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The prevalence of serum neutralizing antibodies to infectious bovine rhinotracheitis virus in Scotland

By P. M. MSOLLA, A. WISEMAN and I. E. SELMAN
University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow

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SUMMARY

The prevalence of serum neutralizing antibodies to infectious bovine rhinotracheitis virus in 1152 serum samples from adult cattle in 114 dairy and beef herds in different regions of Scotland was 12%. In the Grampian region, the number of seropositive cattle in the self-contained herds was significantly less ($P < 0{\cdot}01$) than in the 'other' herds. Holstein cattle had been introduced into five of these 'other' herds, and significantly more ($P < 0{\cdot}01$) of the samples from these five herds were seropositive compared with the samples from the rest of the 'other' herds in the same region into which recently purchased cattle had been introduced. The introduction of Holstein cattle was also a major factor in the association between the prevalence of antibodies and herd size in the Grampian region. The prevalence of serum neutralizing antibodies to infectious bovine rhinotracheitis virus was significantly greater ($P < 0{\cdot}001$) in this survey than in those previously undertaken in the United Kingdom.

INTRODUCTION

Following the recognition of infectious bovine rhinotracheitis (IBR) in Britain (Dawson *et al.* 1962; Darbyshire & Shanks, 1963), Dawson & Darbyshire (1964) undertook a serological survey to determine the occurrence and distribution of antibodies to the IBR virus in the United Kingdom. Serum neutralizing (SN) antibodies were detected in only 42 (2·1%) of 2000 sera obtained either from abattoirs (1000) or from suspected incidents of mucosal disease (1000). More recently, Kirby, Martin & Waring (1978) examined 2368 sera from dairy herds in southern England taken during the brucellosis eradication campaign. Using the indirect haemagglutination (IHA) test, they found antibodies in 162 sera (6·8%). It can be deduced, therefore, that IBR virus infection was widespread throughout the country although the prevalence of disease would appear to have been low, since in neither survey was infection known to have been associated with clinical signs. Until 1978 the available published evidence indicated that IBR was a relatively mild, economically unimportant respiratory disorder.

During the winter housing period of 1977–8, however, there appeared in certain parts of northern Britain a severe upper respiratory tract disease, which was diagnosed as IBR (Wiseman *et al.* 1978). Many such incidents were subsequently confirmed in the Grampian region of Scotland and the disease has occurred in

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most of the other regions also (Anonymous, 1979; Cuthbertson & Wood, 1979; Imray, 1979; Wiseman *et al.* 1979). Since there had been a dramatic change in the nature of this disorder, it was decided to investigate the prevalence of SN antibodies to IBR virus in different regions of Scotland.

MATERIALS AND METHODS

Herd description. The dairy herds were selected from the following regions: Strathclyde 56 herds, Grampian 40 herds, Central 4 herds, Dumfries and Galloway 3 herds. In addition, samples were obtained from 11 beef suckler herds located either in the Strathclyde (7 herds) or the Highland (4 herds) regions. A 'self-contained' herd has been defined as one into which purchased female cattle were said not to have been introduced for several years. Herds into which purchased female cattle had been introduced recently have been designated 'other' herds.

Blood samples. The blood samples, which were taken from 10 randomly selected, adult cattle in each herd, were collected from the caudal vein into vacutainers containing no additive (Becton Dickinson UK Ltd, England). The samples were stored overnight at 4 °C and, after being spun at 1300*g* for 30 min, the serum was removed and put in a waterbath at 56 °C for 30 min, left to cool and then stored at −20 °C until required.

Infectious bovine rhinotracheitis virus. The 'Strichen' strain of virus, which was used in the SN test, had been isolated from a field incident of severe IBR (Wiseman *et al.* 1978). The virus was grown up in secondary calf kidney cells at 37 °C and was harvested when approximately 90% of the monolayer showed a cytopathic effect (CPE). After a single cycle of freezing and thawing, the resulting fluid was centrifuged at 168*g* for 15 min in a refrigerated GF-6 MSE centrifuge. The supernatant (virus suspension) was put into 0·5 ml ampoules and stored at −70 °C until required. When used in the SN test, the virus was in its third passage in secondary calf kidney cell cultures.

Tissue cultures. Secondary calf testes cell monolayers were used in the SN tests. After dispersal, the cells were suspended in M 199 medium, containing 10% fetal bovine serum, at a concentration of 2×10^5 cells per ml.

Serum neutralization test. The SN tests were carried out in flat-bottomed, tissue culture grade, microtitre plates (Linbro-Cooke-96, Flow Laboratories, Scotland). The serum samples were diluted initially to 1 in 4 with sterile PBS, and then serial twofold dilutions were made.

