VIRAL INFECTIONS AND RESPIRATORY DISEASE IN DAIRY-BRED CALVES.

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Introduction

After salmonellosis respiratory disease probably remains the most important condition of market purchased dairy bred calves. Treatment rates for respiratory disease have been found to exceed 30% during the first three months of the rearing phase (1) and this disease is therefore responsible for significant losses in terms of animal performance, veterinary costs and labour irrespective of the implications for the welfare of the young calf.

The association between respiratory virus infection and clinical respiratory disease has been demonstrated in weaned beef calves and in home reared dairy calves, but in calves less than three months of age there was little evidence of viral involvement (2 and 3). However serum monitoring on one calf rearing unit led to the observation that despite good response to antibiotic therapy, the outbreaks of sudden onset high morbidity respiratory disease seen in calves less than three months of age, had a possible viral aetiology. To explore this further the following study was carried out.

Material and Methods

The study at a commercial calf unit involved an entire year's throughput. Calves were purchased in batches of 30-40 from markets; they were milk fed until 6 weeks after purchase when they were weaned and moved to hardening-off pens. Clinical disease and treatments were recorded by the calf rearer according to an established protocol drawn up by the unit's vet. Blood samples were collected from calves within two days of arrival; at six weeks after arrival and at the end of the calf-rearing phase, twelve weeks after arrival. Serum was removed from each sample and split into two aliquots, one of which was frozen at -20°C and stored until all samples had been collected before immunoglobulin testing was carried out. The other sample was examined for the presence of BVD virus within one week of collection.

An enzyme-linked immunosorbant assay (ELISA) which combined 5 viral antigens, bovine herpes virus 1 (BHV-1), bovine respiratory syncytial virus (BRSV), parainfluenza 3 (Pi3), bovine adenovirus 1 (BAV-1) and bovine viral diarrhoea virus (BVDV), was used to measure virus specific antibody. Results were expressed in optical density units (ODU) and a rise in optical density of >0.2 ODU between successive samples was taken to indicate seroconversion.

Incidents of respiratory disease that occurred during the first four weeks on the unit were related to seroconversion between samples collected at arrival and at six weeks. Incidents of respiratory disease that occurred during weeks five to ten after purchase were related to seroconversion between samples collected at six and twelve weeks after purchase. In individuals that received treatment for respiratory disease in both periods the second incident was only included in the analysis if it followed the first by a period of more than one week.

The immunoperoxidase test (IPT) was used to detect the presence of BVD virus. The zinc sulphate turbidity test (ZST) was used to measure total immunoglobulin concentration of the initial blood sample from each calf.

Fisher's exact test was used to examine the association between disease incidence and seroconversion; the chi-squared test for equality of proportions was used to examine the relationship between disease occurrence and both initial ELISA values and ZST units; the test used for the trend in proportions of calves treated for respiratory disease with increasing ELISA values was that of Cochran (1954). The correlation coefficient statistic was used to measure the relationships between immunoglobulins as measured by the ZST test and the linear combinations of the ELISA values for specific immunoglobulin against the five viruses (sum of ELISA values) at the initial sampling.

Results

Of the 549 calves involved in the study 13 died, ten in the first six weeks after arrival and three in the second six weeks. One calf from each of these two periods died from pneumonia, but the chronic nature of the lung changes found at autopsy prevented a more specific diagnosis. Other causes of

death were Salmonella dublin septicaemia (4 calves), rumenal tympany (1 calf), chronic enteritis of unknown aetiology (1 calf) and no diagnosis was reached on one occasion.

Of the remaining 536 calves 238 (44%) were treated in weeks 1-4 and 100 (19%) were treated in weeks 5-10. These included 59 (11%) which received treatment in both periods. Several batches of sera were inadvertently destroyed during storage leaving 389 calves with full serological data and 62 calves with serological data for only the first period. Removal of those calves that lacked serological results gave treatment rates of 44% in the first period and 20% in the second period.

Serology results confirmed the presence of active infection with all five viruses under study (tables 1 and 2). During the first period a positive association between seroconversion and disease was seen for all viruses except Pi3 and BVDV. In weeks 5-10 no such relationship was found. Two calves were found to have a BVD viraemia without subsequent seroconversion.

Table 1

The relationship between viral seroconversions and the occurrence of respiratory disease in 451 calves in the first four weeks after arrival.

