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ANTIBODIES IN DANISH CATTLE AGAINST MYXOVIRUS PARA-INFLUENZA TYPE 3 (STRAIN KO-23)

By

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In a previous paper (8), we reported on the isolation of a virus from a cow with an illness of the mucosal disease complex. This virus (strain KO-23) has later been identified as the bovine type of myxovirus para-influenza type 3 (PI-3) by the hemagglutination inhibition test (9).

This paper deals with the presence of antibodies in Danish cattle against PI-3 virus, strain KO-23, as calculated from the neutralizing capacity of serum.

MATERIALS AND METHODS

Virus. Strain KO-23 was passed in calf-kidney tissue culture and stored in sealed glass ampoules at —20°C until use. The infectivity titer of various stocks ranged from $10^{5.8}$ to $10^{7.1}$ TCD_{50} per 0.1 ml.

Tissue cultures. The tubes were prepared from freshly trypsinized calf kidney. The growth medium used was T. C. Medium 199 (Difco) with 10 per cent calf serum. The maintenance medium was Eagle's medium plus 10 per cent horse serum.

Neutralization tests. Serial 10-fold dilutions of virus-infected tissue-culture fluid were prepared in Eagle's medium. Sera were diluted 1/10 in Hank's BSS and equal volumes (0.3 ml.) of virus dilution and serum were mixed and placed at 37°C for 30 minutes. From each mixture 0.1 ml. was inoculated into each of three calf-kidney cultures. Final readings were made 12 days after inoculation.

Titration of the virus was calculated by the Kärber method, and the index represents the difference in the titer of the virus without serum and in the presence of the test serum. The index

expresses the logarithm of TCD_{50} neutralized by the particular serum.

Sera. The sera were collected during the period from October 1961 to June 1962, and convalescent sera were taken 3 to 10 weeks after the acute specimen. All sera were heated to 56°C for 30 minutes and kept at —20°C until use.

Kaolin treatment of sera. Kaolin was used for the removal of nonspecific serum inhibitors to myxoviruses. Only a few sera were examined. The technique is described by *Spence* (10). To one volume of serum are added four volumes of borate-saline buffer at pH 9.0 and five volumes of a 25 per cent suspension of acid-washed kaolin in borate-saline buffer. The mixture is allowed to stand at room temperature for 20 minutes; it was then centrifuged and the supernatant used.

RESULTS

The results of the neutralization tests are given in Fig. 1. Of the 80 animals studied, only 10 showed a significant increase in antibodies, i.e. the convalescent serum was capable of neutralizing at least 100 TCD_{50} more than the acute-phase serum (values above the broken line).

The use of kaolin seems to have little effect on the neutralization test as indicated in Table 1.

Table 1.
Kaolin treatment of sera for removal of nonspecific inhibitors.

Cow	Difference between neutralization index for serum 2 and serum 1	
	Before kaolin	After kaolin
FH T10	— 0.4	— 0.4
FH T21	— 1.6	— 2.0
Kalø 124	0.3	0
Kalø 186	1.0	0.7
Kalø 226	2.3	2.3
Kalø 136	5.3	4.3
Kalø 167	4.0	3.3
R K1	2.7	2.3
AN K17	1.7	1.7
JM K2	2.3	2.0
JM K14	4.3	4.5
SS K1	3.3	3.8
SS K2	4.0	3.0
SS K3	3.4	3.0

The acute and convalescent sera (80 pairs) were obtained from cattle in herds which suffered, or had suffered, from the mucosal disease complex. An overwhelming majority of the acute-phase sera (serum 1 in Fig. 1) contained neutralizing antibodies against strain KO-23 of the PI-3 viruses indicating a wide distribution of this virus among Danish cattle.

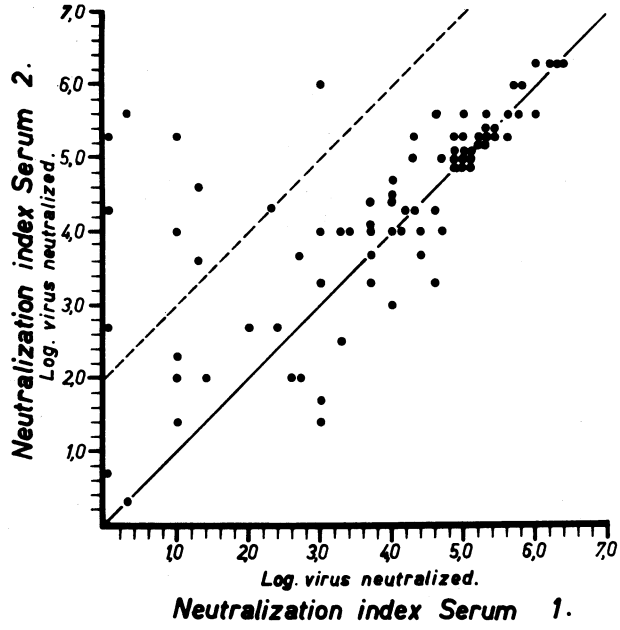

Fig. 1. Neutralization tests on 80 paired sera from cattle.
Solid line: Neutralization index is the same for both sera.
Broken line: Values 100 TCD_{50} higher for serum 2 (convalescent) than for serum 1 (acute).

As already mentioned, 10 animals had an increase in antibodies during the course of the disease. From a clinical point of view these 10 animals can be divided into two groups: seven animals in group I and the remaining three in group II.

Group I. The clinical picture of this group was very similar to that observed in the cow from which strain KO-23 was isolated in January 1961 (8). The morbidity was moderate — 10 to 30 per cent — the mortality very low, and both young and older animals were attacked.

