

DETECTION OF FOOT AND MOUTH DISEASE -VIRUS INFECTION ASSOCIATED ANTIBODIES IN SHEEP SERA IN BASRA BY AGID TEST

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ABSTRACT

This study was performed to detect the antibodies against the virus-infection-associated antigen (VIAA) in previously diseased sheep in Basra. The test is valuable in epizootiological surveys because only infected animals with foot and mouth disease virus will give positive reaction.

Seventy five sheep sera were re-tested by AGID test to VIA antibodies from 241 sheep sera examined by ELISA-VIAA test, collected from 13 suspicious infected sheep flocks with FMD from two areas in Basra.

It was found -by AGID test that 56% of the total positive tested sheep sera to ELISA build specific VIA antibodies against FMD virus, and that 96% of the negative ELISA samples were negative to AGID test.

The results indicated that ELISA technique was more sensitive than AGID technique.

INTRODUCTION

Foot and mouth Disease (FMD) is the most contagious disease of the cloven-hoofed animals and has a great potential for causing severe economic losses in susceptible cloven-hoofed animals, of the domestic species, cattle, pigs, sheep, goats and buffalo are susceptible to FMD, in addition many species of cloven- hoofed wildlife such as deer, antelope and wild pigs may become infected (1).

Infection of susceptible animals with FMD virus lead to the appearance of vesicles on the feet, in and around the oral cavity and on the mammary gland in females, the

severity of the clinical signs varies with the strain of virus, the exposure dose, the age and breed of animal, the host species and its degree of immunity (2).

There are seven serotypes of FMD virus namely O, A, C, SAT₁, SAT₂, SAT₃, and Asia₁, infection with any serotype dose not confer immunity against other (3).

In Iraq, the serotypes A, O, and Asia₁ were recorded in years 1952, 1957, and 1975 respectively (4). In the period between the end of 1998 to the beginning of 1999 an sever outbreak of FMD occurred in Iraq, it affected cows, buffalos, sheep and goats and may be other animals, the virus isolated from cow, buffalo, and sheep, the disease is still endemic in Iraq (5). FMD is still prevalent in many parts of the world as emphasized by the 2001 epidemics in the European Union, southern Africa, Asia and South America (6).

(7) reported the finding of third antigenic component associated with infection with Foot and Mouth Disease Virus, called virus infection associated antigen (VIA), which reacted with the sera from convalescent animals but not with sera from vaccinated animals, VIA antigen is specific for FMD but is not virus type-specific, as antiserum to six of the seven virus types gave positive agar gel diffusion precipitin (AGID) reaction with VIA antigen prepared from type A infected tissue culture fluids.

Antibody against VIA was originally demonstrated only in sera from animals infect with FMDV and not in sera from animals immunized with inactivated vaccines (8).

VIA antibodies in sera from animals convalescent with FMD have been detected by Immunodiffusion in agar gel (AGID) test (9).

The antibodies against the VIA antigen were detected between the second week and 13th or 14th week after infection (10).

The detection of antibodies to virus infection associated antigen was done in Iraq by (11), the survey was done on Iraqi cow sera, collected from Mosul slaughter house in Mosul Government. By the Agar Gel Immuno-Diffusion, samples were examined.

Objective of the study

1. To detect a previously infected sheep with Foot and Mouth Disease (FMD).
2. To differentiate in vaccinated flocks between antibodies due to vaccination and antibodies due to infection.
3. To compare between the result of ELISA and AGID tests in the diagnosis of FMD.

MATERIAL AND METHODS

Chemicals: The chemical materials used through the work were manufactured by the following companies; Fluka, Sigma and Serva, Pharmacia, BDH, and Difco.

Blood Samples: The investigated samples comprised 75 serum samples, which re-tested by AGID test from 241 sheep blood samples collected from two areas in Basra governorate during the period from March to June 2004 and tested by ELISA-VIA antigen technique [The ELISA technique for measuring the antibody response was established by chaquar – board titration of the antigen, control sera and conjugate. The procedure for conducting the solid phase ELISA was essentially as described by (12).] in the University of Basra, college of Vet. Medicine in the Central Researches Laboratory. The samples were collected from different location in each area. The sheep were in contact with other species such as cow, buffalo, and goats. The studied herds include some animals suffered from some clinical symptom of FMD as lameness, high fever and presence of some vesicles in the oral cavity and hoof. High mortality rate was observed in lambs.

