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THE ABILITY OF GROUP III VIRUSES ASSOCIATED WITH INFECTIVITY IN CATTLE IN SOUTH AFRICA TO INFECT THE RESPIRATORY TRACT

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Maré & van Rensburg (1961) described the isolation of a number of viruses from infertile cattle in South Africa in a preliminary report. These viruses were classified into three main groups and the "FH335" strain of Group III was shown to be pathogenic to cattle. An acute anterior and posterior vaginitis followed the intravaginal instillation of this virus into cows. Bulls infected intra-urethrally or intratesticularly showed acute urethritis, balano-posthitis, orchitis and mild epididymitis. However, intranasal and intratracheal instillation of the virus failed to elicit a febrile reaction or respiratory symptoms.

Reciprocal cross-neutralization between the "FH335" virus strain and the virus of infectious bovine rhino-tracheitis (IBR) and infectious pustular vulvovaginitis (IPV) has been demonstrated (Maré & van Rensburg, 1961).

Two independent teams of investigators in the United States of America have shown that the IBR and IPV viruses are immunologically identical, but that they differ in their pathogenicity (Gillespie, McEntee, Kendrich & Wagner, 1959, and McKercher, Straub, Saito & Wada, 1959.) Whereas both viruses caused a pustular vulvo-vaginitis in cattle infected by the vaginal route, only IBR virus caused a marked febrile response and respiratory symptoms after intranasal infection. IPV virus gave rise to a less marked febrile reaction (up to 104° F) with no respiratory symptoms.

The investigations reported in this paper were carried out to determine the respiratory pathogenicity for cattle of the seven strains of Group III viruses thus far isolated from the genital tract of cattle in South Africa.

Materials and Methods

Tissue cultures

Roller tube cultures were prepared according to the methods described by Youngner (1954) with the modifications suggested by Bodian (1956). The maintenance medium consisted of modified Hank's balanced salt solution (Hanks BSS) (Weiss & Geyer, 1959) with 0·5 per cent lactalbumin hydrolysate, 5 per cent bovine serum, penicillin (200 i.u. per ml), streptomycin (200 μgm per ml) and amphotericin B (2μgm per ml).

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The method of Dulbecco & Vogt (1954) was used for plaque assay. Each of four 70 mm petri dishes with calf kidney monolayers was seeded with 1·0 ml of various dilutions of virus after removal of the nutrient medium. After adsorption of the virus for one hour, the excess fluid was aspirated and the agar overlay was added. The cultures were incubated at 37° C in a cabinet with 5 per cent CO_2 in air and were examined daily under a stereoscopic microscope for evidence of plaques. When distinct plaques became visible in the limiting dilutions on the fourth to the sixth day, the monolayers were stained with a 1 : 10,000 dilution of neutral red.

Virus strains

For the intranasal inoculation of cattle seven different Group III viruses were used at the tissue culture passage levels indicated below.

Strain	Designation	Calf kidney T.C. passage level
1	FH335	9
2	F.B.	3
3	A 464	4
4	H	6
5	X	5
6	Irwin	4
7	C3	5

The prototype strain used as antigen in serum virus neutralization tests was FH335. For the preparation of antigen the virus was passaged in limiting dilution once in roller tubes and again by plaque assay to ensure the purity of the strain. Virus was re-isolated from a single plaque in the highest dilution by aspirating the material with a Pasteur pipette and resuspending it in 1·0 ml Hank's BSS. It was then multiplied through several transfers in roller tube cultures before antigen was finally prepared from the eighth passage level and stored at −80° C.

Neutralization tests

Serum-virus neutralization tests were performed to identify the viruses re-isolated from the experimental animals and also to determine the antibody response of these animals 32 days after inoculation. In the tests ten-fold virus dilutions were mixed in equal volume with undiluted serum. The serum-virus mixtures were kept at 4° C for 18 hours, after which two roller tube cultures of calf kidney cells were seeded with each serum-virus mixture. Cultures were incubated at 37° C and examined daily for cytopathic effects.

Collection and preparation of specimens for virus isolation

Specimens were collected with sterile gauze swabs attached to 8 in. long wire holders from the nasal passages, mouth and rectum of each animal prior to and on the 2nd, 4th, 6th, 9th, 11th, 18th, 25th and 32nd days after inoculation. After collection of the specimens, the swabs were rinsed in 2 cc of Hank's BSS containing penicillin (500 units per ml), streptomycin (500 μgm per ml) and amphotericin B (10 μgm per ml) and left in contact for one hour. The fluid was then seeded into roller tube cultures which were examined daily for cytopathic effects.

Whenever cytopathic changes were noticed during the re-isolation of virus from the experimental animals, a permanent stained preparation was made from one of these cultures according to the method described by De Lange (1959). These preparations were examined for the presence of inclusion bodies.