Early indications of infection were marked depression and

a sharp reduction in milk flow. In the initial phase, the animals had a rough hair coat, rectal temperatures up to 41° or 42°C, pulse rate 100 to 110, a rapid respiration (about 40) and symptoms localized to the mucous membranes.

The eyes were blurred, and there was plenty of lachrymal discharge. The conjunctivae were reddish and swollen. The nasal mucosa was congested, and ample amounts of thin, watery nasal discharge were observed. The oral mucosa was diffusely red, and salivation slight. The vaginal mucosa was also congested. There was marked respiratory distress, and most animals were coughing. The lung area was normal, although now and then moist rales were heard. The lymph nodes were not enlarged and normal. The pharynx was sore when palpated. Feces were liquid, but not watery. Involvement of the central nervous system was not observed, and there were no lesions on the skin.

After 4—5 days, the temperature subsided, and the respiratory and pulse rates decreased. At this time, the lachrymation was mucous and purulent, and eroded patches on the conjunctivae were seen. The character of the nasal discharge had also changed. It was more viscous, darker and purulent. The nostrils were coated with crusts, and small eroded patches were seen on the nasal mucosa. The oral mucosa showed small buccal erosions. The urine yielded a positive Heller's reaction, and examination of the blood showed leukopenia. The formol gel test was negative.

The animals recovered after 10—12 days.

Group II. In all herds of this group the morbidity was 100 per cent. All age groups were attacked, but the mortality was low. The most characteristic symptoms were fever, slight coughing and a violent diarrhea containing mucus and blood. The initial symptoms were a rise in temperature, depression, lack of appetite and slight coughing. Heart beat and respiration were rapid. At an early phase of the disease, a serous to mucous nasal discharge occurred, and the nasal mucosa was red and congested. The salivation was pronounced with long threads of saliva reaching to the manger. When right flank was palpated succussion sounds were heard. After 1—2 days a severe, foul-smelling diarrhea developed. Feces were watery containing mucus and blood. The animals were apathic and the milk production was greatly reduced. In animals of this group, the urine also yielded a positive Heller's reaction, and the blood showed leukopenia. The formol gel test was negative.

The diarrhea may last for 3—5 days followed by recovery, or may progress in severity. The nasal discharge then becomes muco-purulent and blood-stained, and erosions of the nasal mucosa develop. The coughing becomes more painful; the feces contain large blood clots, and the defecation is accompanied by violent tenesmi. At this stage of the disease, dehydration develops. In some animals, it may be so severe that it rapidly leads to death if left untreated.

Several animals had a sore gait, and small eroded patches could be found in the interdigital clefts. When the animals had recovered, the hoofs were marked by ridges running parallel with the coronary margin. In two cases, abortion occurred, and several animals had dermatitis of the nuchal region and the milk shield.

DISCUSSION

PI-3 virus have been isolated from healthy cattle (1) and from cattle with shipping fever (7), mucosal disease (2) and respiratory infection (4). The clinical picture of our group I resembles mucosal disease, whereas group II must be characterized as virus diarrhea (VD).

No attempts were made to isolate virus from the cattle considered in this report, and the diagnostic value of the neutralization test is rather uncertain. Thus, *Dinter* (5) isolated a PI-3 strain from a case of mucosal disease, but later it was shown that the disease was likely to have been due to a dual infection with PI-3 virus and VD virus. In an epizootic of mucosal disease, *Borgen and Dinter* (3) found a significant increase in antibodies against both PI-3 virus and VD virus. *Dinter* (6) also reported on the isolation of PI-3 virus from cattle which during the course of the disease showed an increasing amount of antibodies against VD virus.

The problem whether we are dealing with a dual infection by PI-3 virus and VD virus in the mucosal disease complex, or it is a matter of common partial antigens between the viruses, as in human enterovirus infections, has not yet been solved.

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SUMMARY

Eighty paired sera from cattle with present or previous experiences with the mucosal disease complex were studied for antibodies against PI-3 virus. Ten animals with a significant increase in antibodies during the course of the disease were divided into two groups: one resembling mucosal disease, the other virus diarrhea. The question of dual infection or common partial antigens between the viruses is discussed.

ZUSAMMENFASSUNG

Gegen das Myxovirus Parainfluenza Typ 3 (Stamm KO-23) gerichtete Antikörper bei dänischen Rindern.

Von 80 Rindern wurden doppelte Serumproben entnommen, die auf Gehalt an virusneutralisierenden Antistoffen gegen Parainfluenza Typ 3 (Stamm KO-23) untersucht wurden. Bei 10 Tieren fand sich eine Titersteigerung in zwei Gruppen; die eine Gruppe gleicht mucal disease und die andere kann mit Virusdiarrhöe bezeichnet werden. Das Problem einer doppelten Infektion gegen gemeinsame Antigene bei Virus innerhalb des mucal disease-Komplexes wird diskutiert.

RESUMÉ

Antistof mod myxovirus para-influenza type 3 hos kvæg i Danmark.

Fra 80 kreaturer er der udtaget parrede serumprøver, der er undersøgt for indhold af virus neutraliserende antistoffer mod para-influenza virus type 3 (stamme KO-23). Hos 10 dyr er fundet en titerstigning. Disse 10 dyr kan klinisk inddeles i to grupper: én der ligner mucosal disease og en anden der må betegnes som virus diarrhoe. Spørgsmålet om dobbelt infektion contra fælles antigener hos virus inden for mucosal disease komplexet diskuteres.

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