



Seroprevalence of Orf virus in sheep in Basrah province, Southern Iraq

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Abstract

Orf which is commonly referred to as contagious ecthyma is a viral infectious disease that infects small ruminants. Little is known about the disease status in our geographical region. This study aimed to determine the seroprevalence of Orf virus in sheep in Basrah province, southern Iraq. Serum samples were randomly collected from 380 sheep of different age and sex groups from 4 different regions in Basrah province, which were Zubair, Abulkhasib, Shatt Al-Arab, and Qurnah. The samples were tested by ELISA for the presence of IgG antibodies to the Orf virus. Out of 380 animals screened, 98 animals were found positive for ELISA, which reveals an overall prevalence of 25.7%. There was no significant difference in the seropositivity between male and female animals. On the other hand, the seropositivity was significantly higher in lambs of the age group 1-6 months in comparison with the other age groups (6-12 months and more than 12 months). This finding indicates that the virus is highly prevalent in Basrah province as confirmed by the ELISA test.

Keywords: Orf virus, sheep, ELISA, IgG, Basrah

Introduction

Orf or alternatively called contagious ecthyma is an important viral disease of sheep and goats (1-3). It is one of the zoonotic viral diseases caused by the Orf virus, which is a member of Parapoxviruses belonging to the family of poxviridae (4,5). It causes large tumor-like vascularized lesions which can be removed surgically or treated using antiviral drugs (6,7). It is transmitted from infected animals to healthy ones through direct contact and environmental contamination (8). Orf virus typically gains access to host tissues through injury, abrasions, and breaks in the skin. Replication takes place in the keratocytes of the epidermis and causes swollen and granular inflammation of the skin (9,10). The disease has a major impact on animal welfare as well as economic impacts on farmers around the world. Infected animals look sick, fail to thrive, and are susceptible to secondary bacterial infections that might extend to the internal organs (11). Characteristics of the disease are self-limiting proliferative lesions (3-4 weeks) on the oral mucosa, around the nostrils, and on the skin of the lips (12,13). The disease is of zoonoses, presents a greater occupational hazard to people working with animal attendants (veterinarians, animal attendants, and farmers), and visitors, and is characterized by nodular and papillary lesions mainly on the mouth and face, and hands (14,15,16). Infected animals should be handled carefully by wearing gloves to avoid contact with obvious lesions (17,18). Orf is not usually fatal and usually clears up in 2 to 4 weeks; however, death is possible when

secondary complications such as bacterial infections or myiasis appear (19,20).

The lesions that appear on the affected animal, which usually resemble cauliflower, can be seen most often on the lips, muzzles, nose, mucous membranes of the mouth, ears, eyelids, and teats of nursing animals. The lesions that appear on the udder are usually caused by direct contamination during lactation which causes mastitis in ewes (21,22). Arthritis, moderate to severe enlargement of the lymph nodes, and pneumonia have been described during the infection (23).

The infection is usually diagnosed in animals depending on the clinical signs and visible lesions (24). In addition, the diagnosis can be confirmed by performing electron microscopy of the scabs taken from animals to visualize the virus particle (25,26); however, this approach cannot be used to distinguish between the Orf virus and other members of Parapoxviruses (27). The performing of histopathology to identify the pathological changes in the affected skin is also helpful (28). On the other hand, although virus isolation is not commonly used, it can be tried on embryonated chicken eggs and a variety of cell cultures (29). On the molecular side, polymerase chain reaction (PCR) can be used to identify the virus during the stages of the infection (30,31,32). With regard to serology, ELISA is considered a good tool to determine the incidence or prevalence of the disease on a large scale (33). There is not enough information about the endemic status of the disease in the small ruminants available in the south

of Iraq. This study aims to determine the seroprevalence of the Orf virus in sheep in Basrah province, southern Iraq. This was achieved by detecting IgG antibodies by performing ELISA.

