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Serological Variation of Foot-and-Mouth Disease Virus in Iran (1963-1966)

by

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A study of serological variation of Foot-and-Mouth Disease (FMD) virus is particularly rewarding in a large country where the disease can spread rather freely, more or less unhampered by mass vaccination and sanitary measures, and where a considerable part of the flock owners still lead a nomadic life.

The present communication deals with serological variants of virus types A and O encountered in Iran in the period from November 1963 to May 1966 and the relative merits of complement fixation (CFT) and neutralization tests (NT) in variant detection, which have been a matter of controversy in the last few years.

MATERIALS AND METHODS

Virus strains : All virus strains tested originated from cattle of various breeds, native or foreign. The location of the

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farms where the samples were collected and the time of isolation are as follows :

— A « Teheran », about 20 km south of Teheran, November 1963;
— A « Hayderabad », some 45 km west of Teheran, February 1964;
— A « Khordan », 60-70 km west of Teheran, February 1964.
— A « Shemiran », about 10 km north of Teheran, May 1964.
— A « Razi Institute », 50 km west of Teheran, outbreak in cattle and sheep, June 1964;
— A « Hamadan », Western Iran, June 1964;
— A « Tabriz », North-western Iran, December 1964;
— A « Turkey », supplied from Ankara by Dr. H. C. Girard, January 1965 (identified by Pirbright as A 22);
— A « Tusi », farm near Rey, some 20 km south-east of Teheran, February 1966;
— O « Rey » No. 4, Rey near Teheran, December 1963;
— O « Rey » No. 9, same area, March 1964;
— O « Shiraz », Southern Iran, June 1964;
— O « Meshed », North-eastern Iran, October 1964;
— O « Sepahpoor », some 20 km south of Teheran, December 1965;
— O « Karadj », 40 km West of Teheran, February 1966;
— O « Razi », outbreak among cattle at Razi Institute, May 1966.

Two more strains, obtained from outbreaks near Razi Institute in April and May 1966, proved identical with O « Sepahpoor » and O « Karadj ». They are not listed in the tables.

Of Type SAT 1 we have only one strain (« Shiraz ») in our collection. It is very unlikely that this type is still present in the field.

Guineapig immune sera : Immune sera were obtained from guineapigs infected with guineapig adapted virus from the second to fourth passages and hyperimmunized once with the same virus by pad inoculation of large doses one

month later. The sera were harvested 7 days after hyperimmunization, filtered through Berkefeld N candles and heated at 56° C for 30 minutes before use in CFT and NT.

CF tests : The method described by TRAUB and MÖHLMANN (13) was used with minor modifications. Veronal buffer, *p*H 7.4, containing $CaCl_2$ and $MgSO_4$ was used as diluent. Antigens prepared from vesicular epithelium of cattle were 10 percent extracts in VM 3 (10), from which lactalbumin hydrolysate was omitted. In the majority of the tests, tissue culture (TC) antigens were used. They consisted of TC fluid from the first or second passages of the respective field strains in baby hamster kidney cells (BHK cell line). Such cultures contained VM 3 as maintenance medium, which was developed (11, 10) in an effort to eliminate the phosphate buffer system which damages FMD virus on freezing and thawing. When cytopathic effect (CPE) was complete, they were deep-frozen at — 25 or — 40° C and cleared by centrifugation after thawing. The supernatant fluid represented the antigen. Complement was titrated in the presence of undiluted antigen and heated normal guineapig serum 1 : 10. As a rule, twice the minimal amount effecting complete lysis (2 units) was used in the test. Occasionally, when an antigen was weak, the complement dose was reduced to 1.5 units. In the CF tests recorded in Tables I-IV, the tubes were incubated in a waterbath at 37° C for 30 minutes, for both fixation and lysis. In special tests (Table V), 17 hour fixation at 5° C was used.

Two-fold serial serum dilutions (1 : 10 to 1 : 320 or 1 : 1,280 in tests with over-night fixation) were tested against undiluted antigen. The recorded titres represent the highest serum dilutions giving complete (4+) fixation. The mean fixation obtained by serum dilutions 1 : 10 to 1 : 320 was recorded, reading being done visually without the aid of a spectrophotometer. For the differentiation of variants, however, visual reading is quite adequate.

Neutralization tests : All NT were made in monolayer cultures of calf kidney cells or, exceptionally, in ovine embryo kidney cells (Test No. 2 in Table III). The cells were grown in Hanks medium with 10 percent heated normal bovine serum, which was later replaced by VM 3 as maintenance medium.

