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A Serological Study on Foot and Mouth Disease in Cattle from the Dhofar Governorate of Oman

Rashid M. Al-Busaidi[1]*, Ahmed Al -Jassasi[2], and Eugene H. Johnson[1]

[1]Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 34, Al-Khod -123, Sultanate of Oman

[2]Ministry of Agriculture and Fisheries, P.O. Box 467, Muscat 113, Sultanate of Oman

دراسة مصلية عن انتشار مرض الحمى القلاعية في الأبقار في محافظة ظفار بسلطنة عمان

راشد البوسعيدي وأحمد الجساسي ويوجين ه. جونسون

الخلاصة: الحمى القلاعية مرض فيروسي شديد العدوى والانتشار وله تأثير كبير على الانتاج الاقتصادي والثروة الحيوانية في البلدان المتضررة. تم إجراء هذه الدراسة في منطقة ظفار في سلطنة عمان في الفترة بين أغسطس 2003 ومارس 2004 بهدف تحديد انتشار مرض الحمى القلاعية في الأبقار الظفارية و تم جمع عينات مصلية من مجموع 395 بقرة في 19 قطيع من قرى ظفار وقد تم استخدام و 11.6% (O) اختبار الاليزا لفحص العينات. كان الانتشار المصلي العام لمرض الحمى القلاعية 52.1 ٪ (العدد = 206) بواقع 40.5% للعترة من إجمالي العينات الإيجابية. 22,3% (A) ما نسبته 77,7% والعترة (O) من إجمالي العينات (395).وشكلت العترة (A) للعترة أشارت الدراسة لعدم وجود تباين كبير في معدل الانتشار المصلي تبعا للجنس والسن ولكن كان هناك اتجاه الى ارتفاع معدلات في منطقة ظفار O و A الاصابة مع تقدم الحيوان في العمر. بالإضافة إلى ذلك أوضحت هذه الدراسة وجود حمى قلاعية من عترة

كلمات مفتاحية: الحمى القلاعية ، الأبقار ، عمان ، العترة A وO.

ABSTRACT: Foot and mouth disease (FMD) is a highly contagious viral disease that has a significant impact on the economy and livestock productivity of affected countries. Based on unpublished reports, serotype O has been incriminated as the sole serotype present in Oman. The present study was conducted in the Dhofar region of the Sultanate of Oman between August 2003 and March 2004 with the objective of determining the seroprevalence against FMDV in Dhofari cattle. Sera samples were collected from 395 cattle in 19 different herds. Sera underwent screening using a Liquid Phase Blocking ELISA to determine the antibody response to the serotypes O, A, C, Asia 1, Sat1, Sat 2, and Sat 3. The overall seroprevalence of FMD was 52.1% (n=206). Of the seropositive animals, 77.7% were positive for type O and 22.3% for type A. There was no significant variation in seroprevalence among different sex and age groups; however, there was a tendency towards a higher incidence of seropositive in older animals. This study confirmed that FMDV is widespread in the Dhofar region. Furthermore, it is the first report of serotype A and an animal testing seropositive for O and A in the Sultanate of Oman.

Keywords: Foot and mouth disease, cattle, Oman, Serotypes O and A.

Introduction

Foot and Mouth Disease (FMD)

FMD is the most contagious disease of cloven-hoofed domestic animals, such as cattle, sheep, goats and pigs (Brown, 2003). The disease is of great concern to veterinarians, farmers, and animal product traders, because of three main factors: its highly contagious nature, its ability to persist in carriers, and the fact that there is no cross protection between the serotypes of the virus (Alexandersen *et al*., 2003).

There are 7 immunologically and serologically distinct types of FMDV: A, O, C, and South African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1. Within each serotype there are several subtypes (Alexandersen *et al*., 2003; Sanson, 1994). Infection with one type does not cross-protect against infection with the other types (Aggarwal and Barnett, 2002; Davies, 2002; Sanson, 1994).

In 1987, an epidemiological study was carried out by the Central Diagnostic Laboratory and revealed that FMD was endemic in the Sultanate of Oman (Anonymous, 2004). Virus type O was the only serotype documented (unpublished data). Representative outbreaks of FMD in the Sultanate of Oman indicate an incidence of the disease of over 100 cases per year since 1995.

Dhofar is located in the southern region of the Sultanate of Oman and is one of the main cattle rearing areas in the country. It has a large animal population due to presence of extensive areas of green pastures. Nearly two thirds of the population depends on animal husbandry for their livelihood. FMD presents a serious problem for Dhofari farmers due to

*Corresponding author/E-mail: rashid99@squ.edu.om

its devastating effect on the overall income of farmers. Therefore, the present study was an endeavor to determine the seroprevalence of FMD in cattle in the Dhofar region of Oman, and to ascertain what serotypes they have been exposed to.

