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Prevalence of antibodies to four bovine rotavirus strains in different age groups of cattle

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ABSTRACT

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Neutralizing antibody titers to four bovine rotavirus strains, representing three serotypes, were measured in 160 sera from cattle of different age groups. Age-specific seroprevalence analysis revealed serotype 6, represented by bovine rotavirus (BRV) NCDV, as the predominant rotavirus serotype infecting German cattle and serotype 10, represented by BRV V1005, as the least prominent. Infections with serotype 8, represented by BRV 678, occurred with intermediate frequency. Antibodies of young calves distinguished between NCDV and UK virus, two serotype 6 BRV strains differing in VP4 antigen.

INTRODUCTION

Rotaviruses are important worldwide causes of acute diarrhoea in humans and in many domestic animals, cattle in particular (McNulty, 1978). Unlike man, calves are usually affected by rotavirus-associated diarrhoea within the first 2 weeks of life (Woode and Bridger 1975; Acres et al., 1977; de Leeuw et al., 1980). At least three serotypes of bovine rotaviruses have been isolated from calves (Ihara et al., 1983; Murakami et al., 1983; Woode et al., 1983; Bridger and Brown, 1984; Snodgrass et al., 1984; Brüssow et al., 1987; Bellinzoni et al., 1989; Snodgrass et al., 1990).

The vast majority of rotaviruses isolated from calves with clinical signs belonged to one serotype (Snodgrass et al., 1984), but adult cows generally showed serum antibodies to different serotypes (Brüssow et al., 1988). The seroepidemiology of rotavirus infection in cattle has not been studied. In the present report we have analyzed the age development of neutralizing antibodies to four bovine rotavirus strains belonging to three serotypes.

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MATERIALS AND METHODS

Bovine serum samples

Rotavirus antibodies were quantitatively measured in a total of 160 bovine sera. Of these, 143 were sent to the Institute of Medical Microbiology, Munich, F.R.G., in the years 1979 to 1983 (before the introduction of dam vaccination, Eichhorn et al., 1983) for diagnostic purposes unrelated to this study. Cases of calf diarrhoea were excluded from the study. Cattle, aged 4 days to 9 years, came from dairy farms situated 50 km around Munich. Animals more than 6 months old were almost exclusively females, whereas about half of the calves up to this age were males, January to March is the main calving season around Munich. The 160 sera were arranged into eight groups according to the age of the animals (see Table 2). Each serum sample represented a different animal. Only the seven commercial lots (Flow, Seromed, Boehringer) of fetal sera were pooled sera from many fetuses. Ten additional fetal serum samples were collected from individual bovine fetuses at the abattoir in Munich.

Guinea pig antisera

Hyperimmune sera against four bovine rotavirus strains were prepared as described by Brüssow et al. (1987).

Viruses

Nebraska calf diarrhoea virus (NCDV) has been isolated in the United States (Mebus et al., 1969) and represents the prototype of serotype 6 rotavirus in the serotype numbering system of Hoshino et al. (1984). Bovine rotavirus UK has been isolated in Great Britain (Bridger and Woode, 1975); it shares VP7, the major neutralization antigen, with NCDV rotavirus (Glass et al., 1985), but if differs from NCDV rotavirus with respect to VP4, the minor neutralization antigen (Hoshino et al., 1985; Kantharidis et al., 1988; Nishikawa et al., 1988). It is classified to as serotype 6 rotavirus in the numbering system of Hoshino et al. (1984) or alternatively as bovine rotavirus serotype 1 (BRV 1) in the numbering system of Snodgrass et al. (1984). Bovine rotavirus 678 was isolated in Great Britain and represents a second serotype of bovine rotavirus (BRV 2) (Snodgrass et al., 1984). Snodgrass et al. (1990) showed that the latter shares neutralization specificity with human rotavirus 69M (Matsuno et al., 1985), classified as serotype 8 in the numbering system of Hoshino et al. (1984). Bovine rotavirus V1005 was isolated in Western Germany (Bachmann and Hess, 1981) and is serologically different from UK rotavirus (Brüssow et al., 1987). Snodgrass et al. (1990) showed that it shares neutralization specificity with bovine rotavirus B223 (Woode et al., 1983) which has been referred to as a third bovine rotavirus serotype

(BRV 3) in the numbering system of Snodgrass et al. (1984) or serotype 10 rotavirus in the numbering system of Hoshino et al. (1984).

