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A survey of virus infections of the respiratory tract of cattle and their association with disease

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SUMMARY

A total of 1590 calves were investigated between May 1972 and December 1975. Twenty-two per cent were treated for respiratory disease and 2·5% died of pneumonia. Almost 80% of the respiratory illness occurred in six sharp outbreaks. Samples for virology were collected routinely from 127 healthy calves and from 354 calves treated for respiratory signs and comprised 1143 nasopharyngeal swabs and 1069 sera. Virus infections were detected on 540 occasions including 135 by parainfluenzavirus type 3 (Pi-3), 78 by respiratory syncytial virus (RSV), 103 by rhinovirus, 49 by bovine virus diarrhoea virus (BVDV), 29 by adenoviruses, 53 by reoviruses and 88 by enteroviruses. The seasonal and age distribution of infections differed between viruses. Only infections by RSV, Pi-3 and BVDV were significantly associated with disease.

INTRODUCTION

Acute respiratory disease is thought to be a major cause of economic loss in the beef industry. However, its precise incidence, its exact cost and its causes all remain uncertain.

Two approaches have been used extensively in attempts to show that viruses cause respiratory disease in calves: first, animals were investigated during outbreaks of illness for evidence of virus infection and, secondly, viruses isolated from the bovine respiratory tract were inoculated experimentally into susceptible animals (see reviews by Darbyshire & Roberts, 1968; Phillip & Darbyshire, 1971; Mohanty, 1978). Much valuable information was obtained about the range of different viruses which infect the bovine respiratory tract and about their pathogenic potential. However, although investigations of cattle with respiratory disease revealed widespread infection by parainfluenza virus, type 3 (Pi-3), rhinoviruses (RV), bovine virus diarrhoea virus (BVDV), adenoviruses (AD), reoviruses (REO), enteroviruses (ENT) and, more recently, respiratory syncytial virus (RSV), few workers studied the same animals when healthy. Without a clear understanding of the ecology of these viruses and their distribution in normal, healthy cattle, it was difficult to assess their aetiological significance when found in animals with disease.

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Interpretation was further confounded by the finding that several different viruses often circulated simultaneously during an outbreak of disease (Rosenquist *et al.* 1970; Rosenquist & Dobson, 1974). Furthermore although large amounts of virus inoculated intratracheally induced pneumonia in some experimental animals, in many others there was little or no disease and the pathogenicity of such viruses disseminated under natural conditions remained uncertain. Thus, the natural history of these viruses is poorly understood.

This paper, which expands and intensifies our earlier work (Thomas & Collins, 1974), describes an epidemiological investigation over three years in which virus infections were followed in groups of animals during periods of health and disease.

MATERIALS AND METHODS

Population studied

The survey took place on a large beef-rearing farm in the South of England. Calves were collected together within the first 10 days of life from farms within a radius of 200 miles in both England and Wales and reared in groups of approximately 100 animals until slaughter about 18 months later. Records of treatment were kept for each animal and post-mortem examinations were carried out on all animals that died. Seventeen groups born between May 1972 and June 1975 were investigated.

Virological samples

The virological survey covered the period October 1972 to September 1975. Seven or eight animals for routine virological study were selected at random from each group. Nasopharyngeal swabs (NS) collected as described by Thomas & Stott (1975) and blood were taken from these calves every 3 or 4 weeks. Where possible, calves were sampled from arrival at the farm until they were 9 months old. When possible NS and paired serum samples were also collected from animals which were treated for respiratory disease.

Virus isolation

Two tubes of secondary calf kidney (CK) cells and two tubes of primary or secondary calf testis (CTe) cells were each inoculated with 0·2 ml of NS extract. The CK cells were maintained in Eagles' basal medium (Flow Laboratories) containing 2% heat-inactivated fetal calf serum, 5% tryptose phosphate broth, 0·1% sodium bicarbonate, 100 μg/ml ampicillin, 100 μg/ml kanamycin and 50 units/ml fungizone; the pH was adjusted to 7·2 with 25 mmol HEPES buffer and the tubes were rolled at 33°C. Medium for CTe cells (Caul, Jacobs & Clarke, 1974) was adjusted to pH 7·8 with 25 mmol HEPES buffer. The tubes were held stationery at 37 °C. The medium of all cultures was changed 24 h after inoculation and they were then examined microscopically three times each week for evidence of cytopathic effects (CPE). Fluid from cultures showing CPE or non-specific degeneration was passed to fresh cultures. Specimens which did not cause CPE after 21 days in CK cultures, or 42 days in CTe cells were considered negative; those which produced transmissible CPE were stored for identification.