	Respiratory Disease	No Respiratory Disease	Total Sero conversion	Significance Probability
BHV-1	7	2	9	0.046
RSV	13	5	18	0.015
Pi3	2	3	5	1.000
BAV-1	59	55	114	0.049
BVD	27	27	54	0.381
Any Virus	83	80	163	0.023
Total Calves	197 (44%)	254 (56%)		

Table 2

The relationship between viral seroconversion and the occurrence of respiratory disease in 389 calves in weeks five to ten after arrival.

	Respiratory Disease	No Respiratory Disease	Total Sero- conversion	Significance Probability
BHV-1	0	1	1	1.000
RSV	4	22	26	0.800
Pi3	2	6	8	0.664
BAV-1	20	73	93	0.767
BVD	4	20	24	0.797
Any Virus	26	106	132	1.000
Total Calves	78 (20%)	311 (80%)		

Tables 3 and 4 detail the amount of specific anti-viral antibody measured by ELISA in the samples collected on entry to the unit. Only 5 and 9 per cent of calves were seronegative (titres <0.1 ODU) for BRSV and Pi3 respectively; 18 per cent were negative for BAV-1; 29 per cent to BVDV and 49 per cent to BHV-1. There was a significant negative association between increasing anti viral immunoglobulin and disease occurrence (p<0.05) for all viruses except BHV-1, during weeks 1-4, but not in weeks 5-10. When total immunoglobulin was measured by ZST test the negative association between respiratory disease in the first period and increasing antibody concentration was again found to be significant (P<0.05).

The multiple correlation between ZST values and the specific antibodies against the five viruses (R = 0.50, P < 0.001) indicated that only 25 per cent of the variation in ZST values could be attributed to antibody against the respiratory viruses.

Table 3

The association between initial ELISA titres to BHV-1 and BRSV and disease occurrence in the first four weeks after arrival in 536 calves.

ELISA titre (ODU)	BHV-1 Number of calves	BHV-1 Proportion treated	BRSV Number of calves	BRSV Proportion treated
<0.1	262	0.49	26	0.50
0.1-0.2	96	0.39	61	0.57
0.3-0.4	34	0.38	84	0.50
0.5-0.6	33	0.39	90	0.47
0.7-0.8	28	0.46	94	0.44
0.9-1.0	30	0.37	95	0.34
> 1.1	53	0.42	86	0.38
x^2		5.63		11.45
Sig. probability		0.466		0.075
Trend probability		0.163		0.003

Table 4

The association between initial ELISA titres to Pi3, BAV-1 and BVDV and disease occurrence in the first four weeks after arrival in 536 calves.

ELISA titre (ODU)	Pi3 No. of Calves	Pi3 Proportion Treated	BAV-1 No. of Calves	BAV-1 Proportion Treated	BVDV No. of Calves	BVDV Proportion Treated
<0.1	48	0.55	96	0.56	156	0.1
0.1-0.2	103	0.54	145	0.48	69	0.42
0.3-0.4	102	0.49	116	0.47	75	0.48
0.5-0.6	93	0.33	73	0.37	63	0.40
0.7-0.8	60	0.45	51	0.29	73	0.51
0.9-1.0	54	0.42	16	0.38	54	0.28
> 1.1	76	0.32	39	0.28	46	0.33
x^2	16.53		17.49		14.50	
Sig. probability	0.011		0.008		0.025	
Trend probability	0.001		<0.001		0.006	

Discussion

The high treatment rate and evidence of active infection with the five viruses throughout the period under study offered maximum opportunity to examine the relationship between viral infection

and clinical respiratory disease in this age group of calves. Of the viral infections under study Pi3 and BHV-1 occurred least commonly; in particular the low Pi3 activity is contrary to the findings of other studies (2 and 3). As Stott and others (2) found most activity in the third month of the rearing phase and the Dutch study (3) commenced when calves were already two months of age, the observed differences may be a consequence of the different age groups involved. The low level BHV-1 activity is in agreement with both these studies, suggesting that this virus is of minor importance in respiratory disease of the young calf.

Infection with BAV-1, BRSV and BHV-1 (as indicated by seroconversion) were all positively associated with clinical respiratory disease. However, since many seroconversions occurred without evidence of clinical disease it would appear that many viral infections resulted in mild or inapparent disease. While BHV-1 infection was associated with disease the importance of this is diminished by the small number of seroconversions. Rather it is BRSV and BAV-1 infections which appear to be of greater relevance to respiratory disease in the young calf. In the former it is probable that the use of serology to identify infection under-estimates the number of infections in calves that have passively acquired antibody. Systemic antibody responses to BRSV have been shown to be suppressed in passively protected calves despite the development of clinical signs and virus shedding (4). This limitation in the study could only have been overcome with difficulty as the isolation of BRSV from clinical cases is notoriously difficult. It is not clear to what extent the existence of passively acquired antibody interferes with seroconversion to other viruses.

