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ISOLATION OF A BOVINE ENTEROVIRUS

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Several viruses have been isolated from cattle during the past decade, and it has been possible to classify some of these strains as bovine adenoviruses, bovine para-influenza viruses etc. Still following the nomenclature of human viruses some strains have been designated bovine enteroviruses, and since a number of these viruses are not associated with disease, they have been called ECBO viruses (Enteric Cythopatic Bovine Orphan) as a parallel to human ECHO viruses.

In the classification of virus isolates we have adopted the scheme of *Hamparian et al.* (1963) using nucleic acid type, ether sensitivity and acid lability as criteria. Thus ether stable RNA viruses can be separated into two groups: Acid stable viruses and acid labile viruses i.e. bovine rhinoviruses. The acid stable viruses in turn can be divided into heat stable viruses, bovine reo-viruses, and heat labile viruses or enteroviruses.

During investigations on the virus diarrhea-mucosal disease complex (VD & MD) a virus was isolated and later classified as a bovine enterovirus.

MATERIAL AND METHODS

Cell cultures. Except for using fetal calf kidney (instead of baby calf kidney) the tissue cultures were prepared and handled as described earlier (*Rindom Schiøtt & Hyldgaard Jensen* 1963 a).

Test for nucleic acid type. Serial ten-fold dilutions of virus were prepared each dilution inoculated into three tubes of primary calf kidney cultures containing 10^{-5} M-5-iodo-2′-deoxyuridine (IDU) and into three tubes without IDU. Each test included vaccinia virus (a DNA virus) titrated in the same way and the test was considered satis-

factory only if the DNA virus was completely or almost completely inhibited by IDU.

Ether sensitivity. Viruses were mixed with 20 % ether and incubated for 18 hrs. at 4°C and control viruses without ether were treated in the same way. After incubation serial ten-fold dilutions were prepared and infectivity titers were determined in calf kidney cultures.

Acid lability. Viruses to be tested were diluted 1/10 in Eagle's medium without bicarbonate and adjusted to pH 3 with 1 N-HCl. The final pH was measured by use of Lyphan paper no. L652 and L656 (G. Kloz, Berlin). After standing at room temperature for four hrs., serial ten-fold dilutions were prepared and the virus titrated in tissue cultures. The control virus was kept at pH 7 and at room temperature. A reduction in titer of one $\log_{10}$ or more of virus kept at pH 3 was interpreted as acid lability.

Thermal stability. Viruses were tested for stability to heat by exposure to 50°C for 30 min. in a waterbath. The control virus was kept at 20°C for the same period of time and then titrated.

Sera. The sera were collected from animals with an illness recorded as belonging to the VD & MD complex. All sera were inactivated at 56°C for 30 min. and kept at — 20°C until use. Immune sera against foot-and-mouth disease virus types O-2, C, and A-5 were obtained from the State Veterinary Institute for Virus Research, Lindholm, and ECHO-10 (Lang), antiserum from the National Foundation for Infantile Paralysis Inc., USA, while healthy donors chosen at random provided human sera.

Neutralization tests. The antibody level in sera was calculated by the constant serum-varying virus method as described in a previous paper (*Rindom Schiøtt & Hyldgaard Jensen* 1963 b). The neutralization index is defined as the difference in titer between virus titrated with and without serum.

Hemagglutination. Red cells from cattle, rhesus monkeys and humans were collected and handled as described by *Rindom Schiøtt & Hyldgaard Jensen* (1963 a), and an 0.25 % suspension was used for the experiments.

CASE HISTORY

The herd in question consisted of ten calves of Red Danish Milkbreed 6—14 months old. For a week the owner had noticed that several calves had rapid breathing and some were coughing. Many had encrusted nostrils, lacrimation and a slight diarrhea. At the end of the week three calves had stopped eating and the veterinarian was notified.

The resulting physical examination revealed the following findings. All three calves had fever (40.5—41.2°C), they had

anorexia and were depressed. Respiration was rapid (40) with some dyspnea. The mucous membranes were reddish and congested, and there was lacrimation and serous to mucous nasal discharge. The lung area was normal on two of three calves, the third (KO-114), however, had bronchial respiration and friction sounds. All three had watery, foul-smelling diarrhea, and KO-114 had shrunken eyes and was dehydrated. Examination of feces revealed no signs of parasites and a bacteriological examination for salmonella bacteria and paratuberculosis was negative.

Calf KO-114 was treated with sulphonamides and appropriate liquids but died after four days. The two other calves received no treatment and recovered in a couple of days. Postmortem examination of calf KO-114 gave the following diagnosis: dehydration, enteritis and pleuropneumonia.

RESULTS

Material from rectal and nasal swabs from calf KO-114 was inoculated into tissue cultures. After two days a cytopathogenic effect (CPE) was observed in all cultures inoculated, and the CPE was complete within four days. The CPE was characterized by very small, round degenerated cells and complete destruction of the tissue culture. In Giemsa stained preparations no inclusion bodies — intranuclear or cytoplasmic — were observed, nor did vacuoles occur in the cytoplasm.

Table 1. Properties which characterize the viruses as enteroviruses.

		Indirect evidence for nucleic acid type			Ether stability		Acid lability	
		infectivity titer			infectivity titer		infectivity titer after exposure to	
Virus strain	Size mμ	with IDU	without IDU	nucleic acid	after ether	without ether	pH 3	pH 7
KO-114N	<50	3.8	3.5	RNA	3.8	3.5	3.5	3.5
KO-114R	<50	4.5	5.1	RNA	5.1	5.1	4.5	5.1

Physico-chemical characteristics. Results on the physico-chemical properties of the two strains are summarized in Table 1. The strain isolated from nasal secretion (KO-114N) and that from feces (KO-114R) seem to be identical, both being RNA viruses resistant to ether and low pH. Both strains were found to

pass Millipore filters with a pore size of 50 mμ. The effect of heating on two strains gave inconsistent results. In some tests the viruses showed total heat stability while in others they were heat labile with a maximum loss of log. 5.0 of virus infectivity titer (Table 2).