Identification of viruses

Preliminary identification of viruses was based on the appearance of CPE and the type of cultures in which it occurred. Parainfluenza virus type 3 was identified by an haemagglutination-inhibition (HI) test with specific antiserum, enteroviruses, adenoviruses and respiratory syncytial virus were identified by neutralization tests with specific antisera. Rhinoviruses were shown to be stable to chloroform, not inhibited by 20 μg/ml of bromodeoxyuridine, inactivated at pH 3 and to pass through a 50 nm Millipore filter.

Virus antibody titration

Sera were inactivated at 56°C for 30 min before use and absorbed overnight with 10% human group 'O' erythrocytes if used in HI tests. Antibodies against Pi-3 and reovirus type 1 (REO) were measured by HI in microtitre plates. Rhinovirus type 1, bovine virus diarrhoea virus and RSV antibodies were titrated in microneutralization tests. Antibodies against infectious bovine rhinotracheitis virus (IBR) were measured by passive haemagglutination (Shimizu *et al.* 1972; Kirby, Martin & Ostler, 1974). A fourfold or greater rise in antibody titre between successive serum samples titrated in the same test was taken as evidence of infection.

RESULTS

Prevalence of respiratory disease

A total of 1590 calves in 17 groups were involved in the survey (Table 1). Between May 1972 and December 1975, 41 animals (2·6%) died from pneumonia and 29 from other causes; 354 (22·3%) required treatment for respiratory disease. The incidence of respiratory disease varied between groups, from only 3% in group 11 to 42% in group 12. Only 23 (7·5%) of 306 calves born in January or February required treatment, whereas 65 (32·5%) of 200 born in March and 167 (31·7%) of 527 born between July and September were treated.

The age at which respiratory disease and death occurred is shown in Table 2. Of 354 cases of respiratory disease 97·5% were animals less than 9 months old and 67·2% were under 5 months old. Only two animals older than 9 months died from pneumonia.

The seasonal distribution of respiratory disease (Fig. 1, top profile) indicated that two outbreaks of illness occurred in each of the three winters. Almost 80% of the 354 cases of respiratory illness detected during the survey occurred during the six outbreaks which occurred in November 1972, April and October 1973, February and December 1974 and March 1975.

Prevalence of virus infections

The routine virological survey was based on 127 animals (Table 1) from which NS and sera were collected at regular intervals. Nasal swabs collected from 185 of the 354 calves treated for respiratory signs were also examined virologically. Viruses were isolated from 194 of the 958 NS collected routinely from animals in the survey and also from 49 of 185 swabs taken from calves undergoing treatment

Table 1. *Population studied and respiratory disease recorded between May 1972 and December 1975*

Group	Breed*	Date of birth	No. of animals	Deaths		Respiratory illness		Routine virology	
				Pneumonia	Other	No.	%	No.	Duration (months)
1	H×F	May 1972	106	1	1	24	23	7	6–11
2	A×F	Aug. 1972	73	4	2	17	23	8	2–9
3	A×F	Sept. 1972	83	0	0	29	35	8	1–9
4	A×F	Nov. 1972	68	0	1	25	37	8	0–6
5	H×F	Mar. 1973	94	2	3	28	30	8	0–9
6	H×F	May 1973	104	0	2	7	7	8	0–9
7	H×F	July 1973	95	0	0	28	29	8	0–9
8	PB	Sep. 1973	101	1	4	33	33	8	0–7
9	H×F	Nov. 1973	104	4	2	20	19	0	—
10	H×F	Jan. 1974	100	0	2	9	9	8	0–9
11	H×F	Feb. 1974	100	0	0	3	3	8	0–9
12	MB	July 1974	79	2	0	33	42	8	0–9
13	MB	Sep. 1974	96	2	1	27	28	8	0–7
14	MB	Nov. 1974	95	10	2	15	16	8	0–5
15	MB	Jan. 1975	106	5	2	11	10	8	0–4
16	MB	Mar. 1975	106	8	5	37	35	8	0–6
17	MB	June 1975	80	2	2	8	10	8	0–3
Totals			1590	41 (2·6%)	29	354	22·3	127	

