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Sero-survey on The Presence of Antibodies Against Foot-and-Mouth Disease in Small Ruminants in the Al-Ahsa Region, Saudi Arabia, During 2023

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Abstract

FOOT AND MOUTH disease is a highly contagious viral disease in cloven-hoofed animals. In contrast to cattle and pigs, Foot and Mouth Disease in small ruminants have been incompletely examined in previous researches. Therefore, this survey was designed to investigate the sero-prevalence of Foot and Mouth Disease among adult sheep at Al-Ahsa Province, Eastern Region of Saudi Arabia during year 2023. A total of 1,356 serum samples from sheep were collected from 16 locations within Al-Ahsa Province, Saudi Arabia. A specific ABC ELISA kits were used to determine the presence of antibodies from the collected serum samples. The data illustrates the distribution of negative, suspect, and positive cases among a total of 1,356 sheep sampled. 162 (12%) sheep were recorded positive, while 85.7% were recorded negative and 31 (2.3%) recorded suspect. The prevalence % of FMD among sheep in 16 regions was 1.2, 47.50, 37.80, 15.0, 9.66, 3.45, 4.88, 6.49, 5.0, 24.59, 7.50, 2.30, 8.20, 8.54, 14.77, and 12.2 respectively. The obtained results concluded that FMD sero-prevalence in the sheep at Al-Ahsa Province, Saudi Arabia, was considered slightly higher recorded than previous years or other regions. In order to update the approach to vaccination for this province district, more detailed investigations of FMD virus serotypes, and regular molecular characterisation is further recommended.

Keywords: Eastern Region, ELISA, FMD, Saudi Arabia, Sheep.

Introduction

Foot and mouth disease (FMD) is one of the most important viral diseases in the middle east and the main way for its control is their detection and sufficient vaccination [1]. All cloven-hoofed ruminants, whether wild or domesticated, are susceptible to foot-and-mouth disease (FMD) which cause a contagious feverish illness. FMD is a member of Picornaviridae family, Aphthovirus genus. FMD genome consisting of 10 nonstructural proteins (NSPs) and four structural proteins which does not have an envelope [2]. There are 7 serotypes of the FMD virus: O, A, C, SAT 1, SAT 2, SAT 3, and Asia-1 which within each serotype considerable antigenic variation occurs. Possessing immunity to a particular serotype does not mean that animals are immune to other serotypes [3, 4].

Generally, small ruminants, sheep and goats, are preferable for Saudi Arabia's livestock owners because they are economic investment and rich source of meat, milk and hides. Foot-and-mouth disease (FMD) is the most contagious viral disease affecting sheep which manifests through symptoms such as fever, loss of appetite, acute vesicular stomatitis, and vesicular dermatitis on the animals' feet and udders. The disease leads to high morbidity and mortality rates, particularly in new-born animals [5].

The prevalence of Foot-and-mouth disease mostly are in South America, Africa and Asia. Serotype O is enzootic throughout the Middle East whilst types A and Asia1 cause sporadic outbreaks of disease. Epizootics due to types C and SAT 1 have occurred in the Middle East but they have been either infrequent or geographically isolated cases. In Saudi Arabia, outbreaks of FMD due to type O virus have been recorded [6].

In sheep, FMD takes three to eight days incubation time. High fever and the formation of oral vesicles, which cause copious salivation, are symptoms of the condition that often appear within two to three days. Rupture of vesicles that form on the snout, teats, or feet can cause lameness. Adults affected by the condition may notice a decrease in

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their body weight and milk output. The disease has a nine-month persistence period in sheep [7]. FMD can be present without symptoms, but can commonly lead to high mortality in young animals. In lambs, the disease is characterized by symptom-less death due to heart failure [8]. The difficulty in identifying the presence of FMD means small ruminants can introduce the virus to disease-free herds [9].

Four serotype O and one SAT 2 FMD outbreaks were recorded in livestock in KSA between July 1999 and June 2004. Although two of the five epidemics affected all types of livestock, including sheep and goats, the other four mostly affected cattle. Two huge epidemics (serotype O) affected all five regions of the country in 2001 (February–April and August–November). Two smaller outbreaks occurred in the central region (serotype O) in 1999 and 2000, in March and April, respectively. In Saudi Arabia, the first case of FMDV serotype SAT 2 infection was reported in March or April of 2000 at Al-Kharj, Riyadh; central region [10].