Rotavirus strains NCDV, UK and V1005 were obtained from P. Bachmann (Munich, F.R.G.) and strain 678 from D. Snodgrass (Edinburgh, Great Britain).

Neutralization test

Sera were tested for neutralizing antibodies to bovine rotaviruses NCDV, UK, V1005 and 678 as described by Gerna et al. (1984).

Briefly, MA-104 cells, which were grown in 96-well microtiter plates, were inoculated with 10^2 50% tissue culture infective doses of the indicated rotavirus strain after the virus had been incubated for 1 h at 37°C with a 1:30, 1:90, 1:270, 1:810, 1:2430 or 1:7290 serum dilution. Cells were incubated for 1 day, fixed with absolute ethanol and then reacted with a rabbit hyperimmune serum sample that was directed against SA11 single-shelled rotavirus particles. Finally a peroxidase-coupled goat antibody to rabbit immunoglobulin G (IgG) was added and intracellular viral antigen revealed by 3-amino-9-ethyl-carbazole.

RESULTS

Serological diversity of bovine rotaviruses

The identity of each rotavirus strain used in this study was confirmed by neutralization with guinea pig hyperimmune sera (Table 1). Guinea pig antisera to BRV NCDV showed 16 to 100 fold lower neutralization titers against the other three BRV strains, including BRV UK. BRV NCDV was not neu-

TABLE 1

Serological characterization with guinea pig hyperimmune sera of the bovine rotavirus strains used in the study

Antiserum to rotavirus	Neutralizing antibody titer to rotavirus strain			
	NCDV	UK	678	V1005
NCDV[1]	12800	400	200	<100
NCDV	3200	200	<100	<100
UK	300	6000	1600	100
UK	1200	6000	1600	300
678	<100	<100	3200	100
678	<100	100	12800	200
V1005	<100	<100	<100	6400
V1005	<100	100	6400	6400
V1005	<100	<100	100	1600

Homologous titers underlined, all preimmune titers < 50.
[1]Results of individual animals.

tralized by antisera to BRV 678 and V1005. Antisera to BRV 678 and V1005 neutralized, with one exception, only the homologous strain, whilst antisera to BRV UK neutralized BRV NCDV and 678, but not BRV V1005.

Age-specific prevalence of rotavirus-specific antibodies in cattle

When a neutralization titer of 90 was taken as the cut-off point for serum positivity, 77% of the calves up to 1 month of age had neutralizing antibodies to BRV NCDV (Table 2). Only 23 to 36% of the sera showed neutralizing antibodies to BRV V1005, UK and 678. Prevalence levels to all BRV strains increased during the next months of life (Table 2).

Since the definition of cut-off points is arbitrary, the geometric mean titers of the neutralizing antibodies were also studied (Table 3). In 1-month-old calves, neutralization titers to BRV NCDV were significantly higher than those to BRV UK (mean titer difference for individual sera: 116 ± 24 (s.e.m.), $P=0.002$ in t-test for paired sera) and to BRV V1005. Geometric mean titers of neutralizing antibodies to all BRV strains increased during the next months of life, e.g. in comparison with 1-month-old calves, antibody titers to BRV NCDV were significantly increased in the 4 to 12 month age group ($P=0.03$).