H×F = Hereford crossed Friesian. A×F = Aberdeen Angus crossed Friesian. PB = Pure breeds: 50 calves were Canadian Holstein, 51 were British Friesian. MB = Mixed breeds, consisting of Hereford, S. Devon, Charolais and Simental.

Table 2. *Respiratory disease according to age*

Month of life	Pneumonia deaths	Treatments	
		No.	%
1	2	36	10·2
2	7	67	18·9
3	8	27	7·6
4	11	108	30·5
5	1	10	2·8
6	3	24	6·8
7	2	33	9·3
8	3	2	0·6
9	2	38	10·7
>9	2	9	2·6
Total	41	354	100

for respiratory illness (Table 3). Most commonly isolated viruses were Pi-3 and ENT. All of the 24 RV isolated were neutralized by antiserum to bovine rhinovirus type 1. One of the 29 AD was type 2 and the remainder type 3. Seventy of the 88 ENT were neutralized by antisera to the LCR4 and M63 strains and therefore belong to bovine enterovirus type 1 (Western Hemisphere Committee, 1975); the other 18 were not neutralized by antisera to strains F226A, M80, M153, T10 or T11F (Huck & Cartwright, 1964).

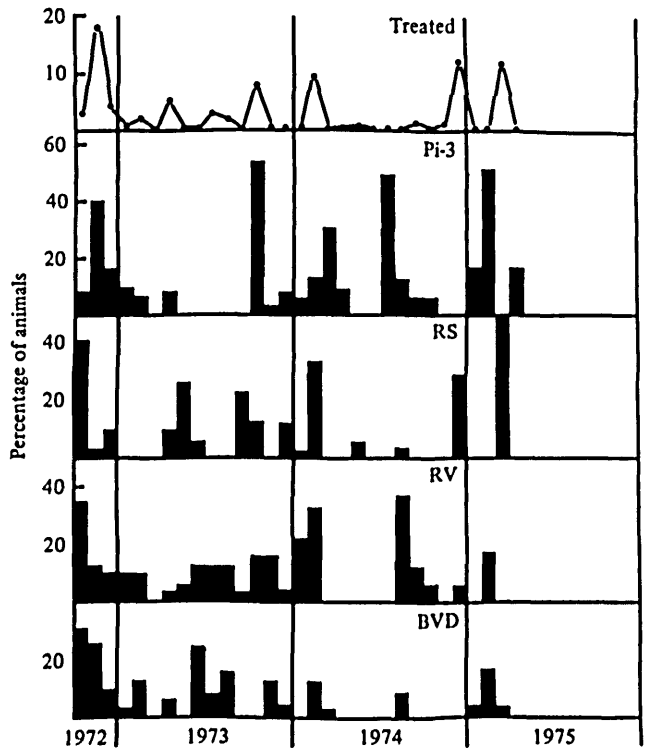

Fig. 1. Monthly incidence of treatments for respiratory disease and virus infections.

Table 3. *Viruses isolated from nasopharyngeal swabs, Warren Farm, October 1972 to September 1975*

Type of animal	N/S examined	No. of indicated viruses isolated*						
		Pi-3	RS	RV	AD	Ent	Untyped	Total
Normal survey	958	61	5	18	22	83	5	194
Treated	185	27	4	6	7	5	0	49
Total	1143	88	9	24	29	88	5	243

* Pi-3 = Parainfluenzavirus type 3. RS = Respiratory Syncytial Virus. RV = Rhinovirus type 1. AD = Adenovirus: 1 strain from a normal animal was type 2, the remainder were type 3. Ent = Enteroviruses.

Virus infections were also diagnosed on 327 occasions from 1069 sera collected from animals in the routine survey (Table 4). Infection by IBR virus was not detected.

