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DETECTION AND CHARACTERIZATION OF FOOT AND MOUTH DISEASE VIRUS, SEROTYPE A IN SAUDI ARABIA IN 2005

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SUMMARY

After elapsing of 10 years on the last recorded cases of Foot and Mouth disease (FMD) caused by the virus of serotype A in Saudi Arabia in 1995, new cases of FMD virus, serotype A were diagnosed in two traditional cattle herds in Al-Hota province, 150 Km south of Riyadh. Clinical signs of fever, vesicular stomatitis and coronitis, with morbidity rate of approximately 90% were reported. FMDV, serotype (A) isolates were identified by indirect sandwich ELISA. Vaccine matching tests (virus neutralization and ELISA), amplification and sequencing of theVP1of the isolates revealed that the isolates were antigenically closely related with A Saudi 95, A 4164 and A22 Iraq 24/64 strains, and genetically identical with isolates of A Iran 2005. Fortunately one of these strains (A22 Iraq 24/64) is already incorporated in polyvalent FMD-vaccine formula in Saudi Arabia.

INTRODUCTION

Foot and mouth disease (FMD) is highly contagious viral disease of cloven hoofed animals including cattle, pigs, sheep and goats. It is characterized by vesicle formation in the tongue, nose and feet. It can kill young animals and infection results in severe production losses in livestock particularly in intensively managed dairy cattle and pigs. FMD is a member of the Aphthovirus genus within the family Picornaviridae. It is positive sense SsRNA, naked and icosahedral virus of approximately 30nm in diameter. There are seven immunologically distinct serotypes of virus, namely type O, A, C, SAT1, SAT2, SAT3 and Asia1. The disease may be clinically confused with other vesicular diseases, and coronitis and

laminitis caused by other agents. Conventionally, diagnosis tests of FMD should be rapid , sensitive and specific. The most common and widely used methods for virus identification are currently virus isolation and neutralization in primary cell cultures, Indirect Sandwich - ELISA and Reverse transcription (RT) ñ PCR. Serological diagnosis of FMD could be done by recently developed ELISA for detection of the viral non- structural proteins (3ABC) group specific antibodies, and by serum neutralization and blocking ñ ELISA for detection of the viral structural proteins (VP1) ñ type specific antibodies in sera of convalescent animals (Remond, et al., 2002).

Vaccination against one serotype of FMD virus does not cross-protect against other serotypes and may be also fail to protect fully against other strains of the same serotype. In Saudi Arabia, control of FMD relies predominantly on vaccination. The currently used inactivated vaccines of FMD in Saudi Arabia are, (1) Polyvalent vaccine contains O1 Manisa/68 and O1 BFS , A22 Iraq 24/64, A Iran 96, A Saudi 23/86 , Asia1 (broad spectrum) and SAT2 Saudi 2000, and (2) monovalent vaccine of serotype (O) contains the two previously mentioned strains. More characterization of the field virus should be done by vaccine matching and sequencing. Saudi Arabia is endemic with FMDV, serotype O, (O1, Manisa strain equivalent), and a limitied outbreaks caused by other serotypes were reported from time to time. This seems to be due to contious importation of livestock from many African a Asian countries (Hafez et al., 1994, Abdel Ba et al., 2005).

This paper described the recent FMD cas caused by serotype A in Saudi Arabia.

MATERIALS AND METHODS

A- Specimens of detached tongue epitheliu scraped vesicles of the mouth and saliva were co lected from two traditional cattle herds (5 a mals) located in Al-Hota province (150 Km sou of Riyadh) during November 2005.

B- Blood samples were collected from the conv lescent cattle herds (20 cattle), two sheep her (20 sheep) raised in between them and from oth two herds of sheep (20 sheep) and one herd goats (10 goats) raised in vicinity of cattle her to get serum for serological tests.

