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NOTES

Serotype 1 and 2 Bovine Noroviruses Are Endemic in Cattle in the United Kingdom and Germany▽

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The genomically and antigenically distinct bovine noroviruses Bo/Jena/1980/DE and Bo/Newbury2/1976/UK have been associated with calf diarrhea. In the present seroprevalence study, both were found to be endemic in cattle from Germany and the United Kingdom, a finding in contrast to previous virus prevalence studies. They were less common than group A rotaviruses, particularly in calves, suggesting a different epidemiology.

Viruses classified within the genus *Norovirus* of the family *Caliciviridae* are an established, widespread cause of gastroenteritis in humans (10). More recently, they were recognized in cattle, in association with calf diarrhea, and classified into a genogroup (III) separate from human noroviruses (1, 17, 19, 22, 23). Two bovine norovirus genotypes have been identified so far and are represented by the Jena virus for genotype 1 and the Newbury2 virus for genotype 2 (8, 14). The two genotypes were found to be antigenically distinct by enzyme-linked immunosorbent assay (ELISA), thus representing two serotypes, Bo/Jena/1980/DE for genotype/serotype 1 and Bo/Newbury2/1976/UK for genotype/serotype 2 (15). Bovine noroviruses have been identified by reverse transcription (RT)-PCR in the United Kingdom, The Netherlands, and the United States (17, 19, 22, 23). However, neither genotype has been found universally. In the Dutch study, where the majority of samples were from diarrheic and nondiarrheic calves aged 3 months or under, only genotype 2 viruses were reported (22). In the United Kingdom study of diarrheic calves mainly aged under 1 month, a few genotype 1 polymerase sequences were identified, but further analysis of one showed it to be a recombinant virus with a genotype 2 capsid gene (16). Similarly, in the United States, the one genotype 1 polymerase sequence was associated with a genotype 2 capsid (12, 19), but, by contrast, genotype 1 and 2 capsid genes were found equally in a second study of 5- to 10-day-old diarrheic calves from the United States (23). In Germany, antibody to the Jena virus was found to be widespread in cattle aged 10 weeks to 9 years (9). The presence of genotype 2 bovine noroviruses in Germany has not been reported. Thus, to clarify the prevalences of these viruses in cattle, their seroprevalences were investigated, for the first time, with bovine sera from Germany and the United Kingdom and compared to the prevalence of bovine rotaviruses.

A total of 400 archived sera or plasma samples were taken from four cohorts of cattle: 100 sera from United Kingdom calves, aged 6 months, from seven farms in the counties of Berkshire, Essex, Somerset, and Sussex during 2004; 100 plasma samples from United Kingdom adult dairy cows from five farms in the counties of Berkshire, Essex, and Nottinghamshire between 1999 and 2000; 100 plasma samples from German calves, aged 6 months, from 12 farms in Thuringia, Germany, during January and February 2002; and 100 plasma samples from German cattle, aged 19 to 98 months, from six of the same farms.

ELISAs were performed as described previously (15). Briefly, virus-like particles of Bo/Jena/1980/DE (genotype/serotype 1), Bo/Newbury2/1976/UK (genotype/serotype 2), or the bovine RF rotavirus (VP2/VP6 [kindly supplied by A. Charpilienne, Unité Mixte de Recherche CNRS-INRA, VMS, 91198 Gif-sur-Yvette, France]) were used as test antigens at a concentration of 5 μg/ml. Supernatants from mock-infected Sf9 cells were used as a negative control antigen. Samples were tested in duplicate at a single dilution of $\log_{10}$ 2.3 to avoid any low-level cross-reactivity between the two bovine norovirus serotypes (15). Positive control sera were from experimental calves given Bo/Jena/1980/DE (K321) (15), Bo/Newbury2/1976/UK (P131) (4), or the bovine group A rotavirus UK (S7) (3). The homologous positive control serum was titrated out on every plate, and endpoints were determined by regression analysis. Heterologous sera were tested at a $\log_{10}$ 1.7 dilution as

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TABLE 1. Positivities and P:N ratios of samples from cattle from the United Kingdom and Germany that were positive by ELISA for antibody to serotype 1 or 2 bovine norovirus or bovine rotavirus[a]