Materials and methods

Sample collection

A total of 380 blood samples were collected from male and female sheep of different age on small local farms during the period from February to June 2019. Of these samples, 185 samples were collected from animals more than 1-year-old, 105 samples from animals between 6-12 months, and 90 samples from animals between 1-6 months. The samples were taken from both sexes (200 males and 180 females). With regard to the study area, the samples were collected from Zubair (107 samples), Abulkasib (95 samples), Shatt Al-Arab (87 samples), and Qurnah (91 samples). The samples were obtained from the animals via jugular vein using a 5 ml sterile syringe for each animal and moved into a pre-labeled test tube. They were then placed in an icebox and transported to the laboratory for serological analysis. Serum was harvested by centrifuging the blood samples at 2000 rpm for 15 min. The serum was transferred into a new microcentrifuge tube and kept in the freezer (-20°C) until subjected to ELISA test.

ELISA test

The Orf IgG ELISA was achieved using a sheep ELISA kit (SunLong Biotech Co., LTD) following the manufacturer's instructions. The samples were classified as positive or negative according to the recommendation of the kit manufacturer.

Briefly, a total of 50 µl of positive and negative samples were moved to the positive and negative control wells, respectively. In addition, 10 µl of serum sample was mixed with 40 µl of sample diluent and loaded into the ELISA plate. The samples were mixed gently, and the plate was sealed with a membrane and incubated at 37°C for 30 min. The membrane was then removed and the samples were discarded. The wells were washed 3 times at intervals of 30 s each. After 3 times of washing, 50 µl of horseradish peroxidase conjugate reagent was added to the wells. The plate was then further incubated and the washing was repeated. After that, 50 µl of chromogen was added to the wells, gently mixed, shaken in a dark place, and incubated at 37°C for 15 min. The reaction was then stopped by adding 50 µl of stop solution into the wells. The results were obtained using an ELISA reader (Biotech) within 10 minutes after adding the stop solution. The cut-off value was determined by using the following formula:

Cut off value = the average value of 2 negative controls + 0.15

According to the optical density readings, values less than the cut-off value were considered negative for anti-Orf antibodies; while optical density values that were equal or more than the cut-off value were considered positive.

Statistical analysis

The data were analyzed using the social science statistical package (SSPS). The significance of the difference between groups was assessed using the Chi-square test (X^2). P-value <0.05 was considered to be significant.

Results

Based on the results gained, a large population (98/380 25.7%) of sheep sampled had antibodies to the Orf virus. The serostatus of the sheep with their locations is shown in table 1. The seropositivity to the Orf virus according to sheep location was not significantly different ($p>0.05$). It was 28/107 (26.1%), 24/95 (25.2%), 24/87 (27.5%), and 22/91 (24.1%) in Zubair, Abulkhasib, Shatt Al-Arab, and Qurnah, respectively.

The relation of the age group of the sheep with their serostatus is shown in table 2. The seropositivity in sheep between aged

6-12 months and more than 1 year was 25/105 (23.8%) and 40/185 (21.6%), respectively. In comparison, the seropositivity was significantly higher in the age group 1-6 months, which was 33/90 (36.6) $P<0.05$.

The seropositivity to Orf virus according to animal sex is revealed in table 3. The results showed that there was no significant difference in seropositivity to Orf virus according to the sex of sheep ($p>0.05$). In the male, the seropositivity was 50/200 (25%) while it was 48/180 (26.6%) in the female.

Table 1: Seropositivity to Orf virus according to the location of sheep

Area	Total tested	Positive (%)	Negative (%)
Zubair	107	28 (26.1)	79 (73.9)
Abulkhasib	95	24 (25.5)	71 (74.5)
Shatt Al-Arab	87	24 (27.5)	63 (72.5)
Qurnah	91	22 (24.1)	69 (75.9)
Total	380	98 (25.7)	282 (74.2)

$P>0.05$

Table 2: Seropositivity to Orf virus according to the age of sheep

Age group	Total tested	Positive (%)	Negative (%)
1-6 months	90	33 (36.6)	57 (63.3)
6-12 months	105	25 (23.8)	80 (76.1)
More than 1 year	185	40 (21.6)	145 (78.3)
Total	380	98 (25.7)	282 (74.2)

