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Scientific Article

Equine respiratory viruses in foals in New Zealand

M Dunowska[*§], CR Wilks[*†], MJ Studdert[‡] and J Meers[*#]

Abstract

AIMS: To identify the respiratory viruses that are present among foals in New Zealand and to establish the age at which foals first become infected with these viruses.

METHODS: Foals were recruited to the study in October/November 1995 at the age of 1 month (Group A) or in March/April 1996 at the age of 4–6 months (Groups B and C). Nasal swabs and blood samples were collected at monthly intervals. Nasal swabs and peripheral blood leucocytes (PBL) harvested from heparinised blood samples were used for virus isolation; serum harvested from whole-blood samples was used for serological testing for the presence of antibodies against equine herpesvirus (EHV)-1 or -4, equine rhinitis-A virus (ERAV), equine rhinitis-B virus (ERBV), equine adenovirus 1 (EAdV-1), equine arteritis virus (EAV), reovirus 3 and parainfluenza virus type 3 (PIV3). Twelve foals were sampled until December 1996; the remaining 19 foals were lost from the study at various times prior to this date.

RESULTS: The only viruses isolated were EHV-2 and EHV-5. EHV-2 was isolated from 155/157 PBL samples collected during the period of study and from 40/172 nasal swabs collected from 18 foals. All isolations from nasal swabs, except one, were made over a period of 2–4 months from January to April (Group A), March to April (Group B) or May to July (Group C). EHV-5 was isolated from either PBL, nasal swabs, or both, from 15 foals on 32 occasions. All foals were positive for antibodies to EHV-1 or EHV-4, as tested by serum neutralisation (SN), on at least one sampling occasion and all but one were positive for EHV-1 antibodies measured by enzyme-linked immunosorbent assay (ELISA) on at least one sampling occasion. Recent EHV-1 infection was evident at least once during the period of study in 18/23 (78%) foals for which at least two samples were collected. SN antibodies to ERBV were evident in 19/23 (83%) foals on at least one sampling occasion and 15/23 foals showed evidence of seroconversion to ERBV. Antibodies to ERAV were only detected in serum samples collected from foals in Group A and probably represented maternally-derived antibodies. Haemagglutination inhibition (HI) antibody titres ≥1:10 to EAdV-1were evident in 21/23 (91%) foals on at least one sampling occasion and 16/23 foals showed serological evidence of recent EAdV-1 infection. None of the 67 serum samples tested were positive for antibodies to EAV, reovirus 3 or PIV3. There was no clear association between infection with any of the viruses isolated or tested for and the presence of overt clinical signs of respiratory disease.

CONCLUSIONS: There was serological and/or virological evidence that EHV-1, EHV-2, EHV-5, EAdV-1 and ERBV infections were present among foals in New Zealand. EHV-2 infection was first detected in foals as young as 3 months of age. The isolation of EHV-2 from nasal swabs preceded serological evidence of infection with other respiratory viruses, suggesting that EHV-2 may predispose foals to other viral infections.

KEY WORDS: *Equine respiratory disease, equine herpesviruses, equine rhinitis viruses, equine adenovirus, infection, foal, horse*

Introduction

Several viruses including equine influenza virus, EHV-1, EHV-4, ERAV (formerly equine rhinovirus type 1), ERBV (formerly equine rhinovirus type 2), reoviruses, PIV3, and EAV have been associated with respiratory disease in horses (Mumford and Rossdale 1980; Jolly et al 1986; Powell 1991; Mair 1996). The results of a recent virological and serological survey of horses sampled following outbreaks of respiratory disease, and yearlings from yearling sales, indicated that EHV-2 and possibly ERBV might be involved in equine respiratory disease in New Zealand (Dunowska et al 2002).

It is generally accepted that foals are protected from viral infections soon after birth by maternally-derived antibodies and subsequently become infected when these maternal antibodies decline at approximately 4–6 months of age (Allen and Bryans

DNA	Deoxyribonucleic acid
EAdV-1	Equine adenovirus 1
EAV	Equine arteritis virus
EHV	Equine herpesvirus
ELISA	Enzyme-linked immunosorbent assay
ERAV	Equine rhinitis-A virus
ERBV	Equine rhinitis-B virus
H&E	Haematoxylin and eosin
HI	Haemagglutination inhibition
PBL	Peripheral blood leucocyte(s)
PBS	Phosphate buffered saline, pH 7.0
PCR	Polymerase chain reaction
PIV3	Parainfluenza virus type 3
RBC	Red blood cell(s)
RK13	Rabbit kidney cell line
SN	Serum neutralisation
Vero	African green monkey (cells)

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1986; Powell 1991). However, some recent investigations have shown that infections can occur earlier than 4–6 months of age, often in the presence of maternal antibody. EHV-1 and EHV-2 infections have been described in foals as young as 30 days of age (Fu et al 1986; Gilkerson et al 1999) and EAdV-1 was isolated from a healthy Clydesdale foal at 3 days of age (Wilks and Studdert 1972).

