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Indirect Complement Fixation in Foot–and–Mouth Disease (*)

I. Study of Antibody Response in Experimental Cattle and Sheep

By

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Introduction

Demonstration of antibodies against *foot-and-mouth disease virus* (FMDV) in cattle serum by direct complement fixation (CF) is rarely successful (8, 6). As far as we know, only MARUCCI (2) reported good results using as antigen bovine vesicular lymph, which is hard to obtain in large amounts and costly. Moreover, antibodies demonstrable by direct CF often persist only for limited periods after convalescence.

Indirect CF tests (ICFT) were successful in diseases of chickens (5, 10, 1). The method was later applied to FMD by PALACIOS and RODRIGUEZ (3) and by RICE and BROOKSBY (6), who reported encouraging results in 1953. The test appeared to be type-specific but ,for unknown reasons, has obviously not established itself as a routine diagnostic procedure.

In countries where foot-and-mouth disease (FMD) is endemic or large-scale vaccination is practised, there is need for a simple and rapid screening test for detecting susceptible animals and for epidemiological studies. Neutralization tests (NT) are cumbersome and their results not always easy to interpret. For these reasons, indirect CF was reinvestigated in the hope that it might be of practical value under certain conditions.

This communication reports results of a study of antibody response in cattle infected in different ways and in vaccinated cattle and sheep, using ICFT and NT comparatively.

Materials and Methods

Bovine serums

The bulk of the bovine serums used originated from cattle 6 to 18 months old, in which tissue-culture passage strains of Type A or O, more or less attenuated for cattle (7), were tested for inocuity and immunizing power. The strains will not be described here in detail. For every animal, one or two pre-inoculation (N) serums, free from neutralizing

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antibodies against the respective virus type, were available. Post-inoculations (PI) serums were obtained 2 to 3 weeks after inoculation and post-challenge (PC) serums 2 weeks after intralingual (i. l.) challenge (for exceptions see tables). Animals infected by contact were bled 3 weeks after onset of the disease. From them, normal serums were available as well.

Bovine serums for A «Turkey», serologically identical with «Middle East A» (A 22 Pirbright), were obtained through the courtesy of Dr. M. Giraud, Institut Français de la Fièvre Aphteuse. They originated from Iranian cattle vaccinated with monovalent «A Middle East» vaccine (Frenkel method).

Ovine serums

Normal and post-vaccination (PV) serums were collected from 14 adult sheep vaccinated twice with formalinized aluminum hydroxide adsorbate vaccine, either non-concentrated or concentrated 4-fold by removal of 75% of the supernatant fluid after low-speed centrifugation. The vaccines were prepared from monolayers of ovine embryo kidney (OEK) cells infected with unmodified Type O virus.

Seven sheep received two subcutaneous inoculations, each with 2 ml. of non-concentrated vaccine at an interval of 59 days, and 7 sheep were treated in the same manner with concentrated vaccine. Normal serum from every animal had failed to neutralize Type O virus. PV serums were taken on day 21 after first inoculation (PV1) and on day 10 after booster (PV 2).

Heating of serum

To decrease anti-complementary effects, both bovine and ovine serums were heated at 58 C. for 60 minutes before use. Such heating did not affect antibody titers. Guinea pig immune (indicator) serums were heated at 56 C. for 30 minutes.

Antigens used in ICFT

At first, virus concentrates prepared with a SPINCO Model L ultracentrifuge from cell-free culture fluid of OEK or BHK-21 monolayers were used as antigens. Antigen concentration was adjusted to give 100% fixation in a dilution of 1:4 with homotypic guinea pig serum (GIPS). Later, this antigen titer was readily reached by non-concentrated culture fluid from well-grown BHK monolayers infected with Types A or O and incubated for 2 days after infection. The cells were grown in Blake bottles with Hanks medium, which was replaced by VM 3 before infection. This maintenance medium allows repeated freezing and thawing of culture virus and has the following composition (gms./1.): NaCl-80; KCl-0.3; $CaCl_2 . 2 H_2O$-0.24; $MgCl_2 . 6H_2O$-0.2; $NaHCO_3$-2.0; glucose-1.8; lactalbumin hydrolysate-5.0; phenol red water-soluble-0.005; penicillin-200,000 units; streptomycin-0.1.