The age of the studied group ranged from more than 2.5 years to 5.5 years. There was marginal predominance of females, over males with overall females to male's ration of 8.5:1.5.

Antigen: Virus infection associated antigen (VIAA). Obtained from the Pan American Foot – and – Mouth Disease Center Aftosa (PAFMDC) (Brazil) as a VIA in activated antigen – Lot 35 through Dr. S. Hasso.

Control Serum: A positive control obtained from PAFMDC (Brazil) – Lot OE.

Agar Gel Immunodiffusion test Techniques

The following technique describes the methodology utilized by the Pan – American FMD Center (PAFMDC) (OPS/OMS), (13).

1 – Preparation o the 2% Noble agar.

2 – Glycine buffer:

3 – Preparation of the plates:

4 – The Titration:

5- Determination of anti – VIA antibodies in sheep sera:

Petri dishes are prepared, and the antigen used as in the suitable dilution was put in the central wells. The control serum, likewise in the pre – established dilution is placed in two opposite wells on the edge of each mold. The sera to be tested are distributed in the four remaining wells.

The remaining details of the techniques are as indicated in the titration method. The test will be regarded as valid only when the controls yield clear precipitation bands.

Statistical Analysis

The results were analyzed by one-way ANOVA test, using a statistical package for the social sciences (SPSS) version 9.0. All data were expressed as Mean \pm Std. Error. Differences between data were compared by X² test (14).

RESULTS

The Agar Gel Immuno- Diffusion Technique Results:

According to the ELISA test, the results of the tested animals are divided into three groups; the high or strong positive (75 samples), weak positive (98 samples) and negative (68 samples) depending on the OD value. Therefore, we selected samples from the three groups; 25 samples from the strong positive, 25 samples from the weak and 25 samples from the negative. These samples re-tested with the Agar Gel Immunodiffusion technique (AGID) Table (1) shows the result of AGID test as follow:

1. The samples which were selected as a positive gave a positive result at rate (68%) (There was cleared precipitation line) and (32%) give a negative result.
2. There are (44%) of the samples which were selected as a weak positive samples gave a positive result and (56%) gave a negative results.
3. Only one sample of the selective negative samples gave a positive result (4%) while the others (96%) gave a negative result.
4. The total positive rate was (38.6%) of all sample were re-tested with the AGID test.

Table (1): The AGID test result

ELISA Results	AGID test Results		
	Ex. No.	Positive %	Negative %
Strong positive (75 samples)	25	17 (68%)	8 (32%)
Weak positive (98 samples)	25	11 (44%)	14 (56%)
Negative (68samples)	25	1 (4%)	24 (96%)
Total samples	75	29 (38.6)	46 (61.3)

$\chi^2 = 54.069$, $df=2$, $p < 0.01$

Determinations of the optimal VIA antigen dilution used in the AGID test:

The optimal VIA antigen dilution used in the AGID test was (1/2) dilution.

Determinations of the optimal serum dilution used in the AGID test:

The optimal serum dilution used in the AGID test was (1/1) dilution according to the titration method of the Agar Gel Immuno Diffusion technique.