Table 2. Test for thermal stability (50°C for 30 min.) of virus KO-114.

	Negative $\log_{10}$ virus titer		
Virus strain	not heated	heated	titer reduction
KO-114N	4.7	4.5	0.2
	3.5	2.8	0.7
	4.5	2.5	2.0
	5.8	0.8	5.0
KO-114R	4.8	1.1	4.7
	5.1	1.8	3.3
	3.5	3.1	0.4
	3.8	4.1	—0.3

Hemagglutination. Virus KO-114 was not able to agglutinate either bovine or human erythrocytes at 4°C or at room temperature. As seen in Table 3, both strains were able to agglutinate red blood cells from rhesus monkeys.

Table 3. Hemagglutination of red blood cells from rhesus monkeys with virus KO-114.

	Temperature		
Virus strain	4°C	20°C	37°C
KO-114R	1/128*)	1/16	<1/2
KO-114N	1/256	1/32	<1/2

*) Highest dilution of virus giving full agglutination.

Neutralization tests. The neutralizing capacity of sera from cattle is shown in Table 4. Very few sera were able to neutralize virus KO-114 and only seven sera had a neutralization index

Table 4. Neutralizing capacity of 103 bovine sera on virus KO-114.

	Number of sera
Neutralization index between —0.5 and 0.5	58
„ „ „ 0.5 and 1.0	32
„ „ „ 1.0 and 2.0	13

higher than 1.5. Unfortunately only acute fase serum was taken from the three calves in question and none of these gave a positive neutralization result when tested. Immune sera against foot-and-mouth disease virus and ECHO-10 virus when diluted 1/8 were unable to neutralize 300 TCD_{50} of virus KO-114. An immune serum against virus-diarrhea had likewise no effect on the virus KO-114. Of 49 human sera examined for antibodies against virus KO-114 all had a neutralizing index below 0.4.

DISCUSSION

The two strains isolated have been classified as bovine enteroviruses for the following reasons. They are RNA viruses, resistant to ether and low pH, and are able to pass filters with a pore size of 50 mμ. They have none of the characteristics of reo-viruses in tissue culture (*Sabin* 1959), and they do not agglutinate human or bovine erythrocytes, as reo-viruses do (*Eggers et al.* 1962). In the heat stability tests findings varied from test to test and in this respect our strains are in accordance with the M-types of ERC viruses (ECHO-28, rhinovirus and coryza virus) (*Ketler et al.* 1962). Rhinoviruses, however, differ from enteroviruses in greater acid lability being quickly inactivated at pH 5.2 for 30 min. (*Bögel & Böhm* 1962).

Virus KO-114 does not seem to be widely distributed among Danish cattle and is not related to the VD & MD complex. *Moscovici et al.* (1961) examined 24 human serum samples for antibodies against three bovine enteroviruses and found that all 24 sera neutralized two of the strains. Only two sera had antibodies against the third virus strain. None of our 49 human sera had this property against the bovine enterovirus KO-114.

The hemagglutinating properties of the two strains of virus KO-114 toward rhesus monkey erythrocytes demonstrate that both strains belong to group I in La Placa's classification of bovine enteroviruses (*La Placa et al.* 1965).

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SUMMARY

During investigations on the virus diarrhea-mucosal disease complex, a virus (KO-114) was isolated from a six-month-old calf, and on the basis of physicochemical characteristics the virus was classified as an enterovirus. The hemagglutinating properties indicate that it belongs to La Placa's group I. Examination of 103 bovine sera for neutralizing capacity against virus KO-114 gave very low titers only 13 % having an index between log. 1 and log. 2. Human sera did not neutralize this virus, which is not related to the virus diarrhea-mucosal disease complex.

ZUSAMMENFASSUNG

Die Isolierung eines bovinen Enterovirus.

Vom einem sechs Monate alten Kalb wurde ein Virus isoliert, welches auf Grundlage der physikalisch-chemischen Verhältnisse als ein bovines Enterovirus klassifiziert wurde. Das Virus agglutiniert Blutkörper von Rhesus-Affen, jedoch nicht von Mensch und Vieh. Es muss daher zu La Placa's Enterovirus Gruppe I zählen. Das Virus wurde von einem Kalb isoliert dessen Krankheit zu dem Mucosal Disease Komplex gerechnet wird. Serologische Untersuchungen zeigen jedoch keine Relation zwischen dem isolierten Virus und dem erwähnten Krankheitsbild. Eine Untersuchung von 103 bovinen Seren auf virusneutralisierenden Antistoff zeigte, dass das betreffende Virus hierzulande kaum weitverbreitet ist.

SAMMENDRAG

Isolering af et bovint enterovirus.

Fra en 6 mdr. gammel kalv isoleredes et virus, der på basis af fysisk-kemiske forhold er klassificeret som et bovint enterovirus. Virus agglutinerer blodlegemer fra rhesus abe, men ikke fra menneske eller kvæg. Det må derfor henregnes til La Placa's enterovirus gruppe I. Virus isoleredes fra en kalv, hvis sygdom må henregnes til mucosal disease komplekset, men serologiske undersøgelser giver intet holdepunkt for nogen relation mellem det isolerede virus og det nævnte sygdomsbillede. En undersøgelse af 103 bovine sera for virusneutraliserende antistof viste, at det pågældende virus næppe har nogen større udbredelse her i landet.

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