Screening for FMD is commonly conducted by use of the 3ABC competition antibody ELISA, which is specific and sensitive [11]. A research on FMD in domesticated ruminants was carried out in the Amhara area of Ethiopia by a cross-sectional serological survey. A seroprevalence of 11.48% (95% CI: 7.52-17.14%) was determined when researchers analysed 1,672 sera samples using a 3ABC-Enzyme Linked Immunosorbent Assay. It was determined that the actual overall prevalence was 12.04%. In comparison to goats (7.10%) and sheep (7.07%), the study found that the seroprevalence of FMD was higher in cattle (14.37%) [12].

Periodical check the prevalence of foot and mouth diseases in restricted area is one of important issue for implementation of an effective control programme using the specific non-structural proteins antigens to differentiate between infected and vaccinated animals. Consequently, the laboratory testing of foot and mouth disease ranked first for their diagnosis as most vesicular diseases cannot easily be clinically differentiated due to their variability in the symptoms [5].

Since 2012, there are global control strategy was endorsed for foot and mouth diseases due to their potential effect on international sheep trade and the substantial economic consequences for sheep producers. FMDV is among the modifiable diseases listed as requiring reporting and investigation to control its spread [4]. Many investigators worldwide has been challenged to search specific mechanisms for controlling the FMD progression based on their prevalence in epidemic area and development of effective antivirals to protect livestock [13]. Therefore, the objective of this study was to run a serological survey on the presence of antibodies against FMDV in sheep in the Eastern Region, Al-Ahsa Province, KSA during year 2023.

Material and Methods

The study population:

This cross-sectional study targeted adult sheep in the Eastern Region, Al-Ahsa Province, of the Kingdom of Saudi Arabia (KSA) during year 2023. About 1,356 serum sheep samples were collected from 16 locations within the study area. Sheep aged six months were selected to avoid including individuals with maternal antibodies to the FMDV.

Serum collection:

Teams of lab workers collected sheep's serum samples under veterinarian supervision. The teeth incisors and owner reports helped estimate their ages **[5]**. Blood was drawn from the jugular vein using gauge 21 needles and 10 mL sterile plain vacutainers. Each sample had a unique area code, location, and animal reference. Samples were left clot in chilly boxes. When the blood clot subsided after 12–24 hours, the vials were centrifuged to collect serum. Two 2 mL cryo-vials with one aliquot each were tagged and stored. Samples were frozen at -20°C at sample sites to retain antigen stability. After sampling, the sample boxes were transported to the College of Veterinary Medicine Virology Laboratory using ice and cool-boxes to regulate temperature. Before testing, the samples heated sera at 56°C for 30 min to eliminate non-specific abs. and inactivate viruses then, stored in a laboratory serum bank at -86°C

ELISA techniques for FMD:

Following the instructions provided by the manufacturer, the CHEKITTM-3ABC-FMD ELISA Kit was used. A 96-well microtitre plate pre-coated with the 3ABC antigen was used to hold two portions of the diluted serum samples, which were divided into two equal halves. On the plate, antigen/antibody complexes were formed when antibodies that were specific to the 3ABC antigen adhered to it, while unbound antibodies were washed away. After that, any leftover antibody/antigen complexes were coupled with a guinea pig anti-bovine IgG conjugate that had been labelled with horseradish peroxidase (HRP). Any unbound conjugate was then rinsed away. The next step was to add a chromogen substrate. A 405 nm laser LED spectrophotometer was used to detect the amount of antibody on the surface of the plates. By averaging the pairs, we were able to determine the final reading for each sample. For each plate, we measured the median of the four control sera—two positive and two negative—that came with the kit

Optical Density (OD) of the Positive Control did not exceed 2.0: True.

OD of the Negative Control did not exceed 0.5: True.

The difference between the Positive and the Negative Control must be 0.4: True.

