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THE IBR-IPV VIRUS-SERUM NEUTRALIZATION TEST

SENSITIVITY AND SIGNIFICANCE OF THE TISSUE CULTURE TUBE TEST

By

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BITSCH, V.: *The IBR-IPV virus-serum neutralization test. Sensitivity and significance of the tissue culture tube test.* Acta vet. scand. 1973, *14*, 683—690. — A modification of the IBR-IPV virus-serum neutralization test (tissue culture tube test) was used in examinations of three groups of cattle: animals at AI centres; selected herds from infected districts; and pregnant cows and heifers from all over the country. In, respectively, 141 samples out of 1335, 215 out of 344, and 46 out of 7928, a virus-neutralizing effect was demonstrated. Within the three groups of samples, respectively, 2.8 %, 29.4 %, and 28.3 % of samples positive by the modified test were negative by the conventional test, even with undiluted serum. The findings gave strong evidence that all positive reactions were results of a preceding infection. All animals with a history of infection responded serologically when examined by the modified test, but still the distribution of the titers recorded in herd examinations indicates the desirability of a further improvement in the sensitivity of the test.

IBR-IPV; infectious bovine rhinothraceitis; infectious postular vulvovaginitis; neutralization test; sensitivity.

A modification of the IBR-IPV (infectious bovine rhinotracheitis — infectious pustular vulvovaginitis) virus neutralization test (M-test) has been employed in Denmark since 1969. With increased amounts of undiluted serum and a lower virus dose than used in the conventional test (C-test), very low levels of virus-neutralizing antibodies (VNA) could be demonstrated (*Bitsch* 1970).

Quantitative studies on the IBR-IPV serum-VNA test have been published by *Mohanty & Lillie* (1965) and *House & Baker* (1971). Studies on the sensitivity of the test and the possibilities of getting false positive reactions, however, can be made only upon animals with a known state of infection or animals that can be divided into two groups, one with and one without the probability of infection. *Huck & Woods* (1972) examined sera of heifers infected experimentally by the vagina and found that the sensitivity of the C-test was not high enough for the detection of VNA in all sera, and even a test modified by increased amounts of serum did not give satisfactory results.

This paper deals with results obtained with the M-test on sera from Danish cattle for estimation of the sensitivity and significance of the M-test and the C-test with the use of tissue culture tubes. (Parts of this material have been reported on in summary previously (*Bitsch*)).

MATERIAL AND METHODS

Animals

Three groups of animals have been examined:

I. Animals at AI centres. In all, 1335 bulls and bullocks were examined 1969—70 (*Bitsch et al.* 1970 a, b), with a view to controlling IBR-IPV at the Danish centres (Table 1).

II. Affected herds. Eighteen herds were represented by 344 samples, originally taken with the intention of studying the presence and spreading of the infection within the herd.

III. Pregnant cows and heifers from the whole country. This group comprises nearly 8000 animals (Table 3) and was examined primarily in order to evaluate the prevalence of the infection in Denmark.

VNA test

Samples were examined according to the routine procedure described previously (*Bitsch* 1970) including the C-test with 100 TCID50 of virus and undiluted serum.

Samples neutralizing 100 TCID50 were subsequently titrated with two-fold serum dilutions and inoculation of two tubes per

F i g u r e 1. Map of Denmark showing the location of IBR-IPV-infected AI centres.

dilution. Titers were given as 50 % end-points (Kärber). Samples neutralizing 10 TCID50 in both tubes but not 100 TCID50 were re-examined with 10 TCID50 and 0.5 ml of serum for confirmation of the result and additionally examined with 10 TCID50 and 0.1 ml of serum to estimate more precisely the antibody level. In the present material all VNA-positive samples that did not neutralize 100 TCID50 of virus in both tubes have been given the same titer value: < 1.

The virus used was the Danish strain HBV1-DaB69.

RESULTS

In all, 141 IBR-IPV-positive animals (139 bulls and two bullocks) were found in Group I. Titers are given in Table 1. Location of centres where the infection was spread — B, Ø, M, T, and also V with only two antibody carriers — is indicated in Fig. 1.

Table 1. Examination of AI centres for IBR-IPV antibody carriers.