Since Type SAT 1 is less cytopathogenic for BHK cells than Types A and O, the maximum antigen titers were 1:3 or 1:2 only. In cultures showing marked CPE, detached cells were sedimented by centrifugation and resuspended in a small portion of the supernatant fluid. Concentrated cell suspensions were frozen and thawed twice and thereafter cleared by centrifugation. Their 4 + titer was adjusted by dilution to 1:4. This procedure eliminated ultracentrifugation and provided suitable antigens.

Procedure in ICFT

Mixtures of equal amounts of heated bovine (BS) or ovine serum (OS) and undiluted antigen of Types A, O and SAT 1 were kept at 37 C. for 60 minutes (6). Guinea pig complement, titrated in the presence of every mixture plus inactivated normal guinea pig serum 1:10, was used in small excess (1.1 to 1.2 units) in the test. Veronal buffer, pH 7.4, containing CaCl2 and MgSO4, served as diluent for all reagents. Mixtures were first tested undiluted against type-specific GPIS 1:10 or 1:20.

Free antigen, not neutralized by serum under test, was then titrated as shown in Table 1. Results were read visually, estimating the amount of fixation effected by individual antigen dilutions 1:1.5 to 1:8. Mean fixation values represent arithmetical means of fixation percentages estimated for individual antigen dilutions. This procedure is called «antigen titration» in the tables.

Serum titrations were carried out when undiluted BS under test neutralized undiluted antigen completely or partially. Such serums were tested in dilutions 1:2 to 1:128 against undiluted antigen, incubating the mixtures at 37° C. for 60 minutes Incubated mixtures were tested against homotypic GPIS. Mean fiaxtion values were calculated for undiluted BS plus dilutions 1:2 to 1:128.

Table 1

Detailed results of ICFT showing procedure and type-specificity of test

Undiluted mixture of BS and antigen					Titration of free antigen in mixtures against homotypic GPIS							BS titration against undiluted antigen using homotypic GPIS as indicator							
BS	Antigen	with GPIS 1:10	A. C. 1	A. C. 2	Dilution of mixtures 1:1.5	1:2	1:3	1:4	1:6	1:8	MF † %	BS dilutions 1:2	1:4	1:8	1:16	1:32	1:64	1:128	MF †† %
4759	A	0	0	100							0	0	6	12	37	87	100	100	43
C	O	100	0	100	100	87	37	12	6	0	49								100
Type A	SAT 1	100	0	100	100	87	12	12	6	0	45								100
2813	A	100*	0	100	100*	100	37	37	12	12	57								100
C	O	0	0	100							0	0*	6	37	75	100	100	100	52
Type O	SAT 1	100	0	100	87*	62	25	25	6	6	44								100
2840	A	100	0	100	100	100	75	50	12	12	64								100
N	O	100	0	100	100	100	100	75	25	12	73								100
	SAT 1	100	0	100	100	62	50	25	12	6	51								100

* estimated percentage of unlysed sheep erythrocytes

\+ mean fixation by undiluted mixture and dilutions 1:1.5 to 1:8

\++ mean fixation in presence of undiluted BS and BS dilutions 1:2 to 1:128

Other abbreviations in this and following tables:

BS = bovine serum
N = normal
C = convalescent
GPIS = homotypic guinea pig immune serum
A. C. 1 = antigen control with complement
A. C. 2 = antigen control without complement

Serum controls not recorded in table.

Neutralization indices and ND50 values were calculated according to the method of REED and MUENCH (4).

Neutralization tests

Neutralization tests with cattle serums were carried out in monolayers of OEK cells according to the constant serum-virus dilution method using undiluted heated serum and unmodified virus from passages 1 to 3 in OEK cells.

Since the experiment with sheep (Table 9) primarily served another purpose, sheep serums were tested in OEK cells according to the constant virus (about 1000 TCID50)-serum dilution method as described elsewhere (9).