A formula was used for the analysis of the samples:

Data calculate the value as a percentage by subtracting the negative optical density (OD) from the samples.

The interpretation that was suggested by the manufacturers was that less than 20% is negative, 20% to 30% is unclear, and more than 30% is positive.

$$\text{Value\%} = \frac{\text{OD (samples)} - \text{OD (negative)}}{\text{OD (positive)} - \text{OD (negative)}} \times 100\%$$

The manufacturers' recommended interpretation was: <20% is negative, 20–30% is suspected and >30% is positive.

Detection of antibodies against antigen in animal sera:

At the time of sampling, none of the sheep herds surveyed showed clinical signs of FMD. Outcomes are shown in table 1; detected by photometer at a wavelength: 450. Table 1 categorizes values into Negative, Suspect, and Positive interpretations based on percentage ranges and corresponding OD readings. Values below 20% are classified as Negative, indicating low OD readings and a lack of significant detection or activity. Suspect values, which fall between 20% and 30%, suggest a need for further investigation, though specific examples are not provided in the table. Positive values, exceeding 30%, show higher OD readings, signifying a strong presence or activity of the substance being tested. This categorization helps in interpreting the results effectively, guiding subsequent actions based on the detected levels. Sero-prevalence of the non-structural proteins (antibodies) of FMDV was assessed for the 1,356 samples. A total of 162 sheep were positive for FMDV giving sero-prevalence of 12%.

Statistical analysis

Data analysis was performed by using SPSS statistical software program (SPSS for Windows version 16, Spss Inc., USA). Data were expressed as mean ± standard error (SE). One-way analysis of variance (ANOVA) with Duncan post-hoc multiple comparisons test. Any significant differences ($P<0.05$) were analyzed by the multiple comparisons procedure of LSD (least significant difference), using a level of significance of alpha = 0.05

Results

The sero-pervelance of FMD in adult sheep using the non-structural proteins antibodies from 16 in the Al-Ahsa Province, Kingdom of Saudi Arabia were reported in Table 2. The data illustrates the distribution of negative, suspect, and positive cases among a total of 1,356 sheep sampled. 162 (12%) of 1356 sheep tested were recorded positive, while 85.7% were recorded negative and 31 (2.3%) recorded suspect. The prevalence % of FMD among sheep in 16 regions was 1.2, 47.50, 37.80, 15.0, 9.66, 3.45, 4.88, 6.49, 5.0, 24.59, 7.50, 2.30, 8.20, 8.54, 14.77, and 12.2 respectively.

Adult Sheep were shown wide range of prevalence of FMDV suggested that there are differing levels of exposure or immunity within the sheep in the studied regions. Some locations in the Eastern Region of the Kingdom of Saudi Arabia exhibited very low prevalence of FMD infection, such as Location 1 (1.22%), meanwhile others Region shown higher prevalence rates, like Location 2, (47.50%) where nearly half of the animals tested positive.

The present study highlighting on the areas within the studied province in the Kingdom of Saudi Arabia that may require more focused on FMD management efforts. The obtained data given important information is for understanding the spread of FMDV in the Eastern Region of the Kingdom of Saudi Arabia to plan target interventions for controlling and prevention of FMD outbreaks within these regions.

Discussion

The study provides information has practical implications for improving FMD control programs in adult sheep at Al-Ahsa Province, Kingdom of Saudi Arabia. The relatively low FMD sero-prevalence in sheep suggests that current vaccination strategies are having a positive impact. However, the detection of FMDV antibodies in prevalence of 12% of sheep indicates that there is still a risk of FMD outbreaks. Regular molecular monitoring and characterization of circulating FMDV strains among sheep could provide valuable information for updating vaccine formulations and ensuring they are effective against the prevalent virus serotypes in the Eastern Region of the Kingdom of Saudi Arabia.