Specificity of the neutralizing antibodies

The specificity of the neutralizing antibodies changed with the age of the cattle (Table 4). The highest frequency of monospecific sera was observed in 1-month-old calves (53%). Sera neutralizing only one BRV strain occurred, with one exception, in cattle up to 1 year of age ($P \leq 0.001$, exact Fisher test). Although $\geq$ 70% of sera taken after 1 year of age neutralized all four BRV

TABLE 2

Prevalence of neutralizing antibodies to four strains of bovine rotavirus in different age groups of cattle

Age group	No. of sera tested	Neutralizing antibodies to indicated bovine rotavirus (serotype)			
		NCDV (6)	UK (6)	678 (8)	V1005 (10)
Fetal	17	0	0	0	0
0–1 month	22	77[1] (100)[2]	27 (59)	36 (77)	23 (36)
1–2 months	19	74 (100)	53 (84)	74 (84)	58 (84)
2–4 months	19	95 (100)	63 (79)	53 (79)	42 (74)
4–12 months	16	94 (94)	62 (81)	62 (88)	56 (69)
1–3 years	27	100 (100)	85 (100)	96 (100)	78 (100)
3–5 years	27	100 (100)	85 (100)	100 (100)	93 (100)
> 5 years	13	100 (100)	100 (100)	100 (100)	100 (100)

[1]% of sera showing neutralizing titers ≥ 90 to indicated rotavirus.
[2]% of sera showing neutralizing titers ≥ 30 to indicated rotavirus.

TABLE 3

Geometric mean titers of neutralizing antibodies to four strains of bovine rotavirus in different age groups of cattle

Age group	No. of sera tested	Neutralizing antibodies to indicated bovine rotavirus (serotype)			
		NCDV (6)	UK (6)	678 (8)	V1005 (10)
Fetal	17	0	0	0	0
0–1 month	22	122	16	42	7
1–2 months	19	152	50	80	48
2–4 months	19	257	56	45	35
4–12 months	16	369	105	113	34
1–3 years	27	603	167	277	131
3–5 years	27	480	227	544	200
> 5 years	13	622	419	578	368

TABLE 4

Specificity of neutralizing antibodies in different age groups

Age group	No. of sera tested	No. of neutralizing sera	No. of sera neutralizing the indicated no. of bovine rotavirus strains			
			One strain	Two strains	Three strains	Four strains
0–1 month	22	17[1] (22)[2]	9[1] (5)[2]	2 (3)	1 (7)	5 (7)
1–2 months	19	17 (19)	1 (1)	5 (3)	6 (0)	5 (15)
2–4 months	19	18 (19)	5 (3)	4 (1)	0 (2)	9 (13)
4–12 months	16	15 (15)	4 (1)	1 (1)	2 (1)	8 (13)
1–3 years	27	27 (27)	1 (0)	1 (0)	6 (0)	19 (27)
3–5 years	27	27 (27)	0 (0)	1 (0)	4 (0)	22 (27)
> 5 years	13	13 (13)	0 (0)	0 (0)	0 (0)	13 (13)

[1]Positive cut-off point set at 1:90 serum dilution.
[2]Positive cut-off point set at 1:30 serum dilution.

strains, only 29% of the sera taken within the first 2 months of life showed similar neutralizing capacity.

Twenty sera neutralized only a single BRV strain. Furthermore 95% of these sera were specific to BRV NCDV. 14 sera neutralized just two BRV strains. 86% of these sera neutralized BRV NCDV in combination with either BRV UK or BRV 678 (Table 5). 19 sera neutralized three BRV strains. The strain not neutralized by these sera was either BRV V1005 or BRV UK. The specificity of the sera was essentially unchanged, when a neutralization titer of 30 was taken as cut-off point (Table 4, 5).

TABLE 5

Specificity of neutralizing antibodies with respect to four individual bovine rotavirus strains

Sera neutralizing one strain, $n=20$ (10)	Sera neutralizing two strains, $n=14$ (8)	Sera neutralizing three strains, $n=19$ (10)
NCDV 19[1] (10)[2]	NCDV+678 6 (5)	not V1005 10 (9)
V1005 1 (0)	NCDV+UK 6 (1)	not UK 9 (1)
	NCDV+V1005 0 (2)	
	678+V1005 2 (0)	

[1]Positive cut-off point set at 1:90 serum dilution.
[2]Positive cut-off point set at 1:30 serum dilution.