$P<0.05$

Table 3: Seropositivity to Orf virus according to the sex of sheep

Sex	Total tested	% Positive (n)	% Negative (n)
Male	200	50 (25)	150 (75)
Female	180	48 (26.6)	132 (73.4)
Total	380	98 (25.7)	282 (74.2)

P>0.05

Discussion

In this survey, the effectiveness of the serological test used is shown to be a good and suggested tool for future applications. The seroprevalence of the Orf virus in Basrah province was evaluated to determine the level of virus exposure in the sheep population. This study employed ELISA for the detection of IgG antibodies to confirm the previous exposure to the Orf virus. The occurrence of Orf illness can be predisposed by numerous factors such as breed, age, sex, farm management, and the location of the farms (34). However, the results gained in this study showed a significant difference in the seropositivity among age groups only. In this study, the overall prevalence of Orf determined in sheep was 25.7%. This prevalence rate was higher compared to that of 12.2% and 16.8% as reported in sheep in Malaysia (35,36), and also in England, which was 19.51% (37). On the other hand, it was relatively lower compared to 52.81% and 60% as reported in lambs in Kars, Turkey (38), and Saudi Arabia (39). The prevalence rate in sheep aged 1-6 months was significantly higher than that of the other age groups. Lambs have a higher probability of infection than adults because lambs' immune system is still

underdeveloped and depends mostly on innate immunity and maternal antibodies to protect against the Orf virus (40). Because vaccination against Orf is not currently applied in the areas studied, both adult sheep and lambs are not protected from Orf virus infection and there is a high chance that lambs will acquire Orf virus and develop clinical disease. It has been speculated that Orf infection in sheep does not confer long-term protection for the next infection, and therefore seasonal outbreaks between flocks are common (41). On the other hand, the study of the spread of the disease in other animals, especially goats, should not be neglected, because goats are susceptible to infection with the virus as in sheep (30,35,36). Moreover, the investigation of the presence of antibodies in humans, especially animal breeders, is necessary to know the extent of transmission of the virus to humans, which often causes local skin lesions (8,42).

Conclusion

It can be concluded that the obtained results in our study show that the overall seropositivity to Orf virus in sheep was 25.7%. There is no significant difference

in the serostatus between sex and geographical distribution. With regard to the age group, sheep between 1-6 months old have the highest seropositivity in comparison with the other age groups, which were 6-12 months and more than 1 year.

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conflict of Interest

The author(s) declared that there is no conflict of interest.

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الانتشار المصلي لفيروس Orf في الأغنام في محافظة البصرة جنوب العراق

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الخلاصة

ان مرض اورف والذي يشار إليه عادة باسم الإكتيما المعدية هو مرض فيروسي معدي يصيب المجترات الصغيرة. لا يُعرف سوى القليل عن حالة المرض في منطقتنا الجغرافية. هدفت هذه الدراسة إلى تحديد الانتشار المصلي لفيروس أورف في الأغنام في محافظة البصرة، جنوبي العراق. تم جمع عينات مصل الدم عشوائياً من 380 رأساً من الأغنام من فئات عمرية مختلفة ولكلا الجنسين من 4 مناطق مختلفة في محافظة البصرة وهي الزبير، أبو الخصيب، شط العرب، والقرنة. تم اختبار العينات بواسطة ELISA للكشف عن الأجسام المضادة نوع IgG لفيروس اورف. من بين 380 حيواناً تم فحصها، تم العثور على 98 حيواناً إيجابياً لاختبار ELISA حيث ان النسبة الاجمالية كانت 25.7 %. لم يكن هناك فرق معنوي في الإيجابية المصلية بين الذكور والاناث. من ناحية أخرى، كانت الإيجابية المصلية أعلى بشكل ملحوظ في حيوانات الفئة العمرية 1-6 شهراً مقارنة بالفئات العمرية الأخرى (6-12 شهر وأكثر من 12 شهراً). تشير هذه النتيجة إلى أن الفيروس منتشر بشكل كبير في محافظة البصرة كما أكدته اختبار ELISA.

الكلمات المفتاحية: الاكتيما المعدية ،الأغنام ، الاليزا ، الاجسام المضادة ، البصرة.