Experimental animals

A batch of sixteen Afrikaner and Jersey crossbred bulls, varying in age from 9 to 15 months, was used. They were housed in a stable and separated from each other by 5 ft high brick walls. Each stall was provided with a separate feed and water trough. For a period of ten days prior to the commencement of the experiment the animals were examined for signs of disease and temperatures were recorded daily. Specimens from the nasal passages, rectum and mouth were collected from each animal before introduction of the virus.

RESULTS

1. *Clinical observations*

No respiratory symptoms were observed in any of the animals during the 30 days following intranasal instillation of virus. Furthermore no indications of orchitis, epididymitis, posthitis or urethritis were encountered and all the cattle appeared healthy throughout the observation period.

The maximum pre- and post-inoculation temperatures of the animals are given in Table 1. The rectal temperatures taken during the ten days preceding virus inoculation were constant The maximum temperatures recorded during this period ranged from 101 to 101·8° F. From the third to the sixth day after instillation of the virus six animals (1328, 1315, 960, 957, 952, 628) showed a slight transient but significant rise in temperature, the maximum ranging from 102·8 to 104° F.

TABLE 1.—*Temperatures recorded before and after intranasal instillation of Group III viruses into cattle*

Identification No. of animal	Virus introduced	Maximum temperature recorded during 10-day observation prior to introduction	Maximum temperature recorded during the 30 days following introduction
		°F	°F
610	Control	101	101·6
624	Control	101	101·4
1328	Virus " X "	101	104
954	Virus " X "	101·8	102·4
1315	Virus " H "	101·2	102·8
959	Virus " H "	101·2	102
960	Virus " Irwin "	101·4	103
639	Virus " Irwin "	101·6	102
958	Virus " F.H. 335 "	101·6	101·8
1326	Virus " F.H. 335 "	101·2	102
630	Virus "A 464 "	101	102
957	Virus "A 464 "	101·2	103·6
952	Virus " F.B."	101·4	103·4
1327	Virus " F.B."	101·4	101·8
628	Virus " C3 "	101·6	102·8
971	Virus " C3 "	101·6	102

From the data presented in Table 1 it is evident that a mild febrile response occurred in some animals on the third to the sixth day following introduction of six of the seven virus strains. The only virus strain which failed to give rise to a definite fever reaction was FH335.

2. *Re-isolation of virus from the experimental animals*

Nasal, buccal and rectal specimens collected from all the animals prior to inoculation failed to yield virus, with the exception of the rectal swab from animal No. 960. From the rectum of this animal a cytopathogenic agent, serologically unrelated to the Group III viruses was isolated. Subsequent investigations showed that this agent was most probably an orphan virus similar to that described by De Lange (1959).

The results of the re-isolation of Group III viruses from the experimental animals are given in Table 2. It is evident from these results that the viruses were isolated

TABLE 2.—*The re-isolation of Group III viruses from experimental cattle infected intranasally*

Animal No.	Virus strain injected	Specimen collected from	Results of re-isolation of virus				
			Before infection	Two days after infection	Four days after infection	Six days after infection	Eight days after infection
610.....	Control	N	Negative	Negative	Negative	Negative	Negative
		R	do	do	do	do	do
		M	do	do	do	do	do
624.....	Control	N	Negative	Negative	Negative	Negative	Negative
		R	do	do	do	do	do
		M	do	do	do	do	
957.....	A 464	N	Negative	Positive	Positive	Negative	Negative
		R	do	Negative	Negative	do	Negative
		M	do	Positive	Positive	do	Positive
630.....	A 464	N	Negative	Positive	Positive	Negative	Positive
		R	do	Negative	Negative	do	do
		M	do	Positive	Positive	do	do
958.....	FH 335	N	Negative	Positive	Positive	Negative	Negative
		R	do	Negative	Negative	do	do
		M	do	do	do	do	do
1326.....	FH 335	N	Negative	Positive	Positive	Positive	Negative
		R	do	Negative	Negative	Negative	do
		M	do	do	do	do	do
954.....	X	N	Negative	Positive	Positive	Positive	Negative
		R	do	Negative	Negative	Negative	do
		M	do	do	do	do	do
1328.....	X	N	Negative	Positive	Positive	Positive	Negative
		R	do	Negative	Negative	Negative	do
		M	do	do	do	do	do

TABLE 2 (*contd.*)