Antibodies sero-prevalence of FMDV in sheep at the Eastern Region of the Kingdom of Saudi Arabia confirmed the previous presence of FMD infections in sheep. The data throw light on sheep health and FMD control strategies in the selected area. Among 1,356 tested sheep in 16 locations in Al-Ahsa Province, KSA, the seroprevalence in sheep was found to be 12%. This prevalence is considered low in comparison to other ruminant-based investigation from other countries. Food and mouth diseases of

sheep recoded in Tanzanian by 48.5% to 52.5% [14, 15], in Myanmar by 42.4% [16], in Kenya by 22.5% [17], in Libya by 19.0% [18], and in Sudan by 14.1% [19]. On the other hand FMD recorded in lower prevalence than the current study in Ethiopia by 4 to 11% [12, 20], and in Israel by 3.7% [21].

In compared to the previous studies conducted in other regions and countries, the sero-prevalence of FMD in the current study is relatively low, which may reflect effective vaccination and disease management strategies implemented in in the Eastern Region of the Kingdom of Saudi Arabia. However, it also underscores the continuous need for monitoring and targeted interventions to prevent outbreaks and limit the spread of FMD among susceptible livestock.

The sero-prevalence variations in FMD rates in sheep between different countries are mainly due to animal's transportation, environmental conditions, and the implementation of vaccination programme. The high levels of sero-prevalence FDM rates are observed in pastoral settings, where sheep are free movement and come in contact with other livestock [17]. This suggests that great dynamism in contacts between animals as during feeding or watering stations can increase the pace of viral spread. In contrast, it worthy to mentioned that more sedentary livestock populations may benefit from different animal health strategies, as localized vaccination campaigns and stricter biosecurity measures.

An study done by Casey-Bryars, [14] confirmed that there are significant difference in the sero-prevalence of FMD between adult (23.6%) and young sheep (10.1%) which reflect the difference in sero-positivity by age due to adult animals face more variation in contact with other animals including in high-risk settings such as animal markets. As a result adult sheep may have previously been exposed to multiple infection strains and serotypes, allowing them to produce antibodies for different virus strains of FMD.

Another factors effect on sero-positivity throughout the animal's lifetime is animal's sex. There was higher sero-prevalence in females sheep (23.8%) than males (21.9%). However, these results vary between various studies, in Ethiopia, FMD were found to be higher prevalence in males (15.7%) than females (8.3%) [22], meanwhile Mesfine *et al.,* [12] recorded higher prevalence of FMD in females sheep (8.9%) than in males sheep (3.0%).

The obtained data has several strengths that contribute to the understanding FMD epidemiology in sheep within the Eastern Region of Saudi Arabia. In the current study, the analysed of 1,356 serum sheep samples collected from 16 different locations at the Eastern Region of Saudi Arabia, ensuring a wide geographical coverage and improving the representativeness of the findings. The use of the ChekitTM-3ABC-FMD ELISA kit, as sensitive and specific diagnostic tool support strengthens on the reliability of the sero-prevalence data obtained. Additionally, the study's focus on adult sheep, which are more likely to have had prolonged exposure to FMDV, provides a more accurate estimate of the disease's prevalence within area under the investigation. However, there is study has a limitations should be concern as the cross-sectional design of this study only provides FMD sero-prevalence in sheep at selected time, and neglect the seasonal variations in FMDV circulation.

Animal vaccination against FMD as endemic disease in KSA, sheep imported for slaughter should be quarantined and vaccinated against FMD. Mahmoud and Galbat, [23] used ELISA for detection of FMDV non-structural (NS) antibodies in serum's sheep from Riyadh, KSA, the results recorded 76% of the serum's sheep tested were positive for FMDV NS viral proteins. It reflectes the importance of the periodical check for the evidence-base demonstrating the virus strains for effective vaccination programmes.

Further investigation using molecular characterization of FMDV strains were still needed to identify the specific serotypes circulating in the region under the study. This information is crucial for guiding vaccine updates and improving the effectiveness of control measures. Additionally, the study highlights the need for further research into the epidemiology of FMD in small ruminants, as their role in the transmission and maintenance of the virus within livestock populations remains underexplored.

Conclusion

FMD sero-prevalence in the sheep at Al-Ahsa Province, Saudi Arabia, was considered slightly recorded than previous years or other regions. In order to update the approach to vaccination for this district, more detailed investigations of FMD virus serotypes, and regular molecular characterisation is further recommended.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Interpretation ranges for foot and mouth disease based on Optical Density (OD) readings.