Results

Antibody response in convalescent cattle

Cases of this sort are recorded in Table 1, which shows an ICFT in detail, and in Table 2. The serum donors were free from neutralizing antibodies against Types A and O at the start of the experiments. They were not tested for antibodies against Type

Table 2

Antibody response in cattle to contact infection with Type O

Cattle No.	Serum	NI +		Antigen titration *			Serum titration **		
		A	O	A	O	SAT 1	A	O	SAT 1
44	N	-0.2	-1.0	53	57	54	100	100	100
	C	0.2	>3.7	44	0	71	100	55	100
2813	N	-0.1	-1.2	53	61	61	100	100	100
	C	0.7	>3.7	48	0	52	100	30	100
2817	N	-1.1	-1.2	50	29	48	100	98	100
	C	0.7	>3.5	56	0	66	100	41	100
2811	N	-0.1	-1.0	68	54	79	100	100	100
	C	1.2	>3.5	56	0	79	100	55	100
2816	N	-0.2	-0.2	49	57	46	100	100	100
	C	1.7	>3.7	6	0	12	94	43	97
2836	N	-0.2	-1.0	65	40	67	100	100	100
	C	0.7	>3.5	67	0	74	100	35	100
2820	N	-0.7	-1.2	62	42	79	100	100	100
	C	0	>3.7	51	0	55	100	39	100

* figures in this and following tables indicate mean fixation (%) by undiluted BS — antigen mixture and dilutions 1 : 1.5 to 1 : 8

** mean fixation (%) by mixtures of undiluted antigen and BS (undiluted to 1 : 128)

\+ neutralization index

SAT 1 because this virus type disappeared from Iran in 1964. Normal serums recorded in Table 2 originated from the same animals as the convalescent serums. It is evident from both tables that the reaction of convalescent serum is essentially type-specific. The only exception is No. 2816 in Table 2, which gave moderate cross-reactions with A and SAT 1 antigens in ICF and with Type A in NT. In all cases, Type O antigen was completely neutralized by convalescent serum in ICFT and mean fixation with O antigen was considerably lower in the serum titration.

Antibody response in cattle inoculated intralingually with tissue-culture passage strains of Types A and O

Since our virus strains subjected to serial passage in different kinds of cells (7) vary considerably in degree of attenuation and antigenicity for cattle, it was possible to obtain sera of widely differing antibody content for comparative ICFT and NT.

Sera obtained from cattle inoculated with passage strains of Type A are listed in Table 3. From every animal, samples of normal, post-inoculation and post-challenge serum were available. N serums were free of antibodies against Types A and O. Control 4794 was bled on days 3, 6, 10 and 14 after challenge to determine when antibodies demonstrable by either test appeared in the serum.

It can be seen in Table 3 that both tests were about equally sensitive with regard

Table 3

Antibody response in cattle inoculated intralingually with tissue-culture passage strains of Type A

Cattle No.	Passage virus and passage No.	Reaction PI *	Reaction PC **	Serum	NT A	NT O	Antigen titration A	Antigen titration O	Antigen titration SAT 1	Serum titration A	Serum titration O	Serum titration SAT 1
4791	I BHK 364	-	+	N	0.2	-0.2	62	54	62	100	100	100
				PI *	1.2		47	48	79	95	100	100
				PC **	>4.7	0.3	0	23	70	13	97	100
4723	II BHK 359	-	+	N	0	0.2	53	51	45	100	100	100
				PI	1.5	0.2	8	27	33	86	94	97
				PC	>5.2	0.8	0	15	48	5	97	100
4797	HL - OEK 134	+	-	N	-0.7	0	65	60	59	100	100	100
				PI	2.7		0	35	79	65	100	100
				PC	>4.7		0	46	75	59	100	100
4755	BK - OEK 243	-	+	N	0.2	0	73	63	68	100	100	100
				PI	1.7		21	62	73	91	100	100
				PC	>5.2		0	28	82	38	100	100
4760	BEL - OEK 243	+ +	-	N	0.2	0.2	66	63	36	100	100	100
				PI	3.9		0	29	46	63	100	100
				PC	>5.4		0	26	53	52	100	100
4795	LT - OEK 205	+	-	N	0.2	0.2	72	70	80	100	100	100
				PI	>5.0	0.8	0	35	77	59	100	100
				PC	>5.0	0.2	0	21	75	41	100	100
4796	BELE - OEK 219	+	-	N	0	0	60	63	63	100	100	100
				PI	2.0		1	60	37	80	100	100
				PC	>4.5		0	54	43	42	100	100
4794	Control for i. l. challenge		+ +	N	-0.8	-0.2	64	75	66	100	100	100
				PC 3†	0		62	58	70	100	100	100
				PC 6	3.8		4	30	68	90	98	100
				PC 10	3.2		4	49	47	87	100	100
				PC 14	>4.7		0	40	69	54	100	100

* post-inoculation
** post-challenge
† serum taken on 3rd day after challenge
− = no reaction
+ = primary lingual vesicle without generalization
++ = generalized FMD