The demonstration of a positive association between seroconversion to BAV-1 should come as no surprise. Adenovirus activity has previously been found in dairy-bred calves (2) and in weaned beef calves (5), however there is little information published on the biology or pathology of this viral infection.

Seroconversion to BVDV was second only to BAV-1 in frequency and yet no significant association was found with respiratory disease. This finding is contrary to that of Stott and others (2), who found significantly more BVDV infections in groups of calves where treatment rates for respiratory disease exceeded 30% compared to those groups with lower treatment rates. In the present study only two groups had treatment rates below 30% during the 10 week period and in one group 27% of the calves seroconverted to BVDV; in the other the figure was 10 per cent. The proportion of persistent BVDV infections, 2 of 549, is lower than the estimated figure of 1% in animals at slaughter (6) but is closer to the figure of 0.4% observed (7) in a survey that examined dairy bred calves.

A marked difference in both disease occurrence and the association between seroconversion and disease was seen between weeks 1 to 4 and 5 to 10. In the first seroperiod 44% of calves were treated and 36% seroconverted, in the second period a treatment rate of 20% was found and 34% seroconverted. Therefore the differences could not merely be attributed to the disease having run its course in the first four weeks. The possibility that many of the treatments in the second period were relapsed cases must be considered as half of the calves treated in the second period had also been treated during the first four weeks. Calves that initially had maternally derived antibody seroconverted when infected with BRSV for the second time without developing clinical disease (8). Some of the seroconversions in the second period may have represented re-infection and therefore be less likely to cause clinical disease.

For the entire first seroperiod calves were milk-fed and housed in one of three enclosed calf sheds, two of which required mechanical ventilation. After six weeks calves were weaned and housed in follow-on accommodation of a mono pitch design, open at one end. For most of the second period calves were ruminating and more resistant to cold, but were also housed in buildings that offered superior air hygiene. The failure to demonstrate an association between seroconversion to any of the viruses under study and respiratory disease in the second period is therefore less surprising.

On the basis of this study it would appear that passively acquired viral antibody does confer protection against respiratory disease in the young calf despite the contention that circulating antibody can offer little protection against infections that do not require a viraemia i.e. respiratory tract infections (9). This finding is in broad agreement with other published literature on the role of the passively acquired antibody in the health of the young ruminant.

It is tempting to suggest that this study has relevance beyond the particular calf unit involved. Calves were purchased from several different markets throughout Britain over the course of one year and the pattern of virus infections experienced in these calves is therefore likely to be similar to that seen in most market-purchased dairy-bred calves. However, it is probable that, because of the

modulating effect of environment on clinical respiratory disease, the scale of the association between viral infections and clinical disease will vary from unit to unit.

Accepting the limitations of a serological survey, BRSV has again been shown to be of importance in the respiratory disease complex. As high levels of passively acquired antibody have been shown to interfere with parenteral vaccination and knowing that many calves have high levels of antibody there is a need to further explore the use of intranasal vaccination against this infection. In addition BAV-1 has been implicated in the respiratory disease complex and the biology of this disease should be further researched

Summary

A total of 549 week old dairy bred calves entering a commercial calf rearing unit were blood sampled at 6 week intervals until 3 months of age. Viral infections were monitored by ELISA for antibodies to bovine herpes virus 1 (BHV-1), respiratory syncytial virus (RSV), parainfluenza 3 (Pi3), bovine adenovirus subgroup 1 (BAV-1) and bovine viral diarrhoea virus (BVDV). The immunoperoxidase test (IPT) was used to detect the presence of BVDV and total immunoglobulin concentration of the initial blood sample was measured by the zinc sulphate turbidity test (ZST). The relationship between clinical respiratory disease, viral seroconversion and initial concentration of serum immunoglobulin was investigated by the use of Fishers exact test, chi-squared techniques and the correlation coefficient.

Respiratory disease treatment rates of 44% in the first period of the study and 19% in the second period were observed. During the first period a positive association between respiratory disease and seroconversion was seen for all viruses except Pi3 and BVDV. In the second period no such relationship was seen. There was a significant negative association between increasing antiviral immunoglobulin and clinical disease in the first period only. Two of 549 calves were found to be persistently infected with BVD virus.