Materials and Methods

Animals

A total of 395 Dhofari cows of both sexes (males n=121, (31%), females n=274 (69%) of different ages were used in this study. The animals were randomly selected from 19 farms from different regions of Dhofar during the period from August to March 2004. None of the farms selected for this study had any vaccination program for their animals. The animals were sorted according to age groups. Group 1: aged less than one year (n=21), Group 2: 1- 2 years (n=29). Group 3: 2- 3 years (n=150), Group 4: 3- 4 years (n=152) and Group 5: 4-7 years of age, respectively (n=43). Cows were from the following areas of Dhofar: Dalkot, Keron Herety, Tawy Ater, Tetam, Madenat Alhaq, Kastwt, Kabrart, Hgeef, Krdhat, Shaba saib, Gadu, Gorokub, Alsan, Sehlanoot, Sadah, Gahnen, Al Deharez, Zeeq and Mqorah. Cattle were fed crop and orchard residues, dates, dried sardines and barley, Rhode grass, agricultural residues and by-products. The cows were maintained in shaded houses and partially closed pens.

Samples

Blood samples were collected from the jugular vein in plain vacutainer tubes for serum isolation. The samples were transported to the laboratory in an icebox (4°C). All serum samples were aliquated and frozen at -20°C.

Liquid Phase Blocking ELISA

Liquid phase blocking ELISA tests, using 96-well polystyrene microtiter plates were conducted following the procedure described by the manufacturer [The Institute for Animal Health (IAH), Pirbright Laboratory, United Kingdom]. The assay is based on specific blocking of a defined amount of FMDV antigen by antibodies in the test sample during the liquid phase. After the test serum is allowed to react with specific FMDV antigen, the test serum/antigen mixture was transferred to an ELISA plate coated with FMDV serotype specific trapping antibodies. The presence of antibodies to FMDV in the serum sample results in the formation of immune complexes and consequently reduces the amount of free antigen trapped by the immobilized rabbit antisera.

Briefly, ELISA plates were coated with 50 µl/well rabbit antisera O_1 monisia, A_{22} and $Asia_1$, and left overnight in a humid chamber at 4°C. The ELISA plates were washed three times with phosphate buffer saline (PBS). In U-shape 96 multiwell plates, 50 µl of a duplicate, control and test serum were prepared at dilution 1/16, resulting in 20 test sera for every serotype/plate. To each well, 50 µl of a constant dose of homologous viral antigen was added and the mixtures were left overnight in a humid chamber at 4°C.

Subsequently, 50 µl of the serum/antigen mixtures (liquid phase) were transferred from the carrier plate to the rabbit-serum coated ELISA plate and incubated at 37°C for 1 hour on a rotary shaker. After washing, 50 µl of guinea-pig antiserum homologous to the viral antigen used in the previous step was added to each well. The plates were then incubated at 37°C for 1 hour on a rotary shaker. The plates were washed and 50 µl of rabbit anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase were added to each well. The plates were incubated at 37°C for 1 hour on a rotary shaker. The plates were washed again and 50 µl of the substrate (orthophenylene diamine containing 0.05% H_20_2) were added to each well. The reaction was stopped after 15 minutes by the addition of 50 µl of 1.25 M sulphuric acid to each well. The plates were read at 492 nm on a spectrophotometer.

The diagnostic threshold for this assay was set at 50 % inhibition (50 PI). If both replicate PI values of a test serum fell below 50 PI then that test serum was considered to be negative. If either, or both replicate PI values of a test serum fell above 50 PI then that test serum was considered to be positive.

Statistical Analysis

The data obtained from this study was analyzed by analysis of variance and least square (LS) mean were used to evaluate the effect of age and sex on the persistence of FMDV infection in Dhofari cows. All of the statistical analysis was performed using the Statistical Analysis System for windows (SAS, 1993).

Results

Serological Results

The results of the serological analysis of the tested animals are given in table 1. 40.5% of the tested sample of cows were positive for FMDV serotype O, 11.6% were positive for serotype A, and 47.9% were negative for all seven serotypes of FMDV (strain O, strain A, strain C, Asia1, Sat 1, Sat 2, and Sat 3) . From the total number of positive samples (n= 206), 160 (77.7%) and 46 (22.3%) were reactive against O and A, respectively (Table 2).

Effect of Age

The results of the serological comparison of different age groups revealed that there were no significant differences ($p>0.05$) between the groups. However, there was a tendency for older animals to exhibit a greater number of seropositive results (Table 3).

Effect of Sex

There was no significant difference ($p>0.05$) in sero-positivity between males and females (Table 4).

Table 1. Serological response of sera collected from 395 cattle to FMDV serotypes.

Serotypes	O	A	C	Sat 1	Sat 2	Sat 3	Asia 1
Seropositive (%)	40.5	11.6	0	0	0	0	0
Number of positive sample	(n=160)	(n=46)	(n=0)	(n=0)	(n=0)	(n=0)	(n=0)

Table 2. Percentage of positive serological responses from the total number of positive samples.

Serotypes	O	A
Seropositive (%)	77.7 (n=160)	22.3 (n=46)

Table 3. The relationship between different cattle age groups and seropositivity against FMD serotypes O and A.