Table 4

Antibody response in cattle inoculated intralingually with tissue-culture passage strains of Type O

Cattle No.	Passage virus and passage No.	Reaction PI	Reaction PC	Serum	NI A	NI O	Antigen titration A	Antigen titration O	Antigen titration SAT 1	Serum titration A	Serum titration O	Serum titration SAT 1
45	BHK 350	-	+	N	-0.2	-0.5	66	59	57	100	100	100
				PI	0	0	52	67	60	100	100	100
				PC	0.3	>3.5	17	0	29	97	43	100
43	HL-OEK 150	-	+	N	-0.2	-1.2	29	66	44	99	100	100
				PI		-0.2	16	7	41	98	89	100
				PC		>3.5	19	0	42	92	27	100
41	BK-OEK 224	+ +	-	N	-0.2	-1.2	57	26	42	100	98	100
				PI		2.9	65	14	73	100	89	100
				PC		>4.0	45	0	57	100	59	100
2814	BEL-OEK 270	+ +	-	N	-0.7	-1.2	39	94	34	100	100	100
				PI		>4.0	33	21	60	100	83	100
				PC		>4.0	18	0	42	94	41	98
39	LT-OEK 200	-	+	N	-0.2	-1.2	55	73	46	100	100	100
				PI		0.5	48	73	46	100	100	100
				PC		>4.0	47	0	41	100	37	100
16	BELE-OEK 172	-	-	N	-0.7	-1.0	80	77	82	100	100	100
				PI		1.2	25	0	48	84	63	87
				PC		>3.5	36	0	43	87	49	87
2812	BELE-OEK 173	+	-	N	-0.7	-1.2	56	51	58	100	100	100
				PI		>4.0	43	0	62	100	29	100
				PC		>4.0	48	0	53	100	19	100
2818	control for i. l. challenge		+ +	N	-0.2	-0.5	46	85	28	100	100	98
				C		>4.5	37	0	37	100	25	100

to demonstration of homotypic antibodies. In the control, antibodies were first detected by both tests on day 6 after i. l. challenge.

Type-specificity of the reaction was more marked in NT than in ICF as shown by serums from cattle 4723 and 4795.

Table 4 gives data on serums from cattle inoculated i. l. with passage strains of Type O. Results are similar to those listed in Table 3. In ICF, PC serum from bovine No. 45 gave a moderate cross-reaction with A antigen and a slight reaction with SAT 1 antigen. In a parallel NT, however, this serum failed to neutralize Type A virus.

That this is not always so is shown by tests listed in Table 5. Cattle D 51 to D 91 were inoculated with passage strains of Type O, two of which were still virulent. In NT against Type A, normal serums from these animals did not give clear-cut results. However, ICF did not indicate past exposure or vaccination immunity to Type A with the possible exception of D 96. Inoculation with Type O virus caused formation of homotypic antibodies demonstrable by both tests. In both ICF and NT, these antibodies crossed slightly over with Type A. Moreover, convalescent serum from D 96 gave also a slight cross-reaction with antigen SAT 1.

Antibody response in cattle carrying heterotypic antibodies

On 6 occasions, cattle carrying A antibodies were fully susceptible to contact

Table 5

Antibody response in cattle inoculated i. l. with passage strains of Type O

Cattle No.	Passage virus and passage No.	Reaction	Serum	NI		Antigen titration			Serum titration		
				A	O	A	O	SAT 1	A	O	SAT 1
D 51	BELE 113	-	N	1.0	1.2	77	78	75	100	100	100
			PI	2.0	> 1.5 > 3.7	30	0	91	99	12	100
D 99	BELE 116	-	N	1.2	0.7	76	89	85	100	100	100
			PI	1.7	> 2.2 > 3.7	28	0	72	100	48	100
D 96	BHK 245	+ +	N	1.7	- 0.2	54	86	70	100	100	100
			PI	2.7	> 3.5 > 3.7	3	0	26	67	8	100
D 91	LT - OEK 122	+ +	N	1.3	0.6	69	72	76	100	100	100
			PI	2.5	> 2.5 > 3.7	28	0	80	94	45	100