C- Laboratory investigation:

Enzyme linked immunosorbent assays (ELISA)

1- Indirect Sandwich (IS)-ELISA: Commerci IS-ELISA test kit produced by the FMD Wor Reference Laboratory (WRL), Pirbright, UK, w used according to the manufacture. The test w developed and validated by Roeder and Le Blan Smith (1987) and Ferris and Dawson., (1988) fo detection and serotyping of FMDV in tissue sam ples and in inoculated cell cultures. Rabbit antise ra specific for different serotypes of FMDV(trap

ping antibodes) passively adsorbed to polystyrene microwells. With the addition of test sample antigen (if present) is trapped by the immobilized antibodies. specific Guinea pig anti-FMDV serotype detecting antibodies are then added which react with the trapped antigen. The bound Guinea pig antibodies are detected by means of the rabbit anti-Guinea pig IgG conjugated to HRPO. With the addition of substrate / chromogen solution, a colour develops with respect to the antigen content of the sample.

2- FMD-3ABC-ELISA: FMD-3ABC-ELISA Kits produced by Bommeli Diagnostic, Liebefeld, Bern, Switzerland was used as described by the manufacture for detection of FMDV-nonstructural proteins (3ABC) antibodies in serum samples to discriminate between infected and vaccinated animals. The test is based upon specific binding between the precoated recombinant FMDV-3ABC viral antigen and the FMDV-3ABC antibodies in tested serum samples. The bound bovine or ovine sera are detected by means of a peroxidase labeled anti-ruminant IgG . With the addition of substrate/chromogen, a color product develops in direct proportion to the antibodies in the sample.

3- Blocking-ELISA: Commercial B- ELISA test kit produced by FMD -WRL, Pirbright, UK was performed as described by Hamblin et al., (1986) and according to manufacture. The test is based upon specific blocking of the FMDV antigen in liquid phase by antibodies in the test serum sample. Rabbit antisera specific for the different serotypes of FMDV are passively adsorbed to polystyrene microwells. After the test serum is allowed to react with the specific FMDV antigen, the test serum/antigen mixture is then transferred to an ELISA plate coated with rabbit antisera. The presence of antibodies to FMDV in the serum samples will result in the formation of immune complex and consequently reduce the amount of free antigen trapped by the immobilized rabbit anitsera. In turn, fewer Guinea pig anti-FMDV detecting antibodies will react in the next incubation steps. After addition of anti-Guinea pig IgG conjugated to peroxidase, then substrate / chromogen solution, a reduction in color development will be observed when compared to controls containing free antigen only.

D-Two detached tongue epithelium samples which given positive results to FMDV, serotype (A) by IS-ELISA were sent to the FMD-WRL, Pirbright. UK. For (1) Vaccine matching by assessment of the antigenic deference between the recent local isolates of the virus and a panel of reference selective Saudi strains and other world strains which incorporated in the formula of polyvalent FMD vaccine in Saudi Arabia and elsewhere in the Middle East . In-vitro virus neutralization test and ELISA were used to determine r_1 value. (r_1 = reciprocal titer of reference

serum against field strain/reciprocal titer of reference serum against the vaccine strain) in accordance with the Standards of the OIE Manual for Diagnostic Test and Vaccine., (2006), and (2) Topotyping by amplification and sequencing of VP1 gene of the recent local isolates of the virus in comparison with many reference Saudi strains and other world (Asian, African and European) strains of the same serotype especially those strains which were isolated recently in Saudi-neighbor-Joining countries. A large sequence database has been used for phylogenetic analysis to aid in recognizing the virus inter-regional spread and monitoring any evolutionary changes which subsequently occur (Knowles et al., 2005 and Kumar et al., 2004).

RESULTS

Epizootiological data:

The reported data revealed that two traditional cattle herds were classically affected with FMD-clinical signs of fever, vesicular stomatitis, vesicular dermatites in coronary band with morbidity rate of approximately 90%, and without mortalities. No clinical signs were observed in herds of sheep and goats raised in between or in vicinity of the affected cattle herds. No history of recent vaccination against FMD in these herds.

Virus detection and typing:

Examination of the detached tongue epithelium, scraped vesicles and saliva samples with indirect sandwich-ELISA revealed 40% positive FMDV serotype A.

Serological results:

60% and 85% of convalescent cattle serum samples were positive to FMDV-nonstructural proteins antibodies 3ABC and FMDV-structural proteins antibodies as detected by FMDV-3ABC ELISA and blocking - ELISA, respectively. Only 25% of serum samples were positive to FMDV structural proteins antibodies in two sheep herds which raised between the affected cattle herds while herds of sheep and goats raised in vicinity of the affected cattle herds were reacted negative to both FMD-3 ABC ELISA and blocking- ELISA, (table) 1.