Undiluted heated (56° C, 30 minutes) guineapig immune serum and two-fold serial serum dilutions in VM 3 were tested against constant amounts of virus (approximately 1,000 $TCID_{50}$). Virus from the first passage of the respective strains in calf kidney cells was used. Mixtures of equal parts of undiluted or diluted immune serum and virus were incubated for 30 minutes in a waterbath at 37° C and then inoculated in 0.2 ml amounts in test-tube cultures containing 1.8 ml VM 3 as maintenance medium, using 6 tubes per mixture, and CPE was recorded daily for 3 days thereafter. Control cultures contained normal guineapig serum instead of immune serum.

The ND_{50}* of immune sera in the presence of different virus strains were calculated according to the method of Reed and Muench (7). In addition, the mean extent of CPE (3rd day) in cultures covering the entire range of serum dilutions was estimated from the records on individual cultures.

Cross-immunity tests in animals have not yet been carried out.

RESULTS

Serological variation within Type A : Table I gives the results obtained with type A strains isolated between November 1963 and January 1965. It shows that a new type A variant appeared in the Teheran area in 1964, which differed in both CFT and NT from the type A strain (« Teheran ») previously prevalent. Representatives of the new variant are strains A « Khordan » and A « Shemiran », both isolated in May 1964. They are closely related to, or identical with, strain A 22 « Turkey » received later (January 1965). In CF tests, A « Teheran » serum reacted only in low dilutions with these strains, but in high dilution with the homologous antigen. In the opposite direction, the reaction between A « Turkey » serum and A « Teheran » antigen was stronger. Such lack of complete reciprocity is frequently encountered in the differentiation of FMD variants. The reactions obtained with A « Tabriz » serum were intermediate between those of A « Teheran » and A « Turkey ».

An interesting (transition ?) strain is A « Hayderabad » obtained in February 1964 from an outbreak at an animal husbandry institute about 45 km west of Teheran. It has

TABLE I

Complement fixation and neutralization tests with A strains isolated 1963-1965.

ANTIGEN of strain	SERUM	CF test with TC antigen		Neutralization tests	
		4+ titre*	Mean fixation** (percent)	ND_{50}	Mean CPE (percent)
A « Teheran » isolated. Nov. 1963	A « Teheran »	1 : 160	83	1 : 6.7	8
	A « Tabriz »	1 : 40	56	1 : 2.1	13
	A « Turkey »	1 : 40	65	1 : 1.7	28
	Normal g. pig	—	0	—	75
A « Hayderabad » Feb. 1964	A « Teheran »	1 : 80	67	1 : 39.9	3
	A « Tabriz »	1 : 20	40	1 : 1.2	28
	A « Turkey »	1 : 80	81	1 : 45.2	1
	normal g. pig	—	0	—	75
A « Khordan » May 1964	A « Teheran »	1 : 5	19	1 : 3.6	38
	A « Tabriz »	1 : 40	62	1 : 5.8	17
	A « Turkey »	1 : 160	85	1 : 24.0	2
	normal g. pig	—	0	—	87
A « Shemiran » May 1964	A « Teheran »	1 : 10	23	1 : 3.0	12
	A « Tabriz »	1 : 40	56	1 : 25.4	4
	A « Turkey »	1 : 160	83	1 : 45.2	1
	normal g. pig	—	0	—	40
A « Razi » June 1964	A « Teheran »	1 : 5	17	1 : 2.5	35
	A « Tabriz »	1 : 40	62	1 : 13.7	3
	A « Turkey »	1 : 80	69	1 : 11.5	10
	normal g. pig	—	0	—	67
A « Hamadan » June 1964	A « Teheran »	1 : 20	40	1 : 2.2	22
	A « Tabriz »	1 : 80	71	1 : 8.0	11
	A « Turkey »	1 : 80	75	1 : 5.6	14
	normal g. pig	—	0	—	62
A « Tabriz » Dec. 1964	A « Teheran »	1 : 20	40	1 : 2.4	30
	A « Tabriz »	1 : 80	81	1 : 14.2	12
	A « Turkey »	1 : 80	73	1 : 5.3	20
	normal g. pig	—	0	—	64
A « Turkey » Jan. 1965	A « Teheran »	1 : 5	17	1 : 1.2	30
	A « Tabriz »	1 : 40	52	1 : 5.6	8
	A « Turkey »	1 : 160	83	1 : 22.1	1
	normal g. pig	—	0	—	50