Table 6

Antibody response to infection with Type O in cattle carrying A antibodies

Cattle No.	Mode of infection	Reaction PI	Reaction PC	Serum	NI		Antigen titration			Serum titration		
					A	O	A	O	SAT 1	A	O	SAT 1
4619	Contact O		+ +	N	> 2.5	- 0.2	0	59	62	76	100	100
				C		3.0	0	0	8	30	41	94
4643	dtto.		+ +	N	> 2.5	- 0.2	6	30	54	86	100	100
				C		2.0	0	0	81	54	59	100
2149	dtto.		+ +	N	3.2	- 0.2	0	43	19	50	100	97
				C		> 3.7	2	0	45	77	38	100
7	BELE - OEK 141 i. l.	-	-	N	1.5	1.0	8	42	38	90	100	100
				PI		> 2.7	0	0	7	46	25	89
				PC		> 4.5	0	0	10	50	26	89
9	BELE - OEK 137 i. l.	-	-	N	4.7	- 0.1	0	47	42	72	100	100
				PI		1.5	0	16	47	72	97	100
				PC		4.2	0	0	44	70	69	100
8	Control for Nos. 7 and 9 (i. l. challenge)		+ +	N	0.3	0.9	55	60	59	100	100	100
				C	1.7	> 4.5	14	0	71	97	10	100
2839	negative control			N	- 1.0	- 1.0	64	72	66	100	100	100

infection with Type O. Three such cases are listed in Table 6 (Nos. 4619, 4643 and 2149). Two animals (Nos. 7 and 9) with A titers were inoculated i. l. with attenuated passage virus of Type O. Inoculation with O virus caused formation of O antibodies detectable by both tests in all 5 animals. In 2 cases (Nos. 2149 and 9), there was no parallel increase of A or SAT 1 antibodies as shown by serum titration. Two cattle (Nos. 4619 and 7) did show such increases, and one animal (No. 4643) experienced an increase of A antibodies but not of SAT 1 antibodies.

Table 7

Average MF values in groups of cattle infected experimentally or by contact

Group No.	No. of Cattle	Mode of infection	Serum	Antigen titration			Serum titration		
				A	O	SAT 1	A	O	SAT 1
1	7	i. l. with passage strains of Type A (see Table 3)	N	64	61	59	100	100	100
			PI	11	42	61	77	99	100
			PC	0	30	64	36	99	100
2	3	i. l. with Type A (unmodified)	N	86	56	42	100	100	100
			C	1	35	48	59	99	100
3	7	i. l. with passage strains of Type O (see Table 4)	N	55	64	52	100	100	100
			PI	40	26	56	97	79	98
			PC	33	0	44	96	39	98
4	5	i. l. with passage strains of Type O	N	63	78	70	100	100	100
			PI	22	0	67	92	36	100
5	4	i. m.* with passage strains of Type O	N	43	66	42	99	100	99
			PI	46	41	47	99	91	100
			PC	23	0	31	91	22	92
6	7	by contact with Type O (see Table 2)	N	57	49	62	100	100	100
			C	47	0	59	99	43	100
7	3	i. l. with Type O (unmodified)	N	61	66	44	100	100	99
			C	32	0	42	99	30	100

* intramuscularly

Table 8

Crosswise indirect CFT with A variants "Teheran" and "Turkey"

Serum donors		Serum	Antigen titration				Serum titration			
No. in test	Cattle No.		A Teh.	A Turk.	O	SAT 1	A Teh.	A Turk.	O	SAT 1
1	4794 (A "Teheran")	C	0	25	44	74	71	98	100	100
2	A 550 / 1021 - 1 / 4 D - 23 (A "Turkey")	PV*	79	25	49	67	100	100	100	100
3	A 550 / 1021 - 1 / 4 D - 7196 (A "Turkey")	PV	41	0	32	62	100	78	100	100
4	A 550 / 1021 - 1D - 7181 (A "Turkey")	PV	15	0	28	72	98	62	100	100
5	A 550 / 1021 - 1D - 7187 (A "Turkey")	PV	24	0	33	70	98	69	100	100
6	A 550 / 1021 - 1D - 7195 (A "Turkey")	PV	32	0	36	56	100	73	100	100
7	A Mo / 7641 - 1D - 305 (A "Turkey")	PV	71	12	62	66	100	95	100	100
8	4797 (negative control)	N	88	70	62	58	100	100	100	100

* post-vaccination

Also listed in Table 6 is the control (No. 8) for i. l. challenge of cattle Nos. 7 and 9. As shown by both tests, this animal did not carry A or O antibodies before infection with unmodified O virus. Its convalescent serum contained much O antibody and a low A titer in both tests, but did not react with SAT 1 antigen in ICF.