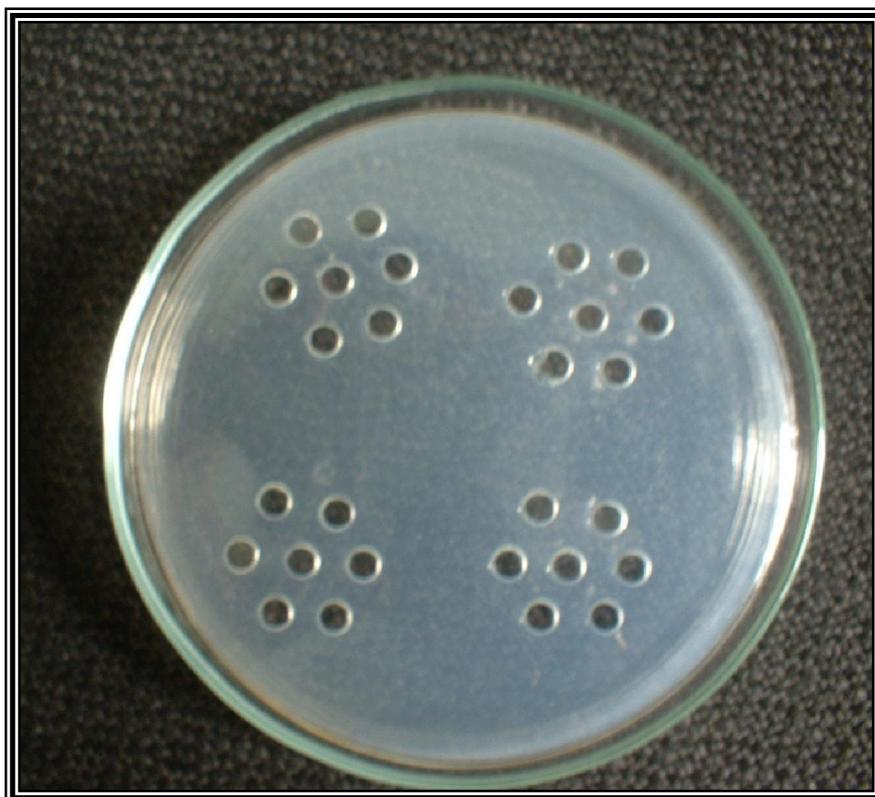


Fig (1): Glass Petri plates measuring 90 mm in diameter in which there are four sets of a seven punch mold used in the AGID test.

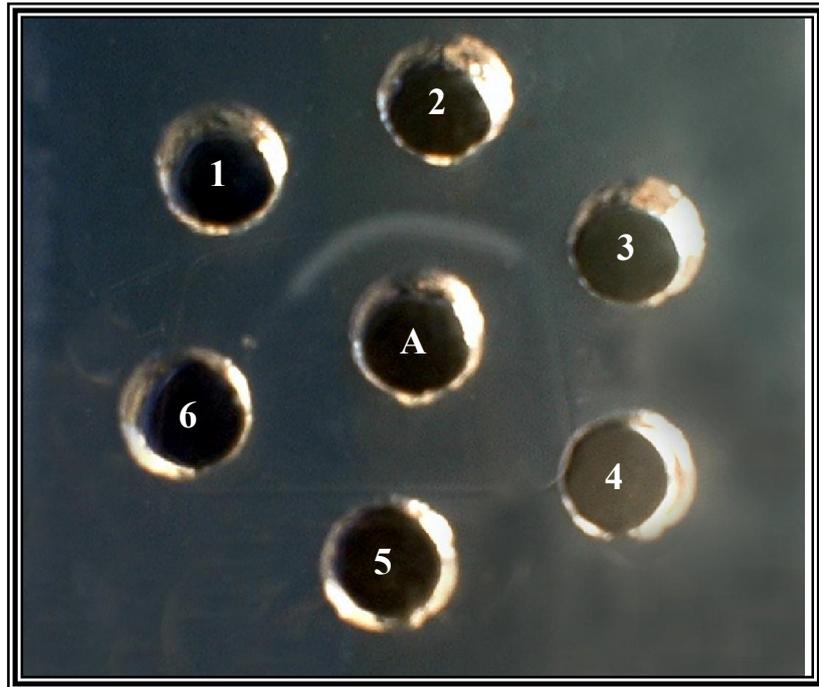


Fig (2): Titration of VIA antigen and control serum.
A = VIA antigen in (1/2) dilution. 1 to 6 = Antigen dilutions 1/1, 1/2, 1/4, 1/8, 1/16 and 1/32 , respectively