Determination of r1 value:

r_1 values by virus neutralization (VN) test and ELISA are shown in table (2).

Mean r_1 values of 0.37, 0.26, 0.16, and 0.12 were obtained by VN in comparison to reference strains of A SAU 95, A22 Iraq 24/64, A MAY 97 and A Iran 96, respectively. r1 values by ELISA were > 1.0, >0.95, > 0.88, 0.54 and 0.16 in comparison to reference strains of A SAU 95, A 4164 A22 Iraq 24/64, A 5925 and A MAY 97, respectively.

Sequencing results:

Dandrogram of the VP1 gene nucleotide sequencing of the recent local isolates of the virus (15/2005 and 16/2005) in comparison to group of the reference strains of FMDV, serotype A revealed that the isolates are genetically typical to A Iran 2005 isolates.

Table (1) FMDV, serotype, A infection in two traditional cattle herds, four sheep herds and one goat herd.

Animal spp.	No. of herds	Clinical signs	FMDV, serotype A in tissue samples detected by IS-ELISA	FMDV, antibodies in convelcsant cattle sera and in sheep and goats sera detected by:		
				3ABC-ELISA	B-ELISA	3ABC-ELISA + B-ELISA
Cattle	2	Fever, anoreoxia, vesicular stomatitis, and dermatitis in cronitis with lamness	2/5 40%	12/20 60%	17/20 85%	60%
Sheep(1)	2	No signs		0/20	5 /20 25%	-
Sheep(2)	2	No signs		0/20	0/20	-
Goat	1	No signs		0/10	0/20	-

-/- : No. of positive samples / test samples

(1) Sheep herds raised with the affected cattle herds.

(2) Sheep herds and goats herds raised in vicinity of the affected cattle herds

IS-ELISA: Indirect Sandwich ELISA

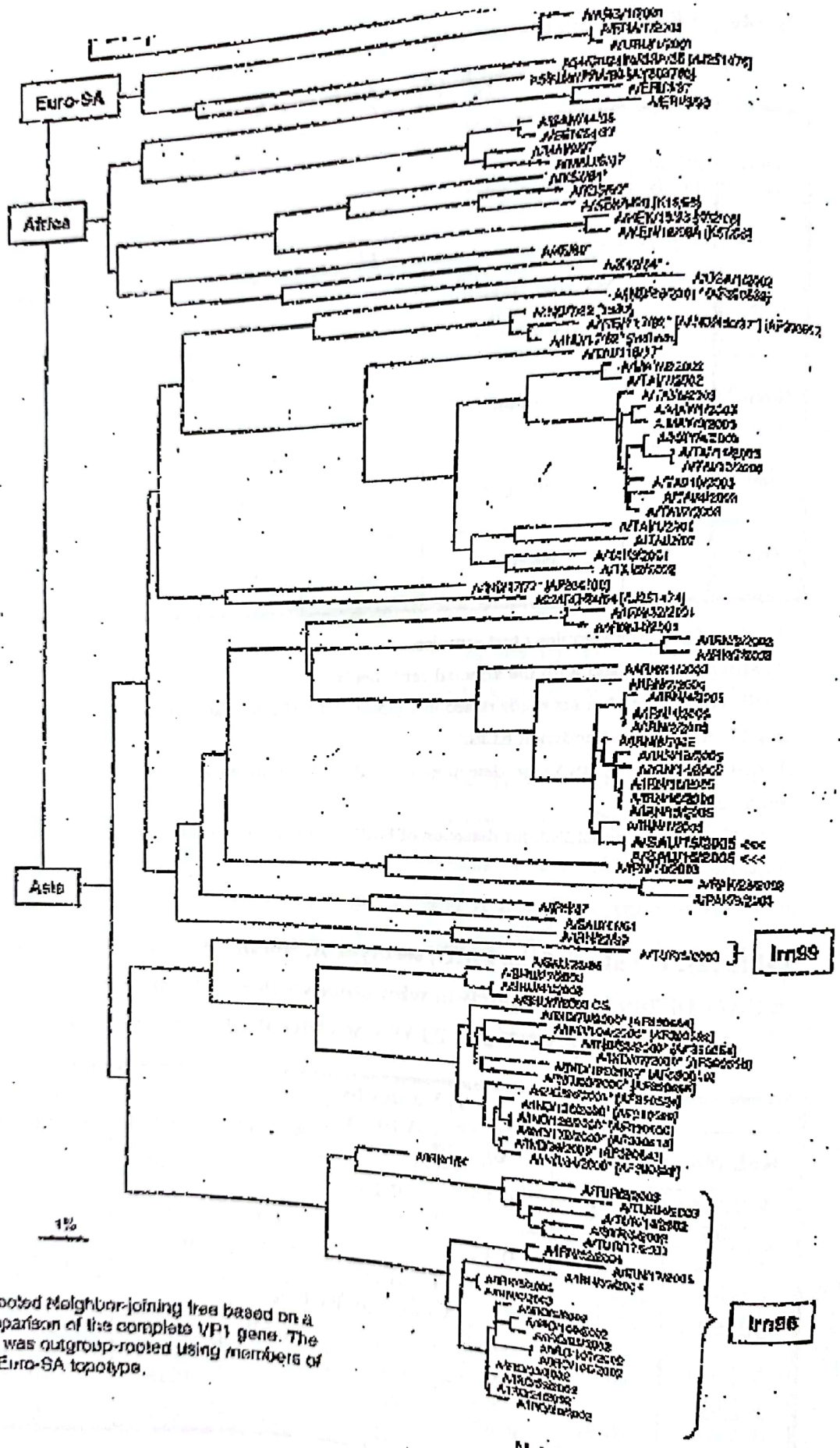
.3 ABC- ELISA: ELISA for detection of FMDV - nonstructural proteins (3ABC)-antibodies

