Serological survey of foot and mouth disease in Saudi Arabia

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Summary: An immunodiffusion test using foot and mouth disease (FMD) virus infection-associated (VIA) antigen was used to detect precipitating antibodies in serum samples collected from non-vaccinated indigenous ruminants raised in different regions of Saudi Arabia. Of 5,985 sheep sera, 1,371 goat sera, 1,052 cattle sera and 694 serum samples from unspecified species of ruminants, precipitating activity was detected in 1,209 (20%), 127 (9%), 172 (16%) and 38 (5%) samples, respectively. In addition, 100 sera showing precipitating activity against VIA antigen originating from 13 different regions were tested for the presence of naturally-occurring neutralising antibodies against the four serotypes of FMD virus (O, A, Asia 1, and C) currently prevalent in the region and incorporated in the vaccine being used. All sera tested gave varying titres against serotypes O, A and/or Asia 1. However, none of the sera showed neutralising activities against serotype C. The results obtained are interpreted with regard to the geographical distribution and epizootiology of FMD in Saudi Arabia.


INTRODUCTION

Outbreaks of foot and mouth disease (FMD) repeatedly occur among cattle, sheep and goats in various regions of Saudi Arabia (13). Planning an adequate control programme requires a thorough understanding of the current epizootiological status of the disease. The population of various ruminant species in Saudi Arabia is estimated at approximately 5,692,000 sheep, 3,350,000 goats, 176,000 indigenous cattle, 87,000 dairy cattle and 390,000 camels (8). In addition, approximately 5,000,000 sheep, 750,000 goats, 30,000 cattle and 6,000 camels are imported annually for slaughter (O. Hashim, personal communication). Many of these animals are imported from countries where FMD is enzootic, and usually a considerable number are mixed with local animals prior to slaughter.

Laboratory diagnosis of field samples from Saudi Arabia was regularly performed at the World Reference Laboratory (WRL) for FMD in Pirbright, United Kingdom. Five serotypes of FMD virus (SAT 1, O, A, Asia 1 and C) have been isolated from Saudi Arabian field samples in the past (4). However, over the last five years, only serotypes O, A and Asia 1 have been isolated. Routine FMD vaccination is performed mainly in dairy cattle, using a commercial quadrivalent vaccine which incorporates serotypes O, A, C and Asia 1.

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Recently, national facilities for the diagnosis of FMD have been established at the Animal Virus Research Laboratory of the National Agriculture and Water Research Centre in Riyadh. It has therefore become possible to carry out long-term investigations on some epizootiological aspects of FMD in Saudi Arabia. The results of a nationwide serological survey are presented below.

**MATERIALS AND METHODS**

**Serum samples**

Blood samples were collected from non-vaccinated indigenous sheep, goats and cattle by district field veterinarians between November 1989 and October 1991. In addition, some sheep sera were provided by commercial sheep farms near Riyadh and in the Northern Region of the country. Animals on these farms are not vaccinated against FMD. The regions from which the samples were collected and the animal species sampled are shown in Figure 1.

![Map of Saudi Arabia showing where serum samples were collected](image)

**Fig. 1**

Map of Saudi Arabia showing where serum samples were collected

Figures indicate percentages of samples in which precipitating antibodies against virus infection-associated antigen were detected.
Virus infection-associated antigen

The foot and mouth disease virus infection-associated (VIA) antigen was prepared from a local serotype O isolate (designated O/SAU/38/89 by the WRL) in baby hamster kidney (BHK)-21 cells, according to the technique described by Fernandez et al. (10).

Positive reference serum

A susceptible ram was inoculated intradermolingually with FMD virus strain O/SAU/38/89. The serum collected 14-30 days post-inoculation formed clear precipitin lines against VIA antigen and was used as reference positive serum for testing the different batches of VIA antigen, and in the routine implementation of the immunodiffusion test.

Immunodiffusion test

The immunodiffusion test was carried out in 100 mm diameter Petri dishes, using 1% agarose in glycine barbital buffer (5) supplemented with 0.01% sodium azide as bacteriostat. Four sets of seven 6 mm diameter wells were punched in each plate, in a hexagonal pattern with one central well. The antigen, positive reference serum and test sera were distributed in wells as described elsewhere (2).