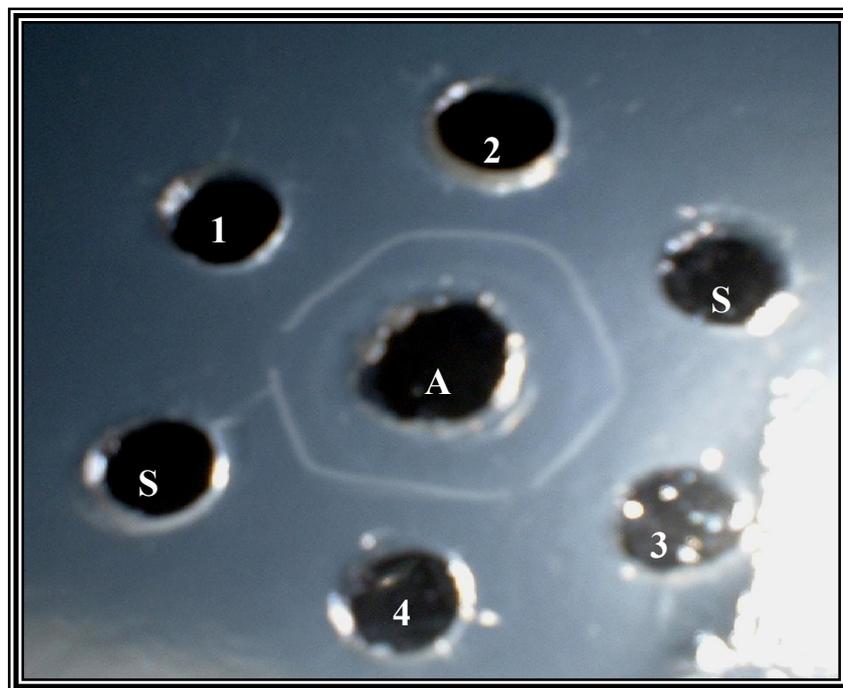


Fig (4): Analysis of sheep sera to identify anti-VIA antibodies by the immuno diffusion agar gel test.
A = VIA antigen in (1/2) dilution.
S = Control serum (VIA positive) in (1/1) dilution.
1 to 4 = Sheep sera under analysis.

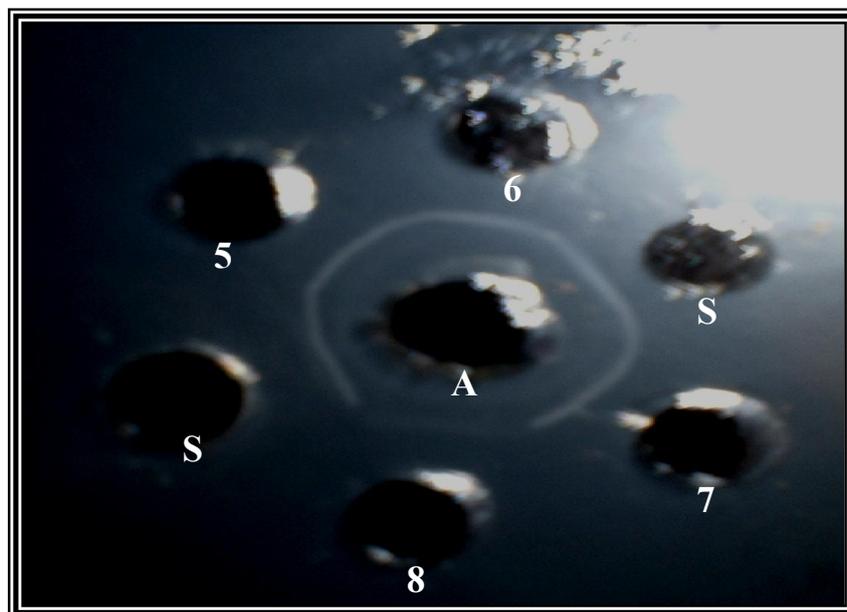


Fig (5): Analysis of sheep sera to identify anti-VIA antibodies by the immuno diffusion agar gel test.

- A = VIA antigen in (1/2) dilution.**
- S = Control serum (VIA positive) in (1/1) dilution.**
- 5 to 8 = Sheep sera under analysis.**