* **Highest serum dilution giving 100 percent fixation with undiluted antigen.**

** **Mean fixation by serum diluted 1 : 10 to 1 : 320 in the presence of undiluted**

TABLE II

Comparison of strain A « Tusi » (1966) with A variants previously prevalent

ANTIGEN	SERUM	CF test with TC antigen		CF test with cattle antigen	
		4+ titre of serum	Mean fixation (percent)	4 + titre	Mean fixation (percent)
A « Teheran » Nov. 1963	A « Teheran »	1 : 160	85	1 : 20	64
	A « Tabriz »	1 : 80	69	1 : 10	50
	A « Turkey »	1 : 40	54	1 : 5	26
	A « Tusi ».	1 : 20	49	1 : 5	25
A « Hayderabad » Feb. 1964	A « Teheran »	1 : 40	64	1 : 20	47
	A « Tabriz »	1 : 20	37	1 : 5	22
	A « Turkey »	1 : 80	69	1 : 40	64
	A « Tusi ».	1 : 5	12	>1 : 5	7
A « Tabriz » Dec. 1964	A « Teheran »	1 : 40	52	not done	
	A « Tabriz »	1 : 160	90		
	A « Turkey »	1 : 80	71		
	A « Tusi »	1 : 40	40		
A « Turkey » Jan. 1965	A « Teheran »	1 : 10	24	not done	
	A « Tabriz »	1 : 40	71		
	A « Turkey »	1 : 160	85		
	A « Tusi »	1 : 20	41		
A « Tusi » Feb. 1966	A « Teheran »	1 : 40	58	1 : 10	40
	A « Tabriz »	1 : 160	85	1 : 40	68
	A « Turkey »	1 : 80	73	1 : 20	53
	A « Tusi »	1 : 80	73	1 : 20	55

not been possible, however, to find any correlation between this outbreak and those at Khordan and Shemiran. There is good evidence that the virus was carried to Khordan by nomads. Strain A « Hayderabad » is still under investigation in an effort to determine whether it represents a true variant or a mixture of two strains.

Strain A « Razi » as well as a second A strain isolated from another outbreak at Razi Institute in July 1964 and

not listed in the table gave similar reactions in CFT as those from Khordan, Shemiran and Turkey. The NT, however, indicated a somewhat closer relationship of the first strain to A « Tabriz ». Strains A « Hamadan » and A « Tabriz » gave almost identical reactions in both tests.

Table II presents data on strain A « Tusi » isolated in February 1966 and which is still prevalent in the Teheran area. This strain was compared in CFT with A « Teheran »,

TABLE III

Complement fixation and neutralization tests with O strains.

Test No.	Antigen of strain	Serum	CF tests with TC antigen		Neutralization tests	
			4+titre of serum	Mean fixation (percent)	ND_{50}	Mean CPE (percent)
1	O « Rey » No. 4 Dec. 1963	O « Rey » No. 4	1 : 80	67	1 : 71.1	6 (60)*
	O « Rey » No. 9 Mar. 1964	dtto.	1 : 80	73	1 : 50.8	5 (60)*
	O « Shiraz » June 1964	dtto.	1 : 80	73	1 : 128	0.2 (48)*
	O « Meshed » Oct. 1964	dtto.	1 : 80	73	<1 : 128	0 (43)*
2	O « Sepahpoor » Dec. 1965	O « Rey » No. 4	1 : 20	45	1 : 3.0	16
		O « Sepahpoor »	1 : 40	64	1 : 4.8	10
		Normal g. pig	—	0	—	44
	O « Karadj » Feb. 1966	O « Rey » No. 4	1 : 20	45	1 : 2.8	17
		O « Sepahpoor »	1 : 40	62	1 : 6.2	13
		Normal g. pig .	—	0	—	42
	O « Rey » No. 4 Dec. 1963	O « Rey » No. 4	1 : 40	60	1 : 5.3	45
		O « Sepahpoor »	1 : 40	59	1 : 1.3	55
		Normal g. pig	—	0	—	100

* Mean CPE in control cultures containing normal serum.

A « Hayderabad », A « Tabriz » and A « Turkey » using TC and, where still available, cattle antigens. The data, as a whole, indicates that A « Tusi » is more closely related to A « Tabriz » than to the other strains tested. The results obtained with TC and cattle antigens are in good agreement.