Virus neutralisation test

The microneutralisation test was applied using BHK-21 monolayers, as described elsewhere (12). One hundred serum samples (39 from sheep, 38 from goats and 23 from cattle) which showed precipitating activity against VIA antigen were tested. These sera originated from 13 geographical locations covering most regions of the country (Fig. 2). Each serum sample was tested against four serotypes of FMD virus, using the virus strains O1/Manisa/68, A/SAU/23/86, C3Pando and Asia 1 Turkey, respectively.

RESULTS

Detection of precipitating antibodies against FMD VIA antigen

In the immunodiffusion test for antibodies against VIA antigen, some sera formed complete lines of identity with those of the positive serum. Other sera produced only spurs from the ends of the adjacent precipitin lines formed by the positive serum, indicating a ‘recruiting’ effect from the reference antiserum. All the serum samples which reacted by the formation of either complete precipitin lines or spurs were considered positive for the presence of precipitating antibodies against VIA antigen.

The results of immunodiffusion tests are shown in Figures 1 and 3. Of 5,985 sheep sera, 1,371 goat sera, 1,052 cattle sera and 694 serum samples from unspecified species of ruminants, 1,209 (20%), 127 (9%), 172 (16%) and 38 (5%), respectively, were positive for the presence of precipitating antibodies against VIA antigen.

The sheep serum samples from Gizan, the goat samples from Madina, Taif and Gizan, the cattle sera from Hail, Riyadh, Washm, Dawadmy, Dawaser and Aflaj, and the sera from unspecified ruminant species collected from Jouf, Riyadh and Baha were all negative when tested for antibodies against VIA. However, a proportion of sera from other species raised in the same regions were positive (Figs 1 and 3). In all regions from which samples were collected, sera from at least one species revealed the presence of precipitating antibodies against VIA antigen, with proportions of positive results ranging from 1% to 78%.
Detection of neutralising antibodies against FMD virus

Various titres (ranging from 1:10 to 1:160) of neutralising antibody against one or more serotype of FMD virus were detected in each of the 100 sera re-tested by virus neutralisation. Antibodies against serotypes O, A and Asia 1 were detected in 92 (38 sheep, 35 goats and 19 cattle), 22 (8 sheep, 7 goats and 7 cattle) and 17 (9 sheep, 5 goats and 3 cattle) samples, respectively (Fig. 4). However, no antibodies against serotype C were detected in any of the sera tested. As shown in Figure 4, some of the tested sera showed neutralising activity against more than one serotype. The geographical distribution of different serotypes of FMD virus as detected by neutralisation tests is shown in Figure 2.

DISCUSSION

Detection of specific antibodies against VIA antigen in sera of different species of animals raised in a certain geographical area is considered a useful tool for epizootiological studies (7, 9, 10, 16, 17, 19, 21). Despite the low sensitivity of the
VIA test compared to other FMD serological techniques (1, 9, 18, 22), this test is still preferred because of its simplicity, group specificity (5), the presence of detectable antibodies for a long period (9, 17) and the ability to differentiate between naturally-infected and vaccinated animals (17). However, it has been shown that sera from animals repeatedly vaccinated against FMD might form precipitating lines against VIA antigen (2, 6, 20).

Despite the low sensitivity of the VIA test, 1,546 of 9,102 (17%) animals tested in Saudi Arabia gave positive results for the presence of specific antibodies. The antibodies were detected in sera from sheep, goats and/or cattle raised in all regions of the country. These results indicate a nationwide prevalence of circulating FMD virus(es). However, clinical FMD had not been recorded in many regions from which the samples were collected (14). Accordingly, the results obtained suggest that certain indigenous animals may have experienced inapparent or subclinical FMD virus infection.

The failure to detect precipitating antibodies against VIA antigen in sera collected from some regions (Figs 1 and 3) may indicate that these animals were not exposed to FMD virus, or that the immunodiffusion test used was not sensitive enough to detect the precipitating antibodies in all previously-infected animals. The detection of antibodies in sera collected from other animal species raised in the same regions is indicative of the prevalence of FMD virus(es) in these regions.