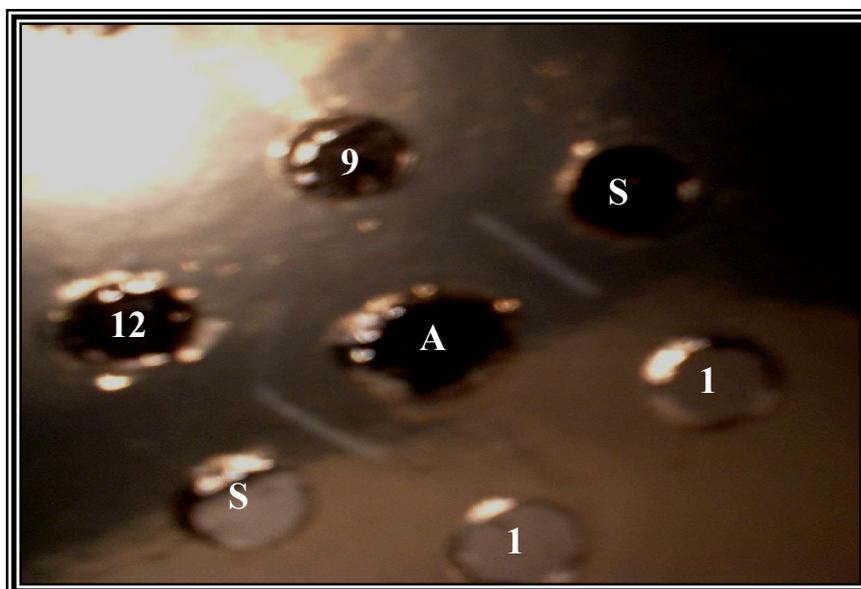


Fig (6): Analysis of sheep sera to identify anti-VIA antibodies by the immuno diffusion agar gel test.

- A = VIA antigen in (1/2) dilution.**
- S = Control serum (VIA positive) in (1/1) dilution.**
- 9 , 10 , 11, 12 = Negative Sheep sera under analysis.**

DISCUSSION

During the end of 1998 and beginning of 1999 an outbreak of FMD disease occurred in Iraq it affected cows, buffalos, sheep, and goats and may be other animals. The virus was isolated from cows, buffalo and sheep (5); the disease is still endemic in Basra.

In this study we conducted a serological survey to detect previously FMD infected sheep in Basra using the virus infection associated antigen employing the agar gel Immunodiffusion test (AGID).

The agar gel Immunodiffusion test was used successfully to detect VIA antibodies to sheep sera; this was on line with the report of (8), (15), (16), (17), and(18).

Nevertheless in this study, the agar gel precipitation technique was less sensitive than the ELISA technique but more specific (Table: 1). The concentration of the VIA antibodies and serum dilutions which were used in AGID were higher than that used in ELISA technique. In ELISA, the antigen dilution was (1/4) and serum dilution was (1/9) while in AGID, the antigen dilution was (1/2) and the serum dilution was (1/1). These results (Table -1) were in agreement with (19) who mentioned that the ELISA test was more sensitive than the AGID test by ten times, and also in agreement with (18) and (20).

Only one sample which was detected by ELISA as a negative sample gave a positive reaction to AGID. This result was not in agreement with (19) and (20) who mentioned that all negative samples also gave negative results by AGID test, such a result occur due to specify of the AGID to the VIA antibodies (8).

التحري عن الأضداد الخاصة بمستضد حمة مرض الحمى القلاعية في أمصال أغنام البصرة بواسطة اختبار الترسيب

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الخلاصة

تكمن أهمية هذه الدراسة في الكشف عن الأضداد المضادة للمستضد المصاحب للخمج بحمة مرض الحمى القلاعية في الاغنام المصابة في البصرة وذلك لما لهذا الاختبار من أهمية في عمليات المسح الوبائي حيث تظهر النتائج الموجبة في الحيوانات المصابة فقط بحمة مرض الحمى القلاعية. تم اعادة فحص ٧٥ عينة من امصال الاغنام بواسطة اختبار الترسيب في الهلام من اصل ٢٤١ عينة دم فحصت باختبار الاليزا للمستضد المصاحب للخمج لحمة مرض الحمى القلاعية والتي جمعت من ١٣ قطيع أغنام مشكوك بأصابتها بالمرض من منطقتين مختلفتين في محافظة البصرة. عند استخدام اختبار الترسيب وجد إن (٥٦%) من مجموع العينات الموجبة المفحوصة احتوت على أضداد مضادة للمستضد المصاحب للخمج بحمة مرض الحمى القلاعية وان (٩٦%) من العينات السالبة المفحوصة أعطت نتائج سالبة. كما أظهرت النتائج أن اختبار الاليزا أكثر حساسية من اختبار الترسيب.

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