Serological behaviour of different O strains : As Table III shows, O strains isolated from December 1963 to October 1964 gave uniformly strong reactions in CFT with immune serum prepared with strain O « Rey » No. 4. The same serum neutralized the other virus strains about as well or even better than the homologous strain. The higher serum titres obtained in NT in the presence of O « Shiraz » and O « Meshed » can be explained by the lower cytopathogenicity of these strains as evidenced by the mean CPE in the controls. There is therefore no indication of serological variation within Type O during the period mentioned.

On the other hand, O strains isolated in December 1965 and in February 1966 (see Test 2, Table III) differed slightly from strain O « Rey » No. 4. In the CFT, antiserum O « Rey » had a lower titre (1 : 20) in the presence of antigens O « Sepahpoor » and O « Karadj » than in the presence of the homologous antigen, but antiserum O « Sepahpoor » reacted equally well (1 : 40) with its own antigen and antigen O « Rey ». Comparative CFT made with two more strains from the same epizootic gave exactly the same results as strains O « Sepahpoor » and O « Karadj ». Strain differences are also evident from the results of the NT.

A CFT carried out with the same sera and cattle antigens (see Table IV) confirmed the results obtained with TC antigens. There can be little doubt, therefore, that slight serological variation occurred also within type O.

Influence of overnight fixation in the cold : Since speed counts in the routine typing of field samples of FMD Virus, the fixation period in CFT is usually kept as short as possible. Weak antigens, however, will sometimes not give conclusive results under such conditions. This is particularly the case with TC antigens made with unadapted virus strains of low cytopathogenicity. It is possible to intensify complement fixation considerably by overnight fixation in the cold instead of incubation at 37° C for 30 minutes. However, the reactions tend to become less type-specific, using overnight

fixation. Different types of FMD Virus share a common antigen which is often not recognizable with short fixation but may become troublesome when overnight fixation is practised. It is possible to control this by appropriate quantitative tests.

Table V gives as an example the result of a CFT made with cattle and TC antigen of A « Hayderabad » comparing fixation for 30 minutes at 37° C with fixation for 17 hours in the cold room at 5° C. The reactions with antisera A « Teheran », A « Tabriz » and A « Turkey » were intensified by overnight fixation and the antigenic character peculiar to the virus strain under study was retained. In this case, there were no marked cross-reactions with type O and SAT 1 antisera as they occurred in other tests with overnight fixation. Usually, non-specific cross-reactions between types A, O and SAT 1 are missing or, at the most, very slight even with highly potent sera when short fixation is

TABLE IV

CF test with O strains comparing TC and cattle antigens.

ANTIGEN	SERUM	TC ANTIGEN		CATTLE ANTIGEN	
		4 + titre of serum	Mean fixation (per cent)	4 + titre	Mean fixation (per cent)
O « Rey » No. 9 Mar. 1964.	O « Rey » No. 4 O « Sepahpoor »	1 : 40 1 : 40	51 54	1 : 20 1 : 10 (3.5 + 1 : 20 and 1 : 40)	47 50
O « Sepahpoor» Dec. 1965	O « Rey » No. 4 O « Sepahpoor »	1 : 20 1 : 40	49 66	1 : 20 1 : 40	60 73
O « Karadj » Feb. 1966	O « Rey » No. 4 O « Sepahpoor »	1 : 40 1 : 40 (3.5 + 1 : 80)	52 64	1 : 20 1 : 40	45 61
O « Razi » April 1966	O « Rey » No. 4 O « Sepahpoor »	1 : 40 1 : 40 (3.5+1 : 80)	54 61	1 : 20 1 : 40	45 64

TABLE V

Comparison of short fixation (30 min. at 37° C). *with overnight fixation* (17 *hrs at* 5° C).

Antigen : A « Hayderabad », first passage in BHK cells.

FIXATION	SERUM (tested in dilutions 1 : 10 to 1 : 320)	ANTIGEN dilution	4 + titre of serum	Mean fixation (percent)
30 min. at 37°C	A « Teheran »	undil. 1 : 2 1 : 4	1 : 40 —* —	62 5 0
	A « Tabriz »	u. 1 : 2 1 : 4	1 : 20 1 : 10 —	34 28 2
	A « Turkey »	u. 1 : 2 1 : 4	1 : 40 1 : 20 —	54 52 10
	O « Rey » No. 4 ..... SAT 1 « Shiraz » ..... normal g. pig.	u. u. u.	— — —	0 0 0
17 hrs. at 5° C	A « Teheran »	u. 1 : 2 1 : 4 1 : 8	1 : 160 1 : 160 — —	84 81 8 0
	A « Tabriz »	u. 1 : 2 1 : 4 1 : 8	1 : 40 1 : 40 — —	66 62 37 3
	A « Turkey »	u. 1 : 2 1 : 4 1 : 8	1 : 80 1 : 80 1 : 80 —	75 81 75 4
	O « Rey » No. 4	u. 1 : 2	— —	1 0
	SAT 1 « Shiraz »	u. 1 : 2	— —	0 0
	Normal g. pig	u.	—	0

practised. With overnight fixation, they are often more marked in a 1 : 10 serum dilution but fade out rapidly in higher dilutions.