To each well, except those of the serum control column, there was added 0·025 ml of virus suspension containing 30 $TCID_{50}$ per ml. The plates were sealed with sterile aluminium foil and incubated at 37 °C for 2 h. At the end of this period, 0·1 ml of the secondary calf testes cell suspension was added to each well. The plates were then incubated at 37 °C in an atmosphere of 5% carbon dioxide.

The test was read on the third day using an inverted microscope to examine the cell sheet in the wells for evidence of a CPE. The titre of the test serum was expressed as the reciprocal of the final dilution of the serum present in the serum–virus mixture at the 50% end-point estimated according to the method of Kärber (1931). A titre of 4 or greater was considered to be positive.

RESULTS

Serum neutralizing antibodies to the IBR virus were detected in 140 (12%) of the 1152 samples and at least one of the animals sampled was seropositive in 58 (51%) of the 114 herds. Although mild IBR had been diagnosed in previous years in seven herds, the proportion of positive sera from these herds (10/68: 15%) was not significantly greater than that from the rest of the herds (130/1084: 12%). Of the 1039 dairy cattle sampled, 117 (11%) had antibodies compared to 23 (20%) of the 113 beef animals sampled; this difference did not achieve statistical significance. There was at least one animal with antibodies in nine (82%) of the 11 beef herds whereas only 49 (48%) of the 103 dairy herds contained seropositive animals.

The prevalence of antibodies in the dairy cattle (Table 1) was virtually the same in the two main regions, Strathclyde (11%) and Grampian (12%). However, in Strathclyde significantly more cows (13%) than heifers (3%) had antibodies ($P < 0{\cdot}05$), although the proportion of seropositive cows (12%) and heifers (11%) in Grampian was similar.

The prevalence of antibodies was significantly less in the self-contained herds than in the other herds in the Grampian region ($P < 0{\cdot}01$), but not in Strathclyde. Animals of the Holstein breed had been introduced into five herds in Grampian and, of the 58 samples examined from these herds, 23 (40%) were positive compared to only 3 (5%) of the 61 sera from the rest of the herds in that region into which cattle had been introduced recently. This difference was highly significant ($P < 0{\cdot}01$).

When the dairy herds in the Grampian and Strathclyde regions were grouped according to their size (Table 2), it was found that the prevalence of antibodies was associated with herd size only in the Grampian region. The proportion of animals with antibodies was significantly greater ($P < 0{\cdot}001$) in the herds in which there were more than 100 cows compared with those in which there were less than 100 animals.

The geometric mean titre (GMT) of the 140 positive sera was 9·6 (Table 3). The GMT of the heifers (13·6) was greater than that of the cows (9·4). Although the number of heifers was small, this difference was statistically significant ($P < 0{\cdot}02$). Within the Grampian region, the GMT in the five herds in which there were Holstein animals was 7·8, compared with 9·2 in the other herds; this difference was not statistically significant.

DISCUSSION

The prevalence of SN antibodies to the IBR virus in adult cattle in certain regions of Scotland was found to be 12%. This is significantly greater ($P < 0{\cdot}001$) than either the 2·1% or the 6·8% reported in previous surveys in Britain by Dawson & Darbyshire (1964) and by Kirby *et al.* (1978), respectively. This difference may have resulted from a considerable increase in the incidence of infection with IBR virus, or from the use of a more sensitive antibody assay system, or as a result of both these factors.

Table 1. *The prevalence of serum neutralizing antibodies to infectious bovine rhinotracheitis virus in dairy cattle in Scotland*

	Total number			No. cows			No. heifers			Type of herd: Self-contained			Type of herd: Other		
Region	Sampled	Pos.	(%)	Sampled	Pos.	(%)	Sampled	Pos.	(%)	Sampled	Pos.	(%)	Sampled	Pos.	(%)
Strathclyde	560	62	(11)	464	59	(13)	96	3	(3)	510	51	(10)	50	11	(22)
Grampian	409	47	(12)	373	43	(12)	36	4	(11)	290	21	(7)	119	26	(22)
Central	40	8	(20)	40	8	(20)	—	—	—	40	8	(20)	—	—	—
Dumfries and Galloway	30	0	(0)	—	—	—	30	0	(0)	30	—	—	—	—	—
Total	1039	117	(11)	877	110	(13)	162	7	(4)	870	80	(9)	169	37	(22)

Table 2. *The effect of herd size on the prevalence of serum neutralizing antibodies to infectious bovine rhinotracheitis virus in dairy cattle in Scotland*

	Size of herd: Less than 100 cows				Size of herd: More than 100 cows			
	No. herds		No. cattle		No. herds		No. cattle	
Region	Sampled	Pos. (%)	Sampled	Pos. (%)	Sampled	Pos. (%)	Sampled	Pos. (%)
Strathclyde	41	19 (46)	410	47 (12)	15	7 (47)	150	15 (10)
Grampian	19	7 (37)	191	8 (4)	21	12 (57)	218	39 (7)
Central	4	4 (100)	40	8 (20)	—	—	—	—
Dumfries and Galloway	3	0 (–)	30	0 (–)	—	—	—	—
Total	67	30 (45)	671	63 (9)	36	19 (53)	368	54 (15)