It is useful to know the age at which foals first become at risk from viral infection for successful prophylaxis, especially by vaccination. The purpose of this investigation was to establish which respiratory viruses were present among foals and at what age foals first became infected under New Zealand conditions.

Materials and Methods

Origin of samples

Three groups of foals were sampled on a monthly basis. The dates of samplings and numbers of foals sampled on each occasion are shown in Table 1. Handlers were asked to observe foals for the clinical signs of respiratory disease such as nasal discharge, cough or lack of appetite.

Group A

Group A comprised seven Thoroughbred foals (A1 to A7) that were kept on a Massey University experimental farm in the Manawatu region in the North Island of New Zealand. The foals were born in October–November 1995 and samples were first collected within the foals' first month of life. Foals were weaned at the end of March and branded in April 1996. Foals were sampled until December 1996. Six foals were sold after weaning, hence only one was available for sampling in November and December 1996. These foals were also utilised for another experiment, which involved frequent (twice weekly) handling from an early age. Their dams had been vaccinated with inactivated EHV-1/EHV-4 vaccine during pregnancy (Donald 1998).

Group B

Foals from Group B (B1 to B19) belonged to a large Thoroughbred breeding farm in the Manawatu region. The foals were born in October–December 1995 and samples were first collected in March–April 1996, at the time when the foals were brought in for weaning and branding at about 4–6 months of age. Samples were collected from 19 foals during the first visit in March. Out of these 19 foals, only 11 remained on the farm and were sampled on the second sampling occasion. Four more foals were lost from the study due to transfer to another farm before the last sampling in December 1996.

Group C

Group C comprised five foals (C1 to C5) from a small stud in the Manawatu region that bred both Thoroughbred and Standardbred horses. Foals C1 and C2 were Thoroughbreds and Foals C3, C4 and C5 were Standardbreds. Foal C1 was born in late October and the remainder were born in November 1995. The foals were branded in March 1996, before the first sampling in April 1996. Foals C3 and C5 were weaned in April 1996 before the first sampling, and Foals C1, C2 and C4 were weaned in May at the time of the second sampling.

Collection of samples

Samples collected from each foal during each visit included a nasal swab (Virocult, Medical Wire and Equipment Co Australasia Pty Ltd), and blood. Blood samples were collected by jugular venepuncture into plain and heparinised vacutainers for the collection of serum and PBL, respectively, with the exception of the first two samplings of Group A foals. From this group of foals only nasal swabs and blood for serology were collected in

Table 1. Sample collection dates, average ages (months) at sampling and numbers (n) of foals sampled for each of the three study groups.

Group A			Group B			Group C		
Date	n	Age	Date	n	Age	Date	n	Age
31/10/95 or 7/11/95	7	<1						
27/11/95	7	1						
9/01/96	7	3						
9/02/96	7	4						
6/03/96	7	5	29/03/96 or 3/04/96[a]	19	5			
3/04/96[a]	6	6	29/04/96	11	6	3/04/96[a]	5	5
7/05/96	3	7	25/05/96	10	7	16/05/96[a]	5	6
						10/06/96	5	7
2/07/96	2	9	10/07/96	9	8	16/07/96	5	8
15/08/96	2	10	20/08/96	9	9	15/08/96	5	9
11/10/96	2	12	14/10/96	9	11	14/10/96	5	11
8/11/96	1	13	18/11/96	8	12	14/11/96	4	12
12/12/96	1	14	17/12/96	7	13	16/12/96	4	13

[a] First sampling after weaning

October and November 1995. Virus isolation from PBL was first attempted from blood samples collected at the third sampling of this group, in January 1996.

All procedures involving the experimental use of animals were approved by the Massey University Animal Ethics Committee (Palmerston North, New Zealand).

Processing of samples

Samples were transferred to the laboratory within 2–6 h after collection and processed. Blood samples were allowed to clot at 4°C overnight, serum separated by centrifugation at 2,000 g for 20 min, and stored in aliquots at –70°C. Nasal swab samples were processed according to the manufacturer's instructions, filtered and either inoculated on to monolayer cell cultures or stored at –70°C. PBL were separated from heparinised blood as described by Gleeson and Coggins (1985). Briefly, buffy-coat-rich plasma was collected from blood that had been allowed to settle for 10–20 min at room temperature and incubated with an equal volume of red blood cell (RBC) lysing buffer (0.85% NH_4Cl, 0.017M Tris, pH 7.4) for 5–10 min. Cells were pelleted by centrifugation at 250 g for 10 min, washed once in phosphate buffered saline (PBS), pH 7.0, and resuspended in 2 ml of PBS.