The combination of virus isolation and serology detected a total of 540 separate infections by at least seven different virus groups during the investigation: 491 in the routine survey of healthy calves and 49 in animals with acute respiratory disease (Table 5).

The method of diagnosis varied considerably for the different virus groups when

Table 4. *Virus infections diagnosed serologically, Warren Farm, October 1972 to September 1975*

	No. infections with indicated virus*					
Sera examined	Pi-3	RS	RV	BVD	REO	Total
1069†	72	69	84	49	53	327

* BVD = bovine virus diarrhoea virus.

† The 1069 sera were collected serially every 3 or 4 weeks from 127 calves in the routine survey. Hence they were equivalent to 942 (1069–127) paired sera.

Table 5. *Method of diagnosis of virus infections, Warren Farm, October 1972 to September 1975*

		No. of infections with indicated virus								
Type of Animal	Method of diagnosis	Pi-3	RS	RV	BVD	AD	REO	ENT	UT	Total
Routine normal survey	Isolation only	36	5	13	0	22	0	83	5	164
	Both isolation and serology	25	0	5	0	ND	0	ND	ND	30
	Serology only	47	69	79	49	ND	53	ND	ND	297
	Total	108	74	97	49	22	53	83	5	491
Treated	Isolation only	27	4	6	0	7	0	5	0	49
Total		135	78	103	49	29	53	88	5	540

both NS and sera were tested. Although 56% of Pi-3 infections and 19% of RV infections were diagnosed by virus isolation, less than 7% of RSV and no BVDV or REO infections were detected in this way (Table 5).

The monthly distribution of the four virus infections shown in Fig. 1 indicated that most infections by Pi-3, RSV and to a lesser degree RV, occurred in sharp outbreaks once or twice per year. Outbreaks involving two or three viruses often occurred in the same month, for example in October 1972, February 1974 and February 1975. Seventy-eight per cent Pi-3, 75% RV, 74% REO and 73% RSV infections were detected during the winter months, October to March. Infections by BVDV and ENT showed no marked seasonal distribution, 55% being detected in the winter months. Most AD infections (77%) were found in the summer months.

The age of calves when infections were diagnosed varied for individual viruses (Fig. 2). There were two peaks of incidence of Pi-3 infections: over 17% were infected in the third month of life and 43% in the seventh month. The incidence of RSV infections remained low until 5–6 months after birth, when 21% of calves were infected; this figure fell only slightly in the succeeding 3 months. Rhinovirus infections were found in 20% of calves in the second month, thereafter the incidence declined to 6% in the fourth month before rising again to 18% in the seventh month. The pattern of ENT infections was quite distinct and showed a single sharp peak of 34% in the fifth month of life. Infections with BVDV, AD and REO did not show any marked trends which could be related to age.

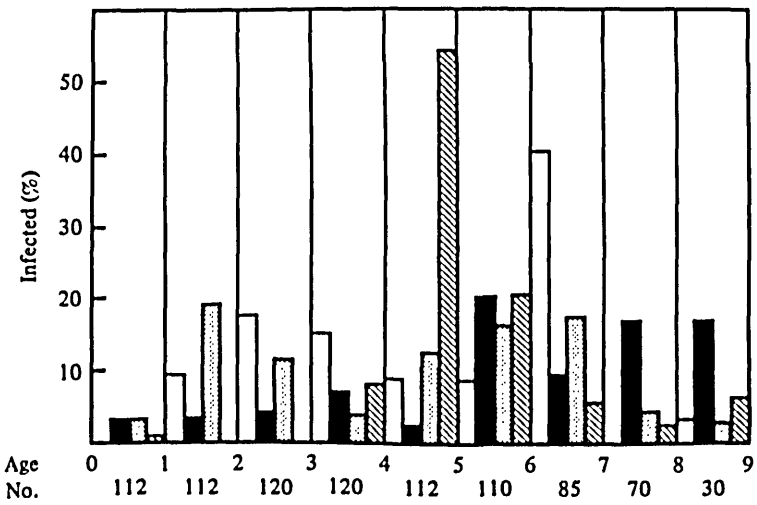

Fig. 2. Proportion of animals infected by Pi-3 (□), RS virus (■), RV (▩) and ENT (▧) according to age.