The detection of neutralising antibodies against FMD virus serotypes O, A and/or Asia 1 but not against serotype C corresponds with the current results of virus isolation tests performed on samples collected in Saudi Arabia (13, 23). Despite the small number of sera examined by neutralisation tests, the results obtained generally confirm...
Detection of neutralising antibodies against foot and mouth disease virus serotypes O, A and Asia 1 in sera from sheep, goats and cattle collected in different regions of Saudi Arabia

Figures above columns indicate the total number of sera tested, while the shading of columns indicates serotypes detected

the present nationwide circulation of serotypes O, A and Asia 1. The failure to detect neutralising antibodies against serotype C is an indirect indication that the examined animals had not been vaccinated. Testing of animals vaccinated with the quadrivalent vaccine used in Saudi Arabia revealed the presence of antibodies against serotypes O, A, C and Asia 1 (unpublished data). Serotype C was last isolated in Saudi Arabia in 1984 (4). No decision has been made to remove this serotype from the vaccine used in Saudi Arabia, owing to the importation of live animals from some countries where this serotype is prevalent.

Although FMD virus serotype SAT 1 was isolated from Saudi Arabia in 1970 (3) and serotype SAT 2 was recently isolated from the neighbouring country of Yemen (11), these two serotypes were not included in the neutralisation tests of the present study for reasons of safety. Some serological tests in which inactivated SAT 1 and SAT 2 viruses
are used as antigens (15) could be safely applied to study the possible prevalence of these serotypes in the field in Saudi Arabia.

The high percentage of positive serological test results in sheep and goats in many regions of Saudi Arabia, in the absence of clinical FMD among these species, indicates the importance of these range animals in transmitting FMD virus between regions within the country. Therefore, these findings emphasise the need to consider small ruminants in future FMD control programmes.

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Résumé : Une épreuve d’immunodiffusion utilisant l’antigène associé à l’infection virale de la fièvre aphteuse (virus infection-associated : VIA) a été appliquée à la recherche d’anticorps précipitants dans des prélèvements de sérum provenant de ruminants d’origine locale, non vaccinés et élevés dans différentes régions d’Arabie saoudite. Sur 5 985 séums d’ovins, 1 371 séums de caprins, 1 052 séums de bovins et 694 séums provenant d’autres espèces de ruminants, le phénomène de précipitation a été observé, respectivement, dans 1 209 (20 %), 127 (9 %), 172 (16 %) et 38 (5 %) des cas. De plus, 100 séums précipitant en présence de l’antigène VIA et provenant de 13 zones différentes, ont fait l’objet d’une recherche d’anticorps neutralisants naturels contre les quatre sérotypes du virus de la fièvre aphteuse (O, A, Asia 1 et C) qui prévalent actuellement dans la région et qui sont intégrés au vaccin employé. Tous les séums étudiés ont présenté des titres différents vis-à-vis des sérotypes O, A et/ou Asia 1. Toutefois, aucun séum n’a neutralisé le virus de sérotype C. Les résultats obtenus sont interprétés en fonction de la répartition géographique et de l’épizootiologie de la fièvre aphteuse en Arabie saoudite.


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Resumen: Se aplicó una prueba de inmunodifusión que utilizaba el antígeno asociado a la infección viral de la fiebre aftosa (virus infection-associated: VIA) para detectar los anticuerpos precipitantes en muestras de suero.
provenientes de rumiantes de origen local, no vacunados y criados en distintas regiones de Arabia Saudí. A partir de 5.985 sueros de ovinos, 1.371 de caprinos, 1.052 de bovinos y 694 sueros de otras especies de rumiantes se observó el fenómeno de precipitación, respectivamente, en 1.209 (20%), 127 (9%), 172 (16%) y 38 (3%) de los casos citados. Por otra parte, en 100 sueros que precipitaron en presencia del antígeno VIA y provenientes de 13 zonas distintas del país, se investigó la presencia de anticuerpos neutralizantes naturales contra los cuatro serotipos del virus de la fiebre aftosa (O, A, Asia 1 y C) que prevalecen actualmente en la región y que habían sido integrados en la vacuna utilizada. Todos los sueros sometidos a la investigación presentaron títulos diferentes respecto de los serotipos O, A y/o Asia 1. Sin embargo, ningún suero neutralizó el serotipo C del virus. Los resultados obtenidos se interpretaron en función de la distribución geográfica y de la epizootiología de la fiebre aftosa en Arabia Saudí.


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REFERENCES