Résumé

Prélèvement d'échantillons de sang était fait à un intervalle de 6 semaines, jusqu'à l'âge de 3 mois, d'un total de 549 veaux laitiers, âgés d'une semaine, entrants une unité d'élevage commercial. Infections à virus furent suivies pour les anticorps à herpes virus 1 (BHV-1), virus syncytial à respiration (RSV), parainfluenza 3 (Pi3), adenovirus bovin sous-groupe 1 (BAV-1) et virus bovin à diarhée (BVDV). L'épreuve d'immunopéroxidase (IPT) était employée pour découvrir la présence de BVDV et la concentration totale de l'échantillon de depart en immunoglobuline était determinée par l'épreuve d'état bourbeux de sulfate de zinc (ZST). Le rapport entre la maladie repiratoire clinique, la séroconversion à virus et la concentration de depart d'immunoglobuline dans le sérum étaient étudies par le moyen d'épreuve précise Fisher, Methode de chi carré et le coefficent de corrélation.

Le taux de traitement de 44% des animaux contre la maladie de respiration était observé dans la première période des recherches, et de 19% dans la deuxième periode. Dans la prémière periode, on a trouvé une association positive entre la maladie de respiration et la séroconversion pour tous les virus, excepté Pi3 et BVDV. On n'a pas observé un tel rapport dans le deuxième période. Il y'avait une association négative importante entre l'immunoglobuline augmentant et la maladie clinique, seulement dans la première période. Deux des 549 veaux furent infectés par virus BVD avec persistance.

Abstracto

Un total de 549 terneros lecheros de 1 semana de edad que ingresaban a una unidad comercial pueron muestreados con intervalos de 6 semanas hasta cumplir 3 meses de edad. Infecciones virales fueron detectadas mediante prueba de ELISA identificando anticuerpos contra herpesvirus bovino

tipo 1 (BHV-1), virus respiratorio syncitial bovino (RSV), parainfluenza tipo 3 (Pi3), adenovirus bovino subgrupo 1 (BAV-1) y diarrea viral bovina (BVDV). La prueba de immunoperoxidasa fué usada para detectar la presencia de BVDV y la prueba de turbidez del suffato de zinc para la concentracción total de immunoglobulinas en muestras de sangres iniciales. La relación entre procesos respiratorios clínicos, seroconversión viral y la concentración inicial de immunoglobulinas fué determinada mediante la prueba exacta de Fisher, chi cuadrado y coeficiente de correlación.

Tratamientos de enfermedados respiratorias fueron realizadas en 44% durante el primer periódo y 19% en el segundo periódo de estudio. Durante el primer periodo se observó una asociación positiva entre enfermedad respiratoria y serconversión para los virus estudiados con excepcion de Pi3 y BVDV. Esta relación no fué observada en el segundo periodo. Además se identificó una correlación negativa significativa entre incremento de immunoglobulina antiviral y enfermedad clínca solo durante en el primer periódo. Infecion persistente con el virus del BVD fueron identificados en 2 de los 549 terneros estudiados.

References

1. Caldow, G.L., White, D.G., Kelsey, M., Peters, A.R., Solly, K.J., (1988) Veterinary Record 122: 63. 2. Stott, E.J., Thomas, L.H., Collin, A.P., Crouch, S., Jebbett, J., Smith, G.S., Luther, P.D., Caswell, R.,(1980) Journal of Hygiene Cambridge, 85: 257. 3. Verheoff, J., van Nieuwstadt, APKMI., (1984) Veterinary Record, 114: 288. 4. Kimman, T.G., Westenbrink, F., Shreuder, B.E.C., Straver, P.J.,(1987) Journal of Clinical Microbiology, 25: 1097. 5. Caldow, G.L., Edwards, S., Nixon, P., Peters, A.R.,(1988) Veterinary Record, 122: 529. 6. Duffell, S.J., Harkness, J.W., (1985) Veterinary Record, 117: 240. 7. Howard, C.J., Brownlie, J., Thomas, L.H., (1986) Veterinary Record, 119, 628. 8. Kimman, T.G., Westenbrink, F., Straver, P.J.,(1989) Journal of Clinical Microbiology, 22: 145. 9. Field, E.W., Smith, M.H., (1984) American Journal of Veterinary Research, 45: 1641.