalence in sheep based on sandwich-ELISA

Season	Animals tested	Positive number (%)	Relative Risk (RR)	Confidence Interval 95%(CI)	P value
Summer	106	26 (24.52)a	1		
Autumn	114	64 (56.14)b	2.288	1.579-3.317	0.000
Spring	132	96 (72.72)c	2.965	2.089-4.207	0.001
Winter	108	85 (78.70)d	3.208	2.265-4.544	0.001

Values bearing different letters within a column are statistically (P<0.05) different.

Babylon Governorate. The prevalence of ORSV infection was significantly greater in the Northern parts of Babylon Governorate 81.14% (RR: 5.049 times, CI: 3.278-7.775) compared with the Eastern, Southern and Western parts of Babylon Governorate which were 72.88, 62.96, and 16.07%, respectively (Table 4).

The prevalence of ORSV infection was considerably affected by the seasons. The prevalence of ORSV infection was significantly greater at Winter and Spring seasons which were 78.70 and 72.72%, respectively (RR: 3.208 and 2.965 times respectively, CI: 2.265-4.544 and 2.089-4.207, respectively) compared to Autumn and Summer seasons which were 56.14 and 24.52%, respectively (Table 5). The results of the statistical analysis of the blood parameters revealed a significant rise in RBC, Hb, and PCV levels in the infected animals with ORSV, while there was a significant drop in the WBC and lymphocyte levels in the infected animals with ORSV in comparison to the control group (Table 6).

The biochemical results revealed a significant elevation in CK, AST, LDH, TP, globulin, total bilirubin and a significant drop in albumin, creatinine and glucose in the infected animals with ORSV in comparison to the control group (Table 7).

DISCUSSION

ORSV is one of the main mortality causes in sheep flocks. As a result of the exposition to adverse climatic conditions, overcrowding, animal displacements and stress, the susceptibility of the animals to viral infections is increasing rapidly (Contreras-Luna et al. 2017). The total prevalence of ORSV infection in Babylon Governorate was 58.91%, in the present study, while other nations reported greater or lower prevalence rates. The prevalence of ORSV infection in the current study was higher than the results of Gulbahar et al. (2002), Contreras-Luna et al. (2017) who reported that the prevalence of ORSV infection in sheep was 3.6% and 47.0% in Turkey and Mexico respectively depending on immunoperoxidase technique, serum neutralization test and RT-PCR. In this study, the prevalence of ORSV infection was more elevated than the results of Sharma et al. (2017), who reported that the seroprevalence of ORSV in sheep was 15%, in Grenada region, West Indies, depending on indirect- ELISA technique.

Table 6: Hematological parameters of the infected animals with ORSV (n=271) and control group (n=25)

Parameters	Units	Control group	Clinical cases
RBC	x 10^6	5.942±0.24a	8.462±0.94b
Hb	g/dL	11.14±0.62a	13.52±0.48b
PCV	%	31.42±2.63a	35.621±3.51b
WBC	x 10^3	8.47±1.31b	6.636±0.96a
Lymphocytes	%	54.3±2.52b	46.7±4.42a

Mean±SE bearing different alphabets in a row differ significantly (P<0.05). RBC (red blood cells), Hb (hemoglobin), PCV (packed cell volume), WBC (white blood cells).

In the present study, the prevalence of ORSV infection was lower than the results of Yesilbag and Gungor (2009) who reported that the prevalence of RSV in sheep was 72.9% in the Marmara region of Turkey depending on virus neutralization test. In the present study, the prevalence of ORSV infection was similar to the results of Goncalves et al. (2011) who reported that the prevalence of RSV in sheep was 58.8% in the region of Botucatu, SaoPaulo, Brazil depending on serum neutralization test. The high variation in the prevalence of the disease between different countries may be attributed to different factors including variations in the geographical regions, disease management, climatic variations and disease control programs. In the current study, there was a significant rise in the respiratory rate, heart rate and rectal temperature of the infected animals with ORSV in comparison with the control group because the viral infection leads to systemic reaction and acute phase response in which the cells involved in the innate immune response releases a large number of inflammatory mediators, such as cytokines which induces a cascade of events leading to appearance of characteristic clinical

Table 7: Biochemical parameters of the infected animals with ORSV (n=271) and control group (n=25)

Parameters	Units	Control group	Clinical cases
AST	U/L	107.31±4.77a	131.42±6.1b
CK	U/L	10.42±1.2a	14.22±1.4b
LDH	U/L	148±8.2a	162±6.4b
Total protein	g/dL	7.4±0.48a	8.6±0.72a
Albumin	g/dL	2.8±0.16b	1.6±0.11a
Globulin	g/dL	4.6±0.49a	6.8±0.36b
Creatinine	mg/dL	1.55±0.11b	0.42±0.04a
Glucose	mg/dL	69.3±4.2b	57.7±3.22a
Total bilirubin	g/dL	0.23±0.1a	1.52±0.2b

Values (Mean±SE) bearing different alphabets in a row differ significantly($P<0.05$). AST (aspartate aminotransferase), CK (creatine phosphokinase), LDH (lactate dehydrogenase).

changes such as fever, polypnea, tachycardia, loss of appetite and weight loss (Gabay and Kushner 1999). In the current investigation, the highest frequency of ORSV infection was seen in animals aged 7 months to 2 years, which is consistent with Al-Sadrani and Abdelsalam (2010) who reported that the higher prevalence of ORSV infection was in growing animals (6-9 months of age) and lower prevalence in young animals due to maternal antibodies.