Table 6. *Reinfection or persistence of viruses*

Type of diagnosis	Viruses							Total
	Pi-3	RS	RV	BVD	AD	REO	ENT	
First	80	64	81	47	20	48	59	408
Second*	19	10	16	2	2	5	24	78
Total	108	74	97	49	22	53	83	486

* Second diagnosis was made when infection with a particular virus was found in two separate investigations of the same animal.

Of the 486 virus infections diagnosed 78 were found in animals that had previous evidence of infection with the same agent (Table 6), suggesting either reinfection or persistence of the virus. Second diagnoses of Pi-3, RSV, RV and ENT infections were most common.

Omitting these second infections, the percentage of animals in each age group with evidence of first infection was calculated. The cumulative percentage for each virus at each month of age is shown in Fig. 3. The number of infections with Pi-3 and RV increased most rapidly in the first 3 months of life, so that at 3½ months almost 40 % of calves had experienced their first infection with these two viruses and by 9 months 85 % and 75 % respectively had been infected. In contrast, only 16 %, 18 % and 9 % respectively had evidence of infection by RSV, REO or ENT at 3½ months. Infections by RSV and ENT were found more frequently after 4 months of age, so that by 9 months RSV had been diagnosed in 70 % and ENT in 56 % of calves.

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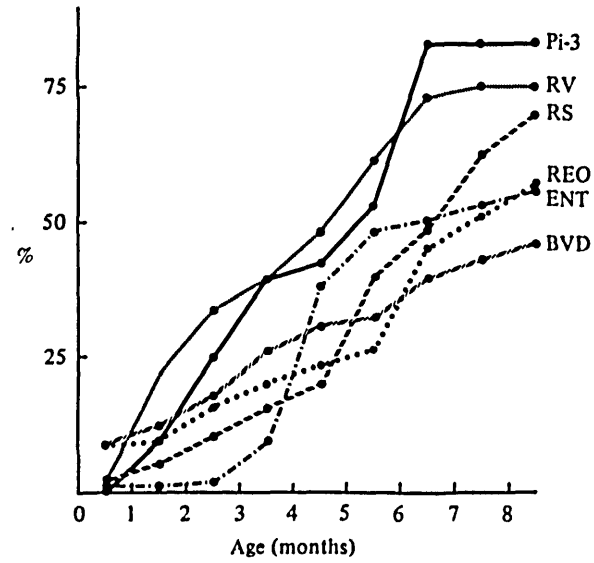

Fig. 3. Cumulative percentage of animals which have experienced their first infection according to age.

Association between respiratory disease and virus infection

There were 6 months during the survey when more than 5 % of the total animals in the farm under 9 months old were treated for respiratory disease (Fig. 1 and Table 7). Out of a total of 942 virological investigations in the whole survey, 204 (21·7 %) were undertaken during the 6 months in which there were outbreaks of disease (Table 7). If virus infections occurred at random and were unrelated to respiratory disease it would be expected that about 20 % of those diagnosed would be detected during the six outbreaks. The percentages for infections by RV (29·9 %), BVDV (18·4 %), AD (22·7 %), REO (28·3 %) and ENT (16·9 %) were not significantly different from the expected 21·7 % ($P > 0·05$). However, 38 % of Pi-3 infections and 58·1 % of RSV infections were diagnosed during outbreaks of disease. These values were significantly different ($P < 0·001$) from the expected 21·7 %, and indicated a significant association between infections with these two viruses and outbreaks of respiratory disease.

The five groups of calves with a low incidence of respiratory disease (10 % or less) were compared (Table 8) with the six groups with a high incidence (30 % or more). Of the animals investigated virologically the proportion infected by Pi-3, RV, AD, REO and ENT were similar for both high and low disease groups. However, significantly more RSV and BVDV infections ($P < 0·01$) were detected in calves from the groups in which 30 % or more were treated for respiratory disease.