Sample source (no. of samples tested)	Serotype 1			Serotype 2			Bovine rotavirus UK		
	No. (%) positive	P:N ratio		No. (%) positive	P:N ratio		No. (%) positive	P:N ratio	
		Median	Range		Median	Range		Median	Range
United Kingdom calves (100)	77	4.1	2.0–18.5	87	10.0	2.2–39.6	99	19.7	2.5–71.8
United Kingdom adults (100)	98	9.0	2.0–77.8	66	5.7	2.0–58.0	100	37.0	10.1–83.4
Totals (United Kingdom calves + adults) (200)	175 (87.5)			153 (76.5)			199 (99.5)		
German calves (100)	66	3.9	2.0–28.0	87	14.0	2.0–92.5	100	26.8	2.5–83.9
German adults (100)	71	6.6	2.0–23.1	94	20.4	2.0–83.6	100	44.0	5.0–71.3
Totals (German calves + adults) (200)	137 (68.5)			181 (90.5)			200 (100)		
Overall totals (400)	312 (78)			334 (83.5)			399 (99.8)		

[a] Medians and ranges of the P:N ratios were determined only for samples that were positive for the test antigen.

negative serum controls. The net absorbance for each test sample was determined by subtracting the mean absorbance value with the negative control antigen (mock-infected Sf9 cell supernatant fluid) from the mean absorbance value with the test antigen. Positive/negative (P:N) ratios were calculated by dividing the net absorbance of test samples by that obtained without test serum. Test samples were considered positive with a P:N ratio of 2 or more. Fisher's exact test, two-tailed, was used to determine significant differences between antibody prevalences. All three ELISAs showed good reproducibility throughout the experimental period. The serotype 1 norovirus antiserum, K321, had a mean titer of $\log_{10}$ 3.5 (n, 6; standard deviation [SD], $\log_{10}$ 0.18); the serotype 2 antiserum, P131, had a mean titer of $\log_{10}$ 3.6 (n, 6; SD, $\log_{10}$ 0.11); and the hyperimmune rotavirus antiserum, S7, had a mean titer of $\log_{10}$ 6.1 (n, 6; SD, $\log_{10}$ 0.10). These sera were negative to the heterologous antigens.

Antibody to both bovine norovirus serotypes was common in cattle but less common than rotavirus antibody, which, as expected, reached almost 100% (Table 1). There was no evidence to suggest that serotype 1 was confined to, or predominant in, Germany or that serotype 2 was confined to, or predominant in, the United Kingdom, as might be predicted from previous virus prevalence studies (9, 17). Cattle from the United Kingdom (combined data from calves and adults) had a 19% higher prevalence of serotype 1 antibody than did German cattle ($P = 0.002$), and German cattle (combined data from calves and adults) had a 14% higher prevalence of serotype 2 antibody than did cattle from the United Kingdom ($P = 0.01$).

The numbers of United Kingdom sera positive for serotype 1 or 2 antibodies differed between calves and adults. More adults had serotype 1 antibody, but fewer adults had serotype 2 antibody. However, differences were less marked between German calves and adults. The percentages of United Kingdom adult sera positive for serotype 1 and German adult sera positive for serotype 2 were close to that found for rotaviruses (100%). In contrast, antibody to both norovirus serotypes was less common in both United Kingdom and German calves than was rotavirus antibody. In United Kingdom and German calves, the prevalence of serotype 2 was higher than that of serotype 1 ($P = 0.0007$). In adults, serotype 1 was predominant in the United Kingdom ($P < 0.0001$) but serotype 2 was predominant in Germany ($P < 0.0001$).

The majority of United Kingdom and German cattle (calves and adults combined) had evidence of infection with one or both norovirus serotypes (Table 2), and many showed evidence of exposure to both norovirus serotypes and rotaviruses. Of those calves that had antibody to a single norovirus serotype, more had antibody to serotype 2 than to serotype 1 in the United Kingdom ($P = 0.025$) and Germany ($P < 0.0002$), reflecting the higher antibody prevalence of serotype 2 than type 1 in United Kingdom and German calves. Of those adults that had antibody to a single serotype in the United Kingdom, none had antibody to serotype 2 but a third had antibody to serotype 1, whereas in Germany the opposite was true, reflecting the antibody prevalence data. P:N ratios were consistently higher for rotavirus than for both norovirus serotypes in the four cohorts of cattle (Table 1). Comparison of P:N ratios for both norovirus serotypes showed complete correlation with antibody prevalence: P:N ratios were higher when antibody prevalence was higher.