AI centre (Fig. 1)	Date of sampling	Number of samples	Animals with VNA	Titer distribution							
				< 1	1—1.4	2—2.8	4—5.6	8—11	16—22	32—45	64—90
B	4/2 1969	47	44	2	5	9	12	6	7	2	1
B1	3/6 1969	37	4				2	1	1		
Ø	14/11 1969	96	64	2	1	7	13	19	17	5	
M	28/2 1970	28	10		1	2	4	3			
T	3/3 1970	29	12		1		1	4	4	2	
V	24/2 1970	60	2				1	1			
5 other centres	Sep. 1969—March 1970	160	5			3	1		1		
remaining 33 centres	Feb.—March 1970	878	0								
Total		1335	141	4	8	21	34	34	30	9	1
Percentage distribution				2.8	5.7	14.9	24.1	24.1	21.3	6.4	0.7

Results from examination of selected herds (Group II) are shown in Table 2 together with the relevant information concerning each herd.

Table 3 gives titers of virus-neutralizing samples found by examination of the 7928 animals in Group III. Of 46 reacting samples 14 were from cows and 32 from heifers. A further very few samples showed virus neutralization at the screening examination, but as there was no serum for a subsequent confirmation they were not recorded as positive.

DISCUSSION AND CONCLUSION

Significance of results

Compared to the C-test, the M-test will theoretically enlarge the titer scale down below 1 by about two steps (*Bitsch* 1970). In this paper the titer designation < 1 has been used for all samples with such low virus-neutralizing effects. The following facts give evidence of the significance of these low titers:

Group I. Of 1335 animals at AI centres only four had titers below 1, and these four animals were stabled at two centres

Table 2. Examination of selected herds for IBR-IPV antibody carriers.

Herd	Related AI centre (Fig. 1)	Date of sampling	Number of samples	Lower age limit (years)	Animals with VNA	Titer distribution							Genital (G) or resp. (R) inf.	Period from inf. in	
						< 1	1—1.4	2—2.8	4—5.6	8—11	16—22	32—45		herd	AI centre
HB	B	31/3 1969	12	2	2			1		1			G	12 weeks	12 weeks
JH	B	25/4 1969	38	1½	19	10	5	1		1	1	(+1 ≥ 1.4)	G	16 „	16 „
TF	B	28/3 1969	27*	2	7	2	1	4					G	12 „	12 „
JJu	B	27/3 1969	24	1	22	8	9	3	1	1			G R?	10—12 „	12 „
PC	B	17/4 1969	57	½	55	17	16	14	5	3			(G?) R	13 „	15 „
PJ	B	3/3 1969	28	1	6	1	3	2					G	9 „	9 „
ON	B	27/2 1969	18	1½	2	1	1						G	8 „	8 „
HP	B	11/4 1969	11	2	1	1							G	14 „	14 „
JoJ	B	8/3 1969	19	1½	1	1							G	9 „	9 „
JJa	B	4/2 1970	28	1	21	7	3	1	5	4	1		G (R?)	8 „	1 year
JJø	B	9/3 1972	16*	½	2	1			1				G	?	2 „
AH	B	9/3 1972	8*	2	6			2	1	1	1	1	R?	?	2 „
KN	B	9/3 1972	16	2	14	1	1	2	3	4	3		R?	?	2 „
SK	M	20/5 1970	20	3	5	1	3			1			G	?	> 2 „
LL	T	27/5 1970	24	2	2						2		G	?	> 2 „
HC	Ø	14/5 1970	19	1½	7	1	2	2	1		1		G (R?)	?	≤ 1 „
RR	V	9/6 1971	31*	2½	23	5	4	7	3	3	1		G (R?)	?	> 1½ „
CH	nat. serv.	13/5 1970	38	2	20	6	4	3	3	3	1		G? (R?)	?	
Total			344		215	63	52	42	23	22	11	1	(+1 ≥ 1.4)		
Percentage distribution						29.4	24.3	19.6	10.7	10.3	5.1	0.5			

* herd partially examined.

Table 3. Country-wide examination of pregnant cows and heifers for IBR-IPV.