DISCUSSION

The prevalence of respiratory disease in beef calves in the United Kingdom is not precisely known. In the current survey, 22·2 % of calves were treated for

Table 7. *Virus infections and outbreaks* of disease, Warren Farm, October 1972 to September 1975*

Outbreak		Animals treated	No. virological	No. infections with indicated virus						
Year	Month	(%)	investigations†	Pi-3	RS	RV	BVD	AD	REO	ENT
1972	Nov.	17·7	47	15	1	4	4	0	7	0
1973	Apr.	5·5	24	2	3	1	1	1	1	2
	Oct.	7·9	31	20	4	6	0	3	6	5
1974	Feb.	9·1	54	4	13	15	4	0	0	3
	Dec.	13·4	24	0	4	1	0	1	1	3
1975	Mar.	11·7	24	0	18	2	0	0	0	1
Total during outbreaks			204	41	43	29	9	5	15	14
Total throughout survey			942	108	74	97	49	22	53	83
% during outbreaks			21·7	38·0	58·1	29·9	18·4	22·7	28·3	16·9
χ^2				18·06	60·58	3·80	0·56	0·02	1·24	1·56
Probability‡				<0·001	<0·001	>0·05	>0·3	>0·8	>0·2	>0·2

* Outbreak = any month in which more than 5% of animals under 9 months required treatment for respiratory disease.
† Investigation = occasion on which both paired sera and NS were collected from an animal.
‡ Calculated using Yate's modification for small numbers.

Table 8. *Infections in groups with high or low incidence of respiratory disease*

	Low disease groups*	High disease groups†
No. calves ...	490	531
No. treated ...	38 (7·8%)	185 (34·8%)
No. investigated‡	40	48
No. infected		
Pi-3	28 (70%)	32 (67%)
RS	9 (23%)	26 (54%)
RV	24 (60%)	28 (58%)
BVD	9 (23%)	22 (46%)
AD	7 (18%)	11 (23%)
REO	14 (35%)	17 (35%)
ENT	19 (48%)	24 (50%)

* Group 6, 10, 11, 15 and 17 (Table 1) in which $\leqslant 10\%$ of calves required treatment for respiratory disease.

† Group 3, 4, 5, 8, 12 and 16 (Table 1) in which $\geqslant 30\%$ of calves required treatment for respiratory disease.

‡ No. of calves in virological survey.

respiratory disease and 2·5% died from pneumonia. Similar figures have been obtained from other surveys (Thomas, 1978). The low incidence of disease (7·5%) in calves born in January and February and the high incidence (32%) in calves born in March, July and September is striking. If this observation is confirmed in larger surveys, it would indicate that the current practice of early autumn calving increases the likelihood of respiratory disease. Our observations that 67·2% of disease occurred in animals under 5 months old and that 80% of cases were found during sharply defined outbreaks in the winter months confirm established beliefs.

Relatively few attempts have been made to survey virus infections in normal cattle; Dawson *et al.* (1966) sampled 20 calves regularly for 18 weeks. No viruses were isolated but six calves had serological evidence of Pi-3 infection when signs of respiratory disease were observed. In Australia 20 calves were continuously studied for 6 months (St George, Hass & Horsfall, 1972). Pi-3 was isolated from 21 nasal swabs from four calves and ENT from 41 faecal swabs of 16 calves. Serological evidence of BVD and Pi-3 infection was also found. A serological survey of 47 calves during 6 months (Thomas & Collins, 1974) revealed frequent infection by Pi-3, BVDV and REO viruses. A smaller survey of 6 calves over 14 months in New Zealand also detected Pi-3 and ENT (Burgess, 1977). Because of the small numbers of animals involved (less than 50), no conclusions were made about seasonal or age distribution of viruses, nor was a statistically significant association between virus infection and respiratory disease established in these surveys. However, an investigation of over 200 calves in Holland revealed a striking correlation between RSV infection and bronchopneumonia of yearling cattle (Holzhauer & van Nieuwstadt, 1976; Holzhauer, 1978).