Age (yrs)	Number of samples	Number of seropositive animals	Percent (%)
1	21	10	47.6
1- 2	29	14	48.3
2- 3	150	77	51.3
3- 4	152	86	56.6
4 - 7	43	25	58.1

Table 4. Comparison of seropositive response to O and A FMD serotypes among male and female cattle.

Serotypes	Males (n=121)	Females (n=274)
O+ A	60 (49.6%)	146 (53.3%)
A	15 (25%)	32 (21.9%)
O	45 (75%)	114 (78.1%)

Serological Response to Multiple Antigens

One animal had a positive serological response to both type A and type O serotypes.

Discussion

This study confirms that FMD is endemic in the Dhofar region of Oman. A total of 395 cattle serum samples were collected from 19 farms, 52% exhibited seropositive responses to one or more FMD viral antigens. The endemic nature of this disease may be due to the husbandry practices of farmers who increase their herd sizes by buying new animals from different markets without quarantining them (Bronsvoort *et al.*, 2004). This practice tends to increase the risk of infection (Esayas *et al.,* 2009). Also, it was noticed that farmers tend to underplay the significance of FMDV as a threat to their herds. This may be due to the farmers' experiences with the less adverse effects of FMDV in the local cattle breed. This attitude towards FMD was also observed in Uganda among local farmers (Clavijo and Kitching, 2003). Tomasula and Konstance (2004) and Balinda *et al*. (2009) reported that FMDV was shed into the milk before dairy cattle show any clinical signs of the disease and therefore, there is opportunity for raw milk to act as a factor contributing to the spread of the disease from farm to farm.

In Dhofar, most farmers keep mixed herds especially with sheep and goats. Barnett and Cox (1999) and Esayas *et al.* (2009) reported on the relationship between mixed herds and the prevalence of FMD among cattle. They found that small ruminants are important reservoirs for FMDV but do not show clinical signs and can remain carriers for long periods of time. It can also not be ruled out that wild animals might be reservoirs of infection for cattle. Indeed, potential sources of infection need to be studied in Oman, as this is absolutely essential in order to develop effective control measures in the Sultanate.

Unpublished results from the Ministry of Agriculture have concluded that serotype O was the only serotype found in the Sultanate. However, the results obtained from the present study confirmed the incidence of serotypes A and O in the Dhofar region with type O accounting for 77.7% and A for 22.3% of the seropositive responses, respectively. The emergence of serotype A may be due to the illegal importation of ruminants across borders with neighboring countries such as Yemen, UAE and Saudi Arabia where FMDV type A exists (Shimshony and Economides, 2006). Further studies are needed to monitor the occurrence of FMDV among illegally and legally imported animals, and the probability of the entrance of other strains of FMDV.

The cattle in this study were categorized according to age. There was no significant difference in the infection rates among these age groups. However, it was noticed that there was a tendency to observe a higher incidence of seroprevalence among animals aged 3 to 7 years old compared to those between 1 to 3 years of age, which is in agreement with the findings of Esayas *et al.* (2009), Molla *et al.* (2010), and Sarker *et al.* (2011), who found

an increasing number of seropositive animals in older animals. This may be due to the farming practices that confine younger animals and prevent them from grazing with older ones, hence decreasing their exposure to FMDV. This would be in agreement with the findings of Rufael *et al.* (2008) and Sarker *et al.* (2011). Also there is a tendency for older cattle to follow seasonal movement patterns in search of good pastures and water where they go for long distances and cover large areas of land, thus giving them a greater opportunity to be exposed to FMD infected animals (Broonsvoort *et al*., 2004).

This study also showed no significant differences in the response to infection between males and females. This is consistent with the findings from other studies that have shown no differences between males and females (Esayas *et al.*, 2009; Mekonen *et al.*, 2011). However, a study by Mazengia *et al.* (2010) reported a significantly higher incidence of FMD in females. The author proposed that this might be the result of higher physiological stressors such as estrus, pregnancy and lactation - factors documented to affect resistance to infection.

The results of the present study showed that one animal aged above 4 years of age was seropositive for both A and O. Molla *et al.* (2010) also found infections with two different serotypes in the same animals. He suggested that this may be due to the fact that animals above 4 years of age have a greater probability of being infected with more than one serotype since they have more likely been exposed to multiple serotypes. This finding draws light on the need for the continuous monitoring of all imported animals to prevent entrance of new strains that do not yet exist in Oman.

In conclusion, the present study provides evidence that exposure to FMDV is widespread in Dhofari cattle. Furthermore, in contrast to unpublished reports of the Ministry of Agriculture, serotype A, in addition to serotype O, is prevalent in Oman. These findings underscore the widespread nature of FMDV in Dhofar and the necessity of establishing appropriate control measures. Studies need to be undertaken to assure that vaccine strategies presently employed in the Sultanate provide adequate protection against clinical outbreaks of the disease. It is obvious from this study that vaccines employed against FMD in Oman should contain serotypes A and O, and all animals in Oman should be targeted for vaccination programs and regular monitoring for development of FMDV infections. Furthermore, this study indicates the need for more efforts to fully characterize isolates of FMD virus from outbreaks in Oman, and to undertake seroprevalence studies in other regions of the country.

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