DISCUSSION

Neutralization tests with guinea pig hyperimmune sera revealed complex serological relationships between four representative BRV strains. BRV 678 has been characterized as a second serotype of bovine rotavirus (Snodgrass et al., 1984) and indeed our antisera to BRV 678 did not neutralize any of the other three BRV strains. There was, however, a one-way crossreaction between BRV UK and 678. BRV V1005 has been characterized as a second BRV serotype (Brüssow et al., 1987), and in fact we showed that it was not neutralized by any of the heterologous antisera tested. Furthermore, with one exception, antisera to BRV V10005 did not neutralize any of the three other BRV strains. Recently it was shown that BRV V1005 was neutralized by antisera to BRV B223 (Woode et al., 1983), a representative third serotype of BRV (Snodgrass et al., 1990).

The guinea pig hyperimmune sera distinguished between BRV NCDV and UK, which have both been assigned to serotype 6. Some antigenic variation between these strains have been previously reported (Ojeh et al., 1984). It is possible that the sera detected differences between the distinct VP4 antigen of the viruses (Hoshino et al., 1985; Kantharidis et al., 1988; Nishikawa et al., 1988), although it is generally believed that animal hyperimmune sera react preferentially with VP7 (Matsui et al., 1989). Alternatively minor sequence variations in the variable regions A, C and E of VP7 (Glass et al., 1985) may account for the serological differences.

The aim of this study was to examine the extent to which the natural host distinguishes between four representative BRV strains. For this purpose we determined the age-specific titers of neutralizing antibodies to all four BRV strains in sera of cattle. One-month-old calves showed a predominance of sera neutralizing only one BRV strain. It seems probable that these monospecific sera reflect a recent rotavirus infection. This interpretation fits with clinical experience (Woode and Bridger, 1975; Acres et al., 1977; de Leeuw et al.,

1980) and a longitudinal study (Reynolds et al., 1985), all showing that a high percentage of calves become infected within the first month of life.

On the other hand, cattle older than 1 year typically showed antibodies neutralizing three or four BRV strains. In the absence of any data on serotypes isolated from individual calves several different interpretations of the serological data are possible. As a calf gets older, it's antibody response to a single rotavirus infection might become broader. It is also possible that a second rotavirus infection induces a broader response than the first. It is not necessary to invoke sequential rotavirus infections with different serotypes to explain the broad neutralizing capacity of cattle older than 3 years. Similar responses occur in cattle following multiple infection with a single serotype of some viruses. However, it is interesting to note that nearly all monospecific sera are specific for BRV NCDV and nearly all sera with dual neutralization specificity neutralize BRV NCDV. In addition, antibody titers to BRV NCDV are substantially higher than titers against BRV UK and V1005. This fits well with epidemiological surveys, where 90% of calf rotavirus isolates belonged to serotype 6 (Snodgrass et al., 1984; Snodgrass et al., 1990). The lowest prevalence and antibody titers were found with BRV V1005, which is also the predominant strain not neutralized by sera neutralizing three other strains. This fits with epidemiological observations, where only 5 to 10% of calf rotavirus isolates belonged to this third BRV serotype (Snodgrass et al., 1984; Snodgrass et al., 1990).

Transmission of passive immunity from the dam to the calf occurs during a brief interval after birth through absorption of colostral antibodies by the neonatal gut, since antibody transfer across the placenta is negligible (Brambell, 1970). Previously we have shown that bovine colostrum commonly shows elevated antibody titers to all BRV strains (Brüssow et al., 1988). It is therefore surprising that we observed such a high percentage of monospecific sera in 1-month-old calves. In these calves as well as in 1-month-old calves lacking rotavirus-specific antibodies, failure of passive immune transfer may have occurred. In one study 30% of calves remained agammaglobulinaemic despite the experimentally controlled ingestion of colostrum (Klaus et al., 1969). On the other hand, this may reflect late administration of colostrum to the calves. Finally, it must be borne in mind, that we studied sera that were sent to us for diagnostic purpose. Calves ingesting insufficient colostrum at birth often do poorly later in life. Consequently, their sera frequently show up in diagnostic laboratories.

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