Interrelationship of Types A, O and SAT 1 as indicated by results of ICF

Arithmetical means were calculated of MF values recorded for individual cattle immunized in a similar way. As Table 7 shows, serums from A-immune animals (groups 1 and 2) cross-reacted slightly with Type O but not with SAT 1. Serums from cattle immune to Type O (groups 3 to 7) gave moderate cross-reactions with A antigen but no or insignificant cross-reactions with SAT 1.

Differentiation of A variants A "Teheran" and A "Turkey" by ICF

These variants or subtypes were differentiated by direct CFT and NT using immune serums from guinea pigs infected with these strains (9).

The result of a crosswise ICFT using bovine convalescent serum A «Teheran» (4794) and 6 post-vaccination serums from cattle vaccinated with A «Turkey» vaccine is presented in Table 8 which shows that the test clearly differentiated the two variants. Antibodies were even detected in cattle vaccinated with ¼ dose of vaccine (low-level in No. 2, considerable in No. 3). The stronger serums (Nos. 3 to 6) cross-reacted moderately with A «Teheran» antigen, while the weaker serums (Nos. 2 and 7) had hardly any effect on this variant.

Again, there were minor cross-reactions with Type O antigen but not with SAT 1.

Time of persistence of antibodies after infection

A group of 6 cattle was held for a period of 49 days after i. l. infection with unmodified Type O virus. In serum titrations against Type O antigen, mean fixation values averaged 29.2% in serums taken on day 14 after infection and 31.7 % in serum samples obtained on day 49. There was thus no significant decrease of antibody within a period of 35 days. All serums did not cross over with A and SAT 1 antigens.

Antibody response in vaccinated sheep

The sheep experiment recorded in Table 9 was carried out at a time when we only had limited experience with ICF. Since the sheep were inoculated with Type O vaccines (see Materials and Methods), their serums were tested with O antigen only.

It can be seen in the table that O antibodies could be demonstrated in vaccinated sheep by both tests. Antibody response to first vaccination was poor as shown by either method, but increased considerably after booster.

While there was no close agreement between results of the two tests in individual animals, calculated mean values given at the foot of Table 9 agree rather well.

Discussion

Antibody demonstrable by ICF appears to react with viral antigen in such a way that it no longer fixes complement in the presence of type-specific guinea pig serum. It is likely that neutralizing antibody is involved, which is known to persist in the blood for considerable periods after convalescence.

According to present information, ICFT and NT are about equally sensitive. It seems, however, that ICF is somewhat less type-specific than NT. This concerns mainly Types O and A which, according to data presented in Table 7, appear to be more interrelated than O and SAT 1 or A and SAT 1. RICE and BROOKSBY (6) observed in ICF moderate cross-reactions between the classical types A, O and C and suspected a common group antigen. In spite of this slight defect in type specificity, ICF was capable of differentiating two serological variants of Type A. It seems possible to make practical use of this whenever new variants or subtypes appear in the field.

It is a disadvantage of ICF that it does not work strictly quantitatively. One reason is that some normal serums contain non-specific inhibitors, which will be dealt with in the following paper. NT, however, are also far from ideal in this respect. In tissue cultures, their results are influenced by differences in cell susceptibility in addition to non-specific inhibitor present in some normal serums. In recent work, normal bovine serums with neutralization indices of 2 log or more were repeatedly encountered. Serum donors proved fully susceptible to contact or experimental infection. With both tests, results cannot be reproduced strictly quantitatively.

Results obtained with serums from cattle used in experimental work have shown that ICF is a useful tool in studies of antibody response. It is easier to carry out and gives results more rapidly than NT.

Information on ICF with sheep serums is still limited. There is reason to believe that they react in a way similar to bovine serums.

Summary and Conclusions

Indirect complement fixation (ICF) in foot-and-mouth disease was reinvestigated using serums from cattle immunized in different ways and from vaccinated sheep. Type A, O and SAT 1 antigens prepared from tissue cultures were emloyed. The method was compared with the neutralization test (NT) with regard to sensitivity and type-specificity.

The results showed that both tests are about equally sensitive. ICF, however, is somewhat less type-specific than NT. This refers only to Types A and O, which are more closely interrelated antigenically than Types A and SAT 1 or O and SAT 1.