Table VI gives data on cross-reactions observed in CFT with a number of antisera of types A, O and SAT 1 when tested with BHK antigens, using short and overnight fixation. The latter procedure markedly increased the serum titres but also led to a moderate increase of the non-specific cross-reactions with antigens of other types.

Prozone phenomena, that is, negative or weak reactions in low serum dilutions and stronger ones in higher dilutions are less frequent with overnight fixation when undiluted TC antigen is used.

TABLE VI

Cross-reaction with other virus types of guineapig immune sera tested with short and overnight fixation.

IMMUNE sera	No. tested*	Fixation for 15 min. at 37° C				Fixation for 17 hrs. at 5° C			
		mean 4+ titre	cross-reaction + with			mean 4+ titre	cross-reaction + with		
			A	O	SAT 1		A	O	SAT 1
A « Teheran »...	9	1 : 68		0	0	1 : 218		20	0
A « Tabriz ». ...	6	1 : 86		0	0	1 : 168		13	0
A « Turkey ». ..	8	1 : 113		7	1	1 : 415		45	7
O « Rey » No. 4.	9	1 : 112	4		0	1 : 508	71		28
O « Sepahpoor »	5	1 : 11	0		0	1 : 184	24		12
SAT 1 « Shiraz »	4	1 : 22	1	1		1 : 380	14	23	

* Sera from single guineapigs or pools from several animals immunized in the same manner.

\+ Mean fixation by serum dilution 1 : 10 (percent).

DISCUSSION

The early appearance in Iran of a variant strain serologically identical with A « Turkey » leaves the possibility open that the virus recently designated as « Near East A » (A 22), was the causative agent of an epizootic of large proportions involving many countries. Type A virus isolated in 1966 differed slightly from A « Turkey » in crosswise CFT.

Our results confirm the previous observation (14, 12) that serological variation is more marked within type A than within type O of FMD Virus.

It has recently been postulated (18, 17) that cross-neutralization tests give a clearer picture of the antigenic character of variants of FMD Virus than do cross CF tests. The results recorded in Tables I and III do not support this conclusion. There was agreement between both tests with regard to A strains (Table I). A concrete example is strain « Hayderabad », which reacted strongly in both tests with antisera A « Teheran » and A « Turkey », but gave weaker reactions with serum A « Tabriz ». Slight serological variation within type O could also be recognized with both methods (see Test 2 in Table III). Our data confirm those of GALLOWAY, HENDERSON and BROOKSBY (3) and BROOKSBY (1) who reported good agreement between the results of CFT and NT in variant detection. Since the antigens and antibodies involved in CFT and NT are not strictly the same, quantitatively identical results cannot be anticipated.

The importance of serological variants or subtypes for vaccination has been a matter of controversy for years. In quantitative vaccination and cross-immunity tests carried out by DINTER and TRAUB (2) in guineapigs, serological strain differences were clearly reflected in the vaccination results. Strain differences within types of FMD Virus and a correlation with immunization were also reported by GIRARD and MACKOWIAK (4), UBERTINI (15) and SCHNEIDER (9). Some investigators, however, considered the antigenic potency of virus strains more important for vaccination than minor serological differences (4, 16). On the other hand, MÖHLMANN (6) and RÖHRER, MÖHLMANN and PYL (8) doubted the significance of variants for vaccination in the field. More recent work by HYSLOP, DAVIE and CARTER (5) is of much value for the settlement of this controversial problem. These authors compared

different variant strains of SAT 1 in CF and neutralization tests as well as quantitative vaccination experiments in large numbers of cattle, which filled a long-existing gap. The results of all three tests were in agreement and the significance of serological strain differences for vaccination was amply demonstrated. It is therefore still the safest procedure for vaccine manufacturers to use the virus actually present in the field at a given time as the production strain.