Value Interpretation	<20% Negative	20-30% Suspect
Interpretation	Value %	Samples OD*
Negative	1%	0.08
Negative	2%	0.094
Negative	5%	0.126
Positive	44%	0.333
Positive	30%	0.25
Positive	159%	1.005

* Photometer at a wavelength: 450

TABLE 2. Sero-prevalence of FMD in adult sheep using the non-structural proteins antibodies from 16 locations in the study area.

Location #	Sheep No.	Negative	Suspect	Positive	Prevalence %
1	82	81	0	1	1.22
2	40	18	3	19	47.50
3	82	48	3	31	37.80
4	40	34	0	6	15.00
5	145	126	5	14	9.66
6	145	135	5	5	3.45
7	82	77	1	4	4.88
8	77	72	0	5	6.49
9	40	37	1	2	5.00
10	122	91	1	30	24.59
11	40	37	0	3	7.50
12	87	85	0	2	2.30
13	122	106	6	10	8.20
14	82	71	4	7	8.54
15	88	74	1	13	14.77
16	82	71	1	10	12.20
Total	1356	1163 (85.7%)	31 (2.3%)	162 (12%)	12.00

*FMD area prevalence = No. of Positive/ No. of Animals in a location

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مدى انتشار مرض الحمى القلاعية في الأغنام بمحافظة الأحساء بالمملكة العربية السعودية خلال عام 2023

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الملخص

يعد مرض الحمى القلاعية من الامراض الفيروسية شديدة العدوى حيث أنه يصيب الحيوانات ذات الظلف المشقوق. وعلى النقيض من الماشية والخنازير، لم يتم فحص مرض الحمى القلاعية في المجترات الصغيرة بشكل كامل في الأبحاث السابقة في المملكة العربية السعودية. لذلك تم تصميم هذا المسح البحثي لمعرفة مدى الانتشار المصلي لمرض الحمى القلاعية بين الأغنام في منطقة الأحساء بالمنطقة الشرقية بالمملكة العربية السعودية خلال عام 2023. وقد تم سحب إجمالي عدد 1356 عينة مصل من الأغنام البالغة من عدد 16 موقعًا داخل منطقة الأحساء، محافظة الأحساء، بالمملكة العربية السعودية. تم استخدام مجموعة ABC ELISA لتحديد وجود الأجسام المضادة في عينات المصل المجمعة. وأوضحت النتائج أن توزيع الحالات السلبية والمشتبه بها والإيجابية بين إجمالي عدد 1356 رأساً من الأغنام التي تم سحب العينات منها تسجيل عدد 162 (12%) من أصل 1356 رأساً من الأغنام إيجابية لمرض الحمي القلاعية، في حين تم تسجيل عدد 85.7% منها سلبية وعدد 31 (2.3%) مشتبه بها. وكانت نسبة انتشار مرض الحمى القلاعية بين الأغنام في عدد 16 منطقة هو نسبة 1.2، 47.50، 37.80، 15.0، 9.66، 3.45، 4.88، 6.49، 5.0، 24.59، 7.50، 2.30، 8.20، 8.54، 14.77، و12.2 على التوالي. وتم ارسال النتائج المتحصل عليها إلى الجهات المعنية لتطبيق برنامج التطعيم النوعي وعلاج الحالات الإيجابية. وخلصت النتائج التي تم الحصول عليها إلى أن الانتشار المصلي لمرض الحمى القلاعية في الأغنام في محافظة الأحساء، بالمملكة العربية السعودية يعتبر مسجلا بشكل طفيف مقارنة بالسنوات السابقة أو المناطق الأخرى. ومن أجل تحديث نهج التطعيم في هذه المنطقة توصى الدراسة بإجراء تحقيقات أكثر تفصيلاً للأنماط المصلية لفيروس الحمى القلاعية والتوصيف الجزيئي المنتظم للمرض.

الكلمات الدالة: المنطقة الشرقية، الاحساء، الحمى القلاعية، المملكة العربية السعودية، أغنام.