In our own survey the optimum method of diagnosis was not the same for all viruses. Although virus isolation and serology were almost equally effective in diagnosing Pi-3 infection, for most other agents virus isolation was relatively

inefficient. Other workers have experienced difficulty in isolating RSV and RV from cattle (Wellemans & Leunen, 1975; Mohanty, Lillie & Ingling, 1976; Lehmkuhl & Gough, 1977; Mohanty & Lillie, 1968). Only cytopathic strains of BVDV would have been isolated in this survey and probably most BVDV infections are by non-cytopathic strains (Kodama *et al.* 1974) and therefore would only have been diagnosed serologically. Furthermore, many BVDV and REO infections may have been primarily enteric rather than respiratory and virus isolation from NS would be inefficient in such cases. The method of diagnosis is important in interpreting the results since, for example, serological diagnosis may be difficult in a young calf with large amounts of maternal antibody.

During the routine survey, 491 virus infections were detected in 942 investigations of 127 apparently healthy calves, indicating that virus infection is not only common but frequently symptomless. This rate of virus infection (52 %) is much higher than the 5–21 % found in similar surveys of children (Bell *et al.* 1961; Chanock & Parrott, 1965; Holzel *et al.* 1965; Cooney, Hall & Fox, 1972). Between 13 % and 18 % of Pi-3, RSV or RV infections were not primary infections and indicate, either virus persistence or failure of a primary infection to give adequate protection against reinfection. Similar repeated infection of children by Pi-3 and RSV is well documented (Chanock, Bell & Parrott, 1961; Beem, 1967; Henderson *et al.* 1979).

Calves were born at intervals of approximately 2 months throughout the survey; there were no late spring or early autumn calving peaks as frequently occur on commercial beef farms. The effect of season and age on the incidence of virus infection can therefore be assessed independently. The marked increase, in the winter months, of infections by Pi-3 and RSV, and their tendency to occur in sharp outbreaks are also similar to findings in children (Chanock *et al.* 1961; Chanock & Parrott, 1965; Kim *et al.* 1973). However, the lack of marked seasonal distribution of ENT infections and the increase in AD infections in the summer contrast with findings in man (Spigland *et al.* 1966; Monto & Cavallaro, 1971).

Primary infections with Pi-3 and RV seemed to occur earlier than with RSV, REO or ENT. This may be because maternal antibody reduced the efficiency of serological diagnosis of RSV and REO or protected calves more efficiently against these viruses than Pi-3 and RV. A third explanation may be a real difference in age susceptibility, although the latter two hypotheses are not supported by data from children (Cooney, Fox & Hall, 1975).

A statistically significant association between virus infections and disease was detected, first, by comparing the incidence of infections during outbreaks of disease with the incidence at other times and, secondly, by comparing the frequency of virus infections in groups with a high incidence of disease with those with a low incidence. In the first comparison, only RSV and Pi-3 were significantly associated with disease. Furthermore, with the exception of RSV, most virus infections (62 % for Pi-3 to 83 % for ENT) were detected when there was no significant disease on the farm. In the second comparison only RSV and BVDV were associated with high incidence of disease. If BVDV has an aetiological role in respiratory disease, it may be through its ability to persist in cells of the reticulo-

endothelial system and thereby suppress immune responses over long periods. If so, its association with disease might not be demonstrated by the first comparison which is a temporal one between acute infections and disease.

It must be stressed that this survey, showing significant association between certain viruses and respiratory disease, does not, and is not intended to prove an aetiological role for such viruses. Proof requires reproduction of disease by the virus or, at least, its prevention by a vaccine against the agent. Nevertheless, our survey considerably strengthens the case for RSV being important in the aetiology of calf respiratory disease and, equally relevant, argues against the overall importance of RV, AD, REO and ENT. These conclusions are again remarkably similar to those derived from studies of lower respiratory disease in young children (Tyeryar, Richardson & Belshe, 1978) and are consistent with a growing number of recent studies in calves highlighting the importance of RSV (Wellemans & Leunen, 1975; Holzhauer & van Nieuwstadt, 1976; Lehmkuhl & Gough, 1977; Bryson *et al.* 1978).

It is not known how far these results derived from one farm apply nationally. However, the calves were derived from many, widely scattered farms all over England and Wales and must represent the national beef herd, at least virologically. Ultimately, this question can only be resolved by similar surveys in other centres on many different farms.

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