It has been known for some time that serological strain differences within types of FMD Virus are often more or less one-sided. This phenomenon has been observed also in the present work. It can be seen in Table I that, in CF tests, antiserum A « Turkey » reacted in higher dilutions with antigen A « Teheran » than did serum A « Teheran » with antigens A « Turkey », A « Khordan » and A « Shemiran », which are closely related or identical strains. Table III shows that antiserum O « Rey » No. 4 reacted in higher dilution with the homologous antigen than with antigens O « Sepahpoor » and O « Karadj » from the 1966 epizootic, while serum O « Sepahpoor » reacted equally well with antigen O « Rey » as with homologous antigen. This has been confirmed by several other tests not recorded in the table. Type O strains isolated in 1966 uniformly showed the same behaviour. Conclusions as to « one-sidedness » cannot be drawn from the neutralization tests, since these could not be carried out at the same time and under exactly the same conditions. It has been our experience that serum titres in NT depend to some extent on the susceptibility of the cells as evidenced by the extent of CPE in the controls. There are great differences in this respect between cells originating from different animals of the same species. An attempt was made to circumvent this difficulty by the use of the BHK cell line, but this was unsuitable for type A because normal guineapig serum completely inhibited CPE in these cells. This was not so with type O.

Overnight fixation in the cold is useful in CF tests for the demonstration of small amounts of antibody or antigen, which would escape detection when short fixation for 30 minutes at 37° C is practised. It may also help in typing A variants, which sometimes differ so much from known A strains that a new virus type might be suspected. In general, however, short fixation is preferable for the study

and classification of variants or subtypes because it brings out strain differences more clearly. A common complement fixing antigen which different types of FMD Virus obviously share is often not recognizable by short fixation but can be demonstrated more readily by overnight fixation.

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SUMMARY

Type A strains isolated in Iran from January 1963 to February 1966 showed considerable serological differences. A variant probably identical with « Near East A », the causative agent of a recent epizootic of great proportions, appeared in the Teheran area in May 1964. Type O strains isolated late in 1963 and in 1964 differed slightly from O samples obtained late in 1965 and in 1966.

These strain differences were apparent in both cross CF and neutralization tests, both of which are suitable for the detection of variants or subtypes. The CFT again proved its value for rapid screening of field strains. Cattle and tissue culture antigens gave identical results.

The relative merits in CF tests of fixation for 30 minutes at 37° C and 17 hours at 5° C are discussed.

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RÉSUMÉ

On trouva des différences sérologiques considérables entre des souches appartenant au type A du virus aphteux qui furent isolées en Iran de janvier 1963 à février 1966. Une variante probablement identique à la variante « A Moyen Orient », agent causal d'une épizootie récente qui prit de grandes proportions, apparut dans la région de Téhéran en mai 1964. Des souches de type O, isolées fin 1963 et en 1964, différaient légèrement des prélèvements de type O isolés fin 1965 et en 1966.

Ces différences entre les souches apparurent à la fois dans les épreuves croisées de fixation du complément et de neutralisation qui conviennent toutes les deux à la détection des

variantes ou sous-types. L'épreuve de fixation du complément confirma sa valeur pour le dépistage rapide des souches trouvées sur le terrain. Les antigènes obtenus sur bovins et en cultures de tissus donnèrent des résultats identiques.

Les mérites relatifs des épreuves de fixation du complément, réalisées pendant 30 minutes à 37° C et pendant 17 heures à 5° C sont discutés.

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RESUMEN

Se encontraron considerables diferencias serológicas entre cepas que pertenecían al tipo A del virus aftoso aisladas en Irán en el período comprendido entre enero de 1963 y febrero de 1966. Una variante, probablemente idéntica a la variante « A Medio Oriente », agente causal de una epizootia reciente que adquirió grandes proporciones, apareció en la región de Teherán en mayo de 1964. Cepas de tipo O, aisladas a finales de 1963 y en 1964, diferían ligeramente de las muestras de tipo O aisladas a finales de 1965 y en 1966.

Dichas diferencias entre las cepas se pusieron de manifiesto a la vez en las pruebas cruzadas de fijación del complemento y de neutralización que se emplean para la determinación de variantes o subtipos. La prueba de fijación del complemento confirmó su valor para el reconocimiento rápido de las cepas encontradas en el campo. Los antígenos conseguidos en bovinos y en cultivos de tejidos proporcionaron idénticos resultados.

Se discuten las ventajas relativas a las pruebas de fijación del complemento realizadas durante 30 minutos a 37° C y durante 17 horas a 5° C.

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