In the current study there was non-significant differences in the diseases frequency in male and female animals, and this result is compatible with the results of Calderon (2011) who reported non- significant differences in the prevalence of ORSV infection between male and female animals. The frequency of ORSV infection was much greater in imported animals than in local animals, which might be due to variations in the breed susceptibility to infection, differences in the disease management, different climatic conditions and due to stress of transportation, and these results are compatible with the results of Timurkan et al. (2019) and Hussain et al. (2019) who reported higher prevalence of the disease in the imported animals than the native animals. In the present study, the prevalence of ORSV infection was significantly more elevated in the outdoor feeding animals than the indoor feeding animals which may be attributed to transfer of the virus from the infected and carrier animals to the healthy animals during grazing, and also may be attributed to direct contact with wild life animals which may be persistently infected with this virus, in addition to the exposure of the animals to the cold weather during grazing which may predispose to the infection with this virus. In the present study, the prevalence of ORSV infection was significantly higher in the large size herds than small size herds and these results are compatible with the results of Rusenova (2009), Saeed et al. (2016) and Navarro et al. (2019) who reported higher prevalence of the disease in large size herds than small size herds, which may be due to overcrowding and close animal contact allowing the virus to spread from carrier to vulnerable animals.

In the present study, the prevalence of ORSV infection was significantly greater in the northern parts of the Babylon Governorate in comparison to other parts and these results are compatible with previous studies by Goswami et al. (2017), Contreras-Luna et al. (2017) and Gaeta et al. (2018) which may be due to many factors such as rearing systems , extensive production systems, maximum sales and purchase of animals, geographical properties, management factors, intensity of breeding and transportation of the seropositive animals from an area to another area may increase the seropositivity due to contagious nature of the disease. In the present study, the prevalence of ORSV infection was significantly higher in the Winter seasons than other seasons and these findings are in agreement with Constable et al. (2017) and Hussain et al. (2019) who reported that the highest prevalence of the clinical disease occurs during winter and autumn seasons due to changes in weather, especially the high drop in the ambient temperatures and atmospheric pressure.

In the current study, there was a significant elevation in red blood cell count (RBC), hemoglobin (Hb) concentration and packed cell volume (PCV) in the infected animals with ORSV in comparison to the control group, which may be attributed to a consequence of adaptation and compensatory processes which reflects pulmonary disease, hypoxia and stimulation of erythropoiesis. The hypoxia for a long period in the infected animals with ORSV may lead to polycythemia, elevation in hemoglobin concentration and packed cell volume (Soltesova et al. 2015), while opposite changes in RBC count and Hb concentration were reported by Fraser et al. (2014) in experimentally infected animals with pneumonia. In the present study, there was a significant reduction in white blood cell count (WBC) which occurred in the infected animals with ORSV, may be due to lymphopenia. The reduction in the lymphocytic count may be due to infection of B-cells (B-lymphocytes) and T-cells (T-lymphocytes) with ORSV after infection corresponding to the peak of viremia leading to a transient immuno-suppression. This transient immunosuppression facilitates systemic dispersal of the virus followed by shedding of the virus to the environment (Nasr El-Deen et al. 2017).

In the current study, there was a significant elevation in the levels of CK, AST and LDH in the infected animals with ORSV compared with the control group and these results are compatible with the results of Nagy et al. (2013), Abdullah et al. (2013) and Soltesova et al. (2015) who reported significantly greater levels of AST, CK and LDH in the infected animals with respiratory diseases. LDH is one of the variables which is regarded as a possible indicator of the lung damage (Drent et al. 1996). AST originates from different tissues, but liver and muscles are major sources for AST production. The higher activity of AST may be originated from the muscles due to higher CK activity, increased breathing rate and increased muscle activity in the long duration of respiratory diseases. Furthermore, the elevation of the activity of AST and CK may be due to decreased feed intake and starvation in respiratory tract infected animals, in addition to the muscle origin due to dystrophic damage of muscles during recumbency for long periods or due to elevated respiratory muscle work in severely respiratory infected animals. In the present study, there was a significant elevation in total protein, globulin and a significant reduction in albumin, and these results are in agreement with Soltesova et al. (2015) who reported a significant increase in total protein, globulin and a significant decrease in albumin in respiratory tract infected animals. These biochemical changes resemble the changes which takes place during acute phase responses. During the acute phase response, the requirement for amino acids increases for synthesis of

acute phase proteins such as haptoglobins which plays important role in alterations in hepatic protein synthesis leading to elevation in globulin levels, thus the production of other proteins such as albumin will be curtailed. The acute phase reaction leads to hyperglobulinemia, hyperproteinemia, hypoalbuminemia and lower albumin-globulin ratio (Tothova et al. 2013).

In the present study there was a significant drop in glucose levels of the infected animals with ORSV, and these results are compatible with Hanzlicek et al. (2010) which may be attributed to inadequate feed intake and energy supply for long periods during the times of sickness.

Conclusion

This is the first investigation of ORSV infection in sheep at Babylon Governorate. This study discovered a significant prevalence of the disease in this Governorates sheep, as well as a number of risk factors related to the ORSV infection.

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Authors Contribution

All authors contributed substantially to this study and are in full agreement with the content of the manuscript.

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