Table 3. ***The distribution of serum neutralizing antibody titres to infectious bovine rhinotracheitis virus in cattle in Scotland***

Reciprocal of titre	No. of cattle	Type		Dairy animals						
				Region			Age of Animal		Type of herd	
		Beef	Dairy	Strath.	Gram.	Cen.	Cows	Heifers	S/Cont.	Other
4	19	1	18	8	9	1	17	1	10	8
6	26	7	19	8	10	1	19	—	11	8
8	21	7	14	4	8	2	14	—	8	6
12	48	5	43	28	12	3	40	3	34	9
16	11	1	10	5	5	—	9	1	5	5
24	12	1	11	8	2	1	10	1	10	1
32	2	—	2	1	1	—	1	1	2	—
64	—	—	—	—	—	—	—	—	—	—
96	1	1	—	—	—	—	—	—	—	—
Total	140	23	117	62	47	8	110	7	80	37
GMT	9·6	9·4	9·6	10·5	8·5	9·5	9·4	13·6	10·4	8·1

Cattle in the Grampian and Strathclyde regions were selected because IBR had been a serious problem for two years in Grampian while the number of outbreaks of severe disease had been comparatively low in Strathclyde. However, despite this marked regional difference in the incidence of severe disease, the prevalence of SN antibodies in the dairy herds in the two regions was virtually the same. One possible explanation for this apparent anomaly is that the recent severe incidents of disease have been predominantly a problem on fattening beef units, not on dairy farms (Wiseman, 1980). On these beef units, the clinical signs of IBR have usually developed initially in animals purchased a few weeks before from a livestock market and, therefore, it can be inferred that the infection had been introduced by these new arrivals.

In view of this, it was not surprising to find that, in both major regions, the prevalence of antibodies was considerably lower in the self-contained dairy herds compared with those into which purchased animals had been regularly introduced. In the latter herds, antibodies were present in 22% of the sera examined from both regions; this would suggest that there had been a similar level of exposure to IBR virus. However, in Grampian the introduction of Holstein cattle into several herds appeared to have been an important factor, as the prevalence of antibodies was significantly higher in these herds than in the rest into which cattle had been recently introduced. In particular, it was the introduction of Holstein females, as all the samples from one herd into which a bull had been imported were seronegative. The 'Holstein' factor was also responsible for the association between the prevalence of antibodies and herd size in the Grampian region, since all five herds into which they were introduced contained more than 100 cows.

In a recent study in France, SN antibodies to IBR virus were found in only 6% of French-born cattle compared with 49% of cattle imported from North America, most of which were of the Holstein breed (Dannacher & Fedida, 1978). In this study, 40% of the samples from herds into which Holstein animals had been imported were positive compared to only 7% of those from the wholly 'British' herds. Although entirely circumstantial, the serological evidence presented here would suggest that the purchase of Holstein females into these herds had also been associated with the introduction of IBR virus.

The prevalence of antibodies in the dairy heifers in Grampian was markedly greater than in Strathclyde, but was similar to the prevalence in the cows of both these regions. In addition, the GMT of the heifers in the Grampian region was much higher than that of the cows. In southern England, Kirby *et al.* (1978) also found a higher prevalence of antibodies and a higher GMT in cows than in heifers and considered that this indicated a stable situation with constant re-exposure in these herds. A similar situation is likely to have been present in Strathclyde, but not in Grampian.

Furthermore, Kirby *et al.* (1978) have suggested that IBR virus (bovine herpesvirus 1) may have been introduced into herds by the use of contaminated semen following artificial insemination, as respiratory signs had not been noted in any of the herds from which their samples had been obtained. However, it is possible neither to confirm nor to refute this suggestion without detailed examination of

semen samples and service records, since both artificial insemination and natural service are used to varying degrees in virtually every commercial dairy herd.

It has been reported that the IHA test is about ten times more sensitive than the SN test at detecting and assaying antibodies to IBR virus (Shimizu *et al.* 1972; Kirby, Martin & Ostler, 1974). Bearing this in mind, it is likely that the prevalence of antibodies in this study would have been considerably higher than the 12% actually recorded had the IHA rather than the SN test been used.

It can be inferred, as a result of this investigation, that the rate of infection with IBR virus in certain areas of Scotland was much higher in 1978 than had been found previously in other parts of Britain, especially as the relatively insensitive SN test had been used. The results from the Grampian region suggest that the pattern of infection with this virus was different from that in Strathclyde as both the prevalence of antibodies and the GMT were particularly high in the heifers. Furthermore, the high prevalence of antibodies in the large herds in the Grampian region was closely associated with the purchase of Holstein female cattle.

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