Two A variants, distinguishable by direct CF and NT, could be readily differentiated by ICF.

ICF does not work strictly quantitatively, but NT in tissue cultures is only slightly superior in this respect. ICF is easier to carry out and gives results more rapidly than NT.

Antibodies demonstrable by ICF first appeared in serum of cattle between 3 and 6 days after intralingual infection and persisted in practically undiminished concentration for at least 49 days after inoculation.

In 3 of 5 cattle carrying A antibodis, infection with Type O effected production of homotypic antibodies and a parallel increase of A antibodies. In ICF, these antibodies crossed over with SAT 1 in 2 animals.

In a test of serums from vaccinated sheep, the results of both methods did not always agree closely in individual animals, but calculated mean values for groups of sheep were in good agreement.

Table 9

Antibody response in adult sheep vaccinated twice with formalinized adsorbate vaccine Type O

Vaccination	Sheep No.	Results of NT and indirect CF	Serums tested N 26 - 6 - 66	PV 1 17 - 7 - 66	PV 2 3 - 9 - 66
Vaccine 1 (not concentrated) 26 - 6 - 66 and 24 - 8 - 66	3942	ND_{50} *	<0	<0	0.436
		MF **	57	70	57
	3937	ND_{50}	<0	0.903	1.730
		MF	78	74	22
	3935	ND_{50}	<0	0.767	1.806
		MF	88	71	29
	3969	ND_{50}	<0	0.827	1.806
		MF	82	73	1
	3956	ND_{50}	<0	<0	1.204
		MF	88	92	19
	3939	ND_{50}	<0	0.301	0.602
		MF	96	87	37
	3963	ND_{50}	<0	<0	1.032
		MF	56	72	43
Vaccine 1C (concentrated 4 - fold) 26 - 6 - 66 and 24 - 8 - 66	3927	ND_{50}	<0	0.201	1.806
		MF	93	74	12
	3934	ND_{50}	<0	0.150	1.204
		MF +	22	14	0
	3944	ND_{50}	<0	~ 0.000	1.505
		MF	68	8	0
	3971	ND_{50}	<0	1.053	2.031
		MF	75	35	2
	3946	ND_{50}	<0	0.990	2.107
		MF	91	65	11
	3952	ND_{50}	<0	1.107	2.107
		MF	81	71	2
	3959	ND_{50}	<0	~ 0.000	1.956
		MF	73	45	1
Mean values	Vaccine 1	ND_{50}	<0	0.400 (1 : 2.5)	1.231 (1 : 17.0)
		MF	78	77	30
	Vaccine 1C	ND_{50}	<0	0.500 (1 : 3.2)	1.819 (1 : 65.9)
		MF	72	45	4

* $-\log_{10}$ of serum dilution

** mean fixation (%) by undiluted serum-antigen mixture and dilutions 1 : 1.5 to 1 : 6

\+ result obtained in another test with weaker antigen

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Zusammenfassung

Indirekte Komplementbindung bei Maul- und Klauenseuche
I. Untersuchung der Antikörper-Reaktion bei Versuchsrindern und -schafen

Die indirekte Komplementbindung (IKB) bei der Maul- und Klauenseuche wurde wurde erneut untersucht, wobei Seren von unterschiedlich immunisierten Rindern und von vaccinierten Schafen verwendet wurden. Als Antigene dienten die Typen A, O und SAT 1 von Gewebekulturen. Die Metuode wurde im Hinblick auf Empfindlichkeit und Typenspezifität mit dem Neutralisations-test (NT) verglichen.

Die Ergebnisse zeigen, dass beide Teste annähernd gleich empfindlich sind. Die IKB ist allerdings etwas weniger typenspezifisch als der NT. Dies bezieht sich jedoch nur auf die Typen A und O, deren Antigenverwandtschaft untereinander grösser ist als gegenüber SAT 1.

2 A-Varianten, die sich durch direkte KB und den NT unterscheiden liessen, konnten auch mittels der IKB differenziert werden.

Die IKB arbeitet nicht streng quantitativ, doch ist ihr der Gewerbekultur- NT in dieser Beziehung nur wenig überlegen. Die IKB ist leichter auszuführen und erbringt schneller Resultate als der NT.

Mittels der IKB waren 3 bis 6 Tage nach intralingualer Infektion im Serum der Rinder zum ersten Male Antikörper nachweisbar. Sie hielten sich mindestens 49 Tage nach der Inokulation in praktisch unverminderter Konzentration.