B-ELISA: Blocking ELISA for detection of FMDV - structural proteins - antibodies

- : Negative

Table (2): r_1 value of the FMD, serotype A, isolates A SAU 15/2005 and A SAU 16/2005 in comparison with other strains of the same serotype, as obtained by VNT and ELISA at FMD-WRL, Pirbright, UK

r_1 Values by VNT						
Ref. No	A22	A IRAN 96	A MAY 97	A 4164	A 5925	A SAU 95
A SAU 15/05	0.28	0.12	0.16	Not tested	Not tested	0.35
A SAU 16/05	0.25	0.12	0.17	Not tested	Not tested	0.39
Mean	0.26	0.12	0.16	--	--	0.37
r_1 Values by ELISA						
A SAU 15/05	>0.88	Not tested	0.17	>0.89	0.50	>1.0
A SAU 16/05	>0.88	Not tested	0.15	>1.0	0.58	>1.0
Mean	>0.88	--	0.16	>0.95	0.54	>1.0



Unrooted Neighbor-joining tree based on a comparison of the complete VP1 gene. The tree was outgroup-rooted using members of the Euro-SA topotype.

N.J. Knowles, 20 February 2006

DISCUSSION

Very limited cases of FMDV, serotype (A) infection were reported by November 2005 in two traditional cattle herds in AL-Hota province (150 Km south of Riyadh KSA). The last reported cases of FMDV, serotype A infection were identified at 1995 in cattle, and sheep (Farag et al., 1998b). Saudi Arabia is endemic with serotype (O), O1 Manisa equivalent virus of FMD, and flair-up of the disease caused by other serotypes or subtypes is expected in any time as a result of the following;(1) The continuous introduction of millions of sheep, goats and cattle from many African and Asian countries where different strains of FMDV may be exist, (2)Vaccination against FMD is mainly applied in dairy cattle, while most of the individual (traditional) herds of cattle, sheep and goats are irregularly vaccinated or not vaccinated, and (3) Local quarantine measures are not strictly applied for infected and contact animals.

Typical clinical signs of FMD including high fever and vesicular stomatitis with aneroxia, and vesicular dermatitis in coronary band with lameness were observed in approximately 90% of cattle.