Animal No.	Virus strain injected	Specimen collected from	Results of re-isolation of virus				
			Before infection	Two days after infection	Four days after infection	Six days after infection	Eight days after infection
959.....	H	N	Negative	Positive	Positive	Positive	Negative
		R	do	Negative	Negative	Negative	do
		M	do	do	do	do	do
1315.....	H	N	Negative	Positive	Positive	Negative	Negative
		R	do	Negative	Negative	do	do
		M	do	do	do	do	do
960.....	Irwin	N	Negative	Positive	Positive	Positive	Negative
		R	*	Negative	Negative	Negative	do
		M	do	Positive	Positive	Negative	do
639.....	Irwin	N	Negative	Positive	Positive	Negative	Positive
		R	do	Negative	Negative	do	Negative
		M	do	Positive	Positive	do	do
971.....	C3	N	Negative	Positive	Positive	Negative	Negative
		R	do	Negative	Negative	do	do
		M	do	Positive	Positive	do	do
628.....	C3	N	Negative	Positive	Positive	Positive	Negative
		R	do	Negative	Negative	Negative	do
		M	do	Positive	Positive	Negative	do
952.....	F.B.	N	Negative	Positive	Positive	Negative	Negative
		R	do	Negative	Negative	do	do
		M	do	Positive	do	do	do
1327.....	F.B.	N	Negative	Negative	Negative	Negative	Negative
		R	do	do	do	do	do
		M	do	do	do	do	do

N = Nasal passages
R = Rectum
M = Mouth
* = Orphan virus isolated

more regularly from the nasal passages of the cattle than from the saliva. These viruses were recovered from the nasal specimens of 13 out of the 14 infected animals on the second and fourth days after infection; from six on the sixth day and from two on the eighth day.

Virus was isolated from the saliva of seven of the infected animals on the second day; six on the fourth day and one on the eighth day.

Group III viruses were not recovered from the faeces of any of the animals or from any of the specimens collected from the control cattle. All the other specimens collected after the eighth day failed to yield virus. With the exception of the orphan described above, all the viruses re-isolated from the experimental animals were neutralized by FH335 immune serum. Examination of stained monolayers of each virus isolate revealed cytopathic changes and large intranuclear inclusion bodies indistinguishable from those encountered with Group III viruses. These findings confirmed that the re-isolated viruses belonged to Group III.

3. *Antibody response of experimental animals*

Serum-virus neutralization tests were carried out on serum samples collected from the experimental animals prior to and 32 days after infection with the seven virus strains.

No antibodies to FH335 virus could be demonstrated in the serum samples collected before commencement of the experiment. The sera of those animals which were infected with virus showed neutralizing indices ranging from 1000 to 30,000 after 32 days, whereas both control animals failed to develop neutralizing antibodies. These results indicated that the seven virus strains used in the experiment were immunologically related to each other and to strain FH335. The failure of the two control animals to develop antibodies showed that it was unlikely that accidental transmission of virus had occurred from one group of animals to another.

Discussion

The versatility of the IBR/IPV group of viruses has become increasingly evident in recent years since Gillespie *et al.* (1959) showed that infectious bovine rhinotracheitis (IBR) and infectious pustular vulvo-vaginitis (IPV) are caused by viruses similar in many respects, if not identical. A number of reports have appeared indicating that viruses of this complex may be responsible for a variety of clinical syndromes. It has been shown that specific conjunctivitis (Abinanti & Plumer, 1961; Quin, 1961; Darbyshire, Dawson & Peterson, 1962), cervico vaginitis, orchitis, balano-posthitis (Bouters, Van de Plassche, Florent, Leunen & Devos, 1960; Marê & van Rensburg, 1961) and mortality in new-born calves (Baker, McEntee & Gillespie, 1960) may be caused by viruses of this complex.

The genital disease produced by the South African virus strains of this group has been compared previously with IPV. It was demonstrated that, whereas IPV infection resulted in a pustular condition particularly of the posterior vagina and the vulva, these viruses caused an acute cervico-vaginitis with no pustule formation. In respect of their pathogenicity to the respiratory tract it has now been shown that the South African virus strains are very similar to IPV. The mild febrile response observed in this investigation and the absence of respiratory disease agree with the findings of both Gillespie and McKercher and their co-workers.

The vaginitis syndrome associated with these viruses has been observed in South Africa occasionally in herds where only artificial insemination is practised and it was evident that some means of transmission other than by coitus must have occurred. From the findings that virus may be present in the nasal secretions and the saliva for up to eight days following nasal infection, it would appear that contact transmission from nose to vagina and *vice versa* could easily have occurred. This form of contact transmission has been described by McKercher and his co-workers in their work on the IBR/IPV complex.

Summary

The pathogenicity to the respiratory tract of seven virus isolates associated with genital disease in cattle was investigated. It was shown that (1) the febrile response was very similar to that encountered after intra-nasal instillation of the IPV virus; (2) virus was present in the nasal secretions and the saliva for eight days after infection, and (3) all the infected animals showed antibodies in their convalescent sera collected 32 days after virus instillation.

The possible role of nasal infection as a means of transmission of the genital disease encountered in South Africa, is discussed.

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