Bei 3 von 5 Rindern mit A-Antikörpern bewirkte die Infektion mit Tyc O die Bildung homotypischer Antikörper und einen gleichzeitigen Anstieg der A-Antikörper. Bei der IKB überkreuzten sich bei 2 Tieren diese Antikörper mit SAT 1.

In einem Test mit Seren von vaccinierten Schafen stimmten die Ergebnisse der beiden Methoden zwar beim einzelnen Tier nicht immer völlig überein, doch waren drei für die Gruppe berechnete Mittelwerte in guter Übereinstimmung.

Résumé

Fixation indirecte du complément lors de fièvre aphteuse
I. Etude de la réaction des anticorps chez les boeufs et des moutons d'expérience

La fixation indirecte du complément (FIC) lors de fièvre aphteuse est soumise à une nouvelle étude, pour laquelle on utilise des sérums de boeufs immunisés de différentes façons et des érums de moutons vaccinés. On emploie comme antigènes les types A, O et SAT 1, obtenus sur cultures de tissus. On compare la méthode avec le test de neutralisation (TN), quant à la sensibilité et à la spécifité des types.

Les résultats montrent que la sensibilité des deux tests est presque identique. La FIC est légèrement moins spécifique du type que le TN. Ceci ne se rapporte pourtant qu'aux types antigéniques A et O, plus étroitement apparentés entre eux qu'avec SAT 1.

Deux variantes A, que l'on peut distingue rpar la fixation directe du complément et le peuvent être différenciées également à l'aide de la FIC.

La FIC ne donne pas des résultats spécialement quantitatifs, mais n'est que légèrement inférieure à la culture de tissus-TN à cet égard. La FIC est facile à réaliser et plus rapide que le TN.

A l'aide de la FIC, on parvient à mettre en évidence les premiers anticorps dans le sérum des bovins 3 à 6 jours après infection intralinguale. La concentration de ces anticorps reste pratiquement constante au moins 49 jours après l'inoculation.

Chez 3 boeufs sur 5, avec des anticorps A, l'infection par le type O induit la formation d'anticorps homotypiques et une augmentation simultanée des anticorps A. Chez 2 animaux, ces anticorps donnent à la FIC des réactions croisées avec SAT 1.

Dans le test avec les sérums de moutons vaccinés ,les résultats des 2 méthodes ne coincident pas toujours ecactement pour un même animal, mais 3 des moyennes calculées pour le groupe concordent bien.

Resumen

Fijación indirecta del complemento en la glosopeda
I. Estudio de la racción de los anticuerpos en reses vacuna. y oveajs de experimentación

De nuevo se estudió la fijación indirecta del complemento (FIC) en la fiebre aftosa, utilizándose sueros sanguíneos de bovinos immunizados de forma diferente y de ovejas vacunadas. Como antígenos se emplearon los tipos A, O y SAT 1 de cultivos hísticos. La ténica se comparó con la prueba de neutralizacíon en atencíon a la sensibilidad y especificidad de tipo.

Los resultados prueban que ambos tests son aproximadamente igual de sensibles. Por cierto, la FIC es algo menos específica de tipo que la PN. Sin embargo, esto solo se refiere a los tipos A y O, cuyo parentesco antigénico es mayor entre sí que frente a SAT 1.

2 variantes A, que se lograron distinguir mediante fijación directa del complemento y la PN, también se lograron diferenciar por medio de la FIC.

La FIC no trabaja de modo cuantitativo muy riguroso, pero la PN del cultivo hístico solo le supera poco a este respecto. La FIC se ejecuta con mayor facilidad y logra unos resultados más rápidos que la PN.

Mediante la FIC, a los 3—6 días tras la infección intralingual, por vez primera se identificaban anticuerpos en el suero sanguíneo de los bovinos. Al menos durante 49 días tras la inoculación se mantenían en concentracíon casi invariable.

En 3 de 5 reses vacunas con anticuerpos A, la infección con el tipo O ocasionaba la formación de anticuerpos homotípicos y el amento simultáneo de los anticuerpos A. En la FIC, estos anticuerpos se entrecruzaban en 2 animales con SAT 1.

En una prueba con sueros sanguínios de ovejas vacunadas no siempre coincidían por completo los resultados de ambas técnicas en el mismo animal, pero tres valores medios calculados para el grupo se hallaban en pervecta armonía.

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