FMDV, serotype A was detected by Indirect Sandwich-ELISA in 40% of the received tissue specimens which collected from the clinically affected cattle. Commercial test kit of IS-ELISA for detection and typing of FMDV manufactured by FMD-WRL, Pirbright , UK is valid to monitor O1 Manisa strain of DMDV at infectivity titre of ≥ 3.8 log10 TCID50 /ml (unpublished data). While 85% of convalescent cattle exhibited positive FMDV, serotype A-antibodies in their serum samples by blocking- ELISA, FMDV, nonstructural proteins- antibodies were detected in 60% of their serum samples by 3ABC-ELISA kit.

On the other hand, no clinical signs of FMD were observed in two herds of sheep raised with the affected herds of cattle. Serological test are particularly useful for the surveillance program during outbreaks to identify silent infection in species like sheep and goats which show little or no signs of disease (Remond et al., 2002). Only 25% of sera collected from these two herds of sheep was reacted positive to FMDV, serotype A antibodies. No positive samples by 3 ABC-ELISA has been found at all. These results supported that the infection of sheep with that virus was likely to be mild. Serological findings indicated that subclinical infection with FMDV in sheep and goats in Saudi Arabia is more common (Farag et al., 1998a). Field sera drawn from two sheep and goats herds raised in vicinity of the affected cattle herds were given negative at all for both Blocking-ELISA and 3 ABC-ELISA. The new virus isolates - vaccine matching test (in-vetro VNT and ELISA) and origin tracing tests (VP1 gene RT-PCR and sequencing) in comparison with other reference isolates of the same serotype originated from district geographical areas (Asian, African and European) (phylogenetic analysis)

were done by FMD-WRL, Pirbright, UK as an essential for disease control. Vaccine matching tests and phylogenetic analysis revealed that the isolated viruses of Saudi 2005 are antigenically closely related to strain A22Iraq24/64 which is ready incorporated in Saudi polyvalent FMD vaccine formula, A SAU95 and A1464; and genetically identical to isolates of A Iran2005. FMDV, serotype A is the most often associated with outbreaks of FMD in Livestock in Iran. Fortunately the current used polyvalent FMD vaccine in Saudi Arabia contains 3 different strains of FMDV, serotype A and no farther cases of FMDV, serotype A has been recorded out side the infected area since that time and until the end of 2007.

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الكشف عن وتوصيف فيروس مرض الحمى القلاعية النوع المصلى (A) فى السعودية العام ٢٠٠٥ م

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بعد مرور عشر سنوات على أخر حالات مسجلة للإصابة بفيروس مرض الحمى القلاعية النوع المصلى (A) فى عام ١٩٩٥ م فى المملكة العربية السعودية تم الكشف عن حالات جديدة للفيروس من نفس النوع (A) فى قطعين تقليديين للأبقار بمحافظة حوطة بنى تميم والتى تبعد حوالى ١٥٠ كم جنوب مدينة الرياض. وقد تم تسجيل أعراض الحمى والتهاب الفم والقدم الحويصلى فى نسبة ٩٠ % منها وتم الكشف عن الفيروس وتحديد نوعه المصلى (A) باستخدام اختبار الاليزا الساندوتشى غير المباشر وذلك بواسطة بمختبر التشخيص البيطرى بالرياض. واجريت على المعزولين اختبارات تماثلها الأنتيجينى مع العديد من عترات الفيروس من نفس النوع منها الداخلية فى تركيبة لقاح الحمى القلاعية المتعدد المستخدم بالمملكة وأخرى مختارة تمثل مناطق عدة من العالم وذلك باستخدام اختبارى تعادل الفيروس والاليزا وتحديد قيم R1 لكل منها مقارنة بغيرها. وكذلك بإجراء اختبار تحديد التسلسل النيوكليوتيدى فى الجين المسؤول عن انتاج بروتين الفيروس VP1 وذلك بمختبر الحمى القلاعية العالمى المرجعى WRL ببربريت بالمملكة المتحدة. وقد وجد ان المعزولين كانا متشابهان أنتيجينياً بدرجة كبيرة مع عترات A Saudi 95 24-64 A22 Iraq, A4104. وكذلك تبين التماثل الجينى التام بين المعزولات والعترات المعزولة فى إيران العام ٢٠٠٥ م.