Sampling period	Number of samples	Related AI centre (Fig. 1)	Animals with VNA	Cows/ heifers	Titer distribution					
					< 1	1—1.4	2—2.8	4—5.6	8—11	16—22
Nov. 1969— July 1973		B	34	13/21	10	9	7	5	2	1
		M	8	1/7	2	3	2	1		
		Ø	3	0/3	1			2		
		V	1	0/1			1			
Total	7928		46	14/32	13	12	10	8	2	1
Percentage distribution					28.3	26.1	21.7	17.4	4.3	2.2

(B and Ø) where in addition great numbers of antibody carriers were found.

Group II. A very great proportion of animals in these 18 herds had titers below 1. Several of these animals had been inseminated (Dec. 24, 1968—Jan. 9, 1969) with semen from acutely diseased bulls (Herds JH, TF, PJ, ON, HP, JoJ) and in several such animals clinical disease was observed. Moreover, a greater number of animals from herds outside the districts B, M, T, Ø, and V (Fig. 1) were examined with completely negative results.

Group III. Of 7928 samples from all over the country 46 showed virus neutralization, 13 with titers below 1. These 13 samples, however, were, just as the remaining 33, all from animals in the districts B, M, Ø, and V.

In conclusion, this lengthy study supports the opinion that any virus-neutralizing effect registered by the M-test is significant of preceding infection*.

* In December 1972 a re-examination of a sample from a one-year-old bull at an AI centre showed an extremely low virus-neutralizing effect. In January 1973 half of the breeder's herd (23 samples) and the whole centre were examined serologically, but with a negative result. From the bull in question samples were still positive in January, but from February up to August all samples were completely negative apart from a not-reproducible result on a sample taken in April. (The very low virus-neutralizing effect in a sample from January was confirmed by H. R. Frey, Hannover, and F. Steck, Bern). The bull was declared not infected. This case is quite unique and cannot influence the conclusions of the present report.

Test sensitivity

That the C-test is not sensitive enough is quite obvious, since it would not have detected 25—30 % of antibody carriers among animals in Groups II and III.

Whether the M-test is sensitive enough is more difficult to estimate. Concerning AI bulls, the titers recorded form a near-normal distribution curve, which gives no ground for assuming that not all infected bulls were found. Subsequent examinations of sero-negative animals at centres B, Ø, M, T, and V during the years 1970—73 have strengthened this view (author's unpublished data).

In Groups II and III VNA titers of sero-positive samples are generally lower, and even if the titer designation < 1 includes abt. two steps of the titer scale, the titer distributions do not convincingly demonstrate that all infected animals have been detected, and an improvement of the sensitivity by another one or two titer steps seems highly desirable. It must be taken into consideration, however, that in the herd examinations animals known to be infected were always found serologically positive, and that when herds were blood-sampled more than once no animal positive at the first examination was later found negative.

The main conclusion is that the modification of the conventional test has given an essential improvement in sensitivity. In Denmark this formed the basis for the control of the IBR-IPV infection at AI centres. Still, however, especially in herd examinations, a need is felt of a further improvement in the sensitivity of the IBR-IPV virus-serum neutralization test.

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SAMMENDRAG

IBR-IPV-neutralisationstestens sensitivitet og signifikans.

En modificeret IBR-IPV-neutralisationstest baseret på vævskulturglas har været anvendt ved undersøgelse af 3 grupper af kvæg: dyr på tyrestationer, udvalgte besætninger fra inficerede områder og drægtige køer og kvier fra hele landet. Virus-neutralisation påvistes i henholdsvis 141 af 1335, 215 af 344 og 46 af 7928 undersøgte prøver. Af de positive prøver i grupperne var henholdsvis 2,8 %, 29,4 % og 28,3 % negative ved den konventionelle test, selv ved anvendelse af ufortyndet serum. Undersøgelserne indicerede, at alle positive resultater opnået med den modificerede test var signifikante som udtryk for en forudgående infektion med IBR-IPV-virus, og alle dyr, som havde vist klinisk sygdom, eller som måtte være blevet smittet, fandtes serologisk positive. Fordelingen af titrene for de fra besætningsundersøgelserne positive prøver påpeger imidlertid ønskeligheden af en yderligere forbedret sensitivitet af testen.

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