Thus, antibody to both bovine noroviruses was common in both United Kingdom and German cattle but less common than rotavirus antibody, which, as expected, was found in nearly all samples tested. The results confirmed previous data showing that serotype 1 noroviruses were endemic in German

TABLE 2. Percentages of samples from calves and adults from the United Kingdom and Germany that were positive to one or more of the three antigens

Sample source	% Positive				
	Bovine norovirus			Rotavirus only	All 3 antigens
	Either serotype	Serotype 1 only	Serotype 2 only		
United Kingdom calves[a]	93	6	17	6	70
United Kingdom adults	98	32	0	2	66
German calves	95	8	29	5	58
German adults	95	1	24	5	70

[a] One serum sample did not have detectable levels of antibody to any of the three antigens.

cattle (9) but provided the first evidence that serotype 2 noroviruses are also endemic in German cattle. The results with the samples from the United Kingdom contrasted with the previous virus prevalence study (17) by showing that antibody to both noroviruses was prevalent in the United Kingdom. The 68.5% seroprevalence of serotype 1 bovine noroviruses in German cattle was lower than that of 99% reported previously (9), but this is likely to be due to the use, in the present study, of higher dilutions of test samples to avoid cross-reactivity between the bovine norovirus serotypes (15).

The high seroprevalence of serotype 1 in cattle from the United Kingdom was unexpected. Only three genotype 1 polymerase sequences were identified in the United Kingdom molecular prevalence study with samples collected in 1999 and 2000 from calves mainly aged under 1 month. More detailed analysis of one sample showed it to be a recombinant virus with a genotype 1 polymerase gene and a genotype 2 capsid gene (16). Genotype 1 capsid genes have yet to be identified in the United Kingdom, but the present study showed antibody to them to be present in the United Kingdom. Similarly, genotype 1 bovine noroviruses have been identified rarely in some studies elsewhere using RT-PCR (12, 17, 19, 22, 23). Reasons for the lack of detection of genotype 1 noroviruses include the use of suboptimal primers to detect them by RT-PCR, the use of primers to the polymerase gene, the existence of chimeric viruses with genotype 2 polymerase genes but genotype 1 capsid genes (23), or differences in the ages of the cattle populations studied. In the published virus prevalence studies (12, 17, 19, 22, 23), most samples came from calves under 6 months of age, with the majority under 1 month of age. However, in the present seroprevalence study, samples came from calves aged 6 months or from cows.

The data suggested different epidemiologies for both bovine norovirus serotypes compared to bovine rotaviruses. Human noroviruses differ in epidemiology from human rotaviruses (reviewed in references 10 and 13): the latter primarily cause childhood diarrhea, whereas the former are more common in adult diarrhea outbreaks. A similar pattern may be true for bovine noroviruses. Further studies are needed to assess whether there are age-associated differences in the prevalence of the two bovine noroviruses.

Calf enteritis causes considerable morbidity and mortality, resulting in significant economic losses (21). Enteropathogens are not found in approximately 30% of calf diarrhea samples (2, 18), but the presence of noroviruses is not routinely tested. The present study showed the endemic nature of bovine noroviruses, and previous experimental studies showed that they are enteric pathogens (4, 11). Thus, there is a case for their inclusion in calf enteric vaccines if both viruses are shown to be implicated in calf diarrhea. Current calf diarrhea vaccines are based on lactogenic immunity, wherein dams are vaccinated to raise colostral antibodies (5–7, 20), but calf diarrhea outbreaks still occur in vaccinated herds (5). Incorporation of bovine noroviruses into calf diarrhea vaccines may increase vaccine efficacy, but it is likely that both bovine norovirus serotypes will need to be incorporated.

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