Virus isolation and detection

A 200 µl quantity of resuspended PBL or nasal swab filtrate was inoculated on to each of three cell cultures: rabbit kidney (RK13), Vero and primary equine foetal kidney monolayers. Viruses were initially identified based on cytopathic effect produced in cell culture. Isolated herpesviruses were typed using a virus-specific polymerase chain reaction (PCR) procedure (Dunowska et al 1999, 2002). PCR using primers specific for EHV-1 and EHV-4 was also performed on all PBL cultures on equine foetal kidney cells, PBL cultures on RK13 cells from samples collected from foals at the time they showed serological evidence of EHV-1 or EHV-4 infection, all cell cultures positive for EHV-5, and directly on all 172 nasal swab filtrates. Additionally, electron microscopy of clarified cell culture lysates (pelleted at 100,000 g for 2 h), and examination of monolayers stained with haematoxylin and eosin (H&E) were performed on several occasions. Cell culture lysates from inoculated Vero and RK13 cells were also tested for haemagglutination activity using human type-O RBC (for Vero cell cultures) or guinea pig RBC (for RK13 cell cultures), to test for the presence of reovirus 3 and PIV3, respectively, that do not produce an obvious cytopathic effect.

Serology

All serological tests were performed at the same time, after all samples had been collected. For each serological test, paired serum samples from individual horses were processed preferentially on the same plate or at least in the same batch to minimise between-test variation. All serum samples were tested in duplicate. Testing for EHV-1 SN, EHV-1 ELISA, ERAV, EAdV-1, and ERBV was performed on 151, 164, 163, 163, and 162 sera, respectively, from 23 foals. Testing for antibodies against EAV, reovirus 3 and PIV3 was performed on 67 serum samples from 23 foals.

The SN test for antibodies to EHV-1 or EHV-4 used EHV-1 Durham (Horner 1981) as the reference virus. Sera were regarded as positive to EHV-1 or EHV-4 if they showed a titre >1:2. Foals were regarded as recently infected with EHV-1 or EHV-4 if they showed a 4-fold rise in the EHV-1 SN titre. Type-specific antibodies to EHV-1 were detected using a blocking ELISA (van de Moer et al 1993; Donald 1998). Samples were regarded as positive if they showed >60% blocking activity compared with a reference negative serum. Foals were regarded as recently infected with EHV-1 if an increase in blocking activity from <60% to >60% or if a 4-fold rise in blocking activity >60% was evident using ELISA. Serological evidence for EHV-4 infection could not be obtained, because EHV-4-specific antibody status could not be determined using these methods in horses positive for EHV-1 antibodies. Serological testing for antibodies against EHV-2 and EHV-5 was not performed.

Antibodies to ERAV and ERBV were detected using SN assays that used ERAV.393/76 (Studdert and Gleeson 1977) and ERBV.P1436/71 (Steck et al 1978), respectively, as reference viruses. Test sera were regarded as positive if they showed titres >1:100 (for ERAV) or >1:2 (for ERBV). Samples collected from foals in Group A that were negative for antibodies to ERAV at a dilution of 1:100 were retested at a dilution of 1:10. Antibodies to PIV3 and EAdV-1 were detected using HI assays that used a commercially available human PIV3 strain (Denka Seiken Co Ltd, Japan) and a New Zealand EAdV-1 isolate (courtesy of GW Horner, National Centre for Disease Investigation, Wallaceville, New Zealand) as antigens, and 0.6% guinea pig RBC or 0.5% human type-O RBC suspension, respectively, as indicator systems. The tests were conducted according to standard laboratory procedures using 4 haemagglutination units of virus antigen per well (Lennette et al 1988). The tests were conducted at room temperature for PIV3 or at 37°C for EAdV-1. Non-specific inhibitory activity in serum samples was minimised by trypsin and periodate treatment (Hsiung 1982) and non-specific agglutinins were removed by prior adsorption with a 50% suspension of the respective RBC for 1 h. Test sera were regarded as positive if the titre was >1:10. A complement fixation test was used to detect antibodies against reovirus 3, using commercially obtained human reagents, according to the manufacturer's instructions (Denka Seiken Co Ltd, Japan). Antibodies to EAV were tested for using a SN assay, and titres >1:4 were regarded as positive. Foals were regarded as recently infected with ERAV, ERBV, EAdV-1, reovirus 3 or EAV if there was a 4-fold rise in antibody titre between successive samples.

Results

Signs of respiratory disease

Except for two foals that showed a slight mucopurulent nasal discharge on one sampling occasion in March or August 1996, all other foals in Group A remained healthy throughout the period of study. Foals in Group B showed clinical signs of respiratory disease during April and May. However, clinical data on individual foals were not available. Foals in Group C showed nasal discharge of varying severity in May and June. The two fillies (Foals C1 and C4) seemed to be more markedly affected than the colts (Foals C2, C3 and C5), Foal C4 showing a white, copious nasal discharge at the time of sampling in June. Also, Foals C1 and C2 showed slight nasal discharge at the time of the sixth sampling, in October 1996.

Virus isolation and detection

The only viruses isolated were EHV-2 and EHV-5 (Figure 1). EHV-2 was first isolated from either PBL or nasal swab samples from foals as young as 3 months (Group A) or 4–6 months (Groups B and C) of age (Figure 1). EHV-2 was isolated from 155/157 (99%) of the PBL samples tested and from 40/172 nasal swabs from 18 foals. All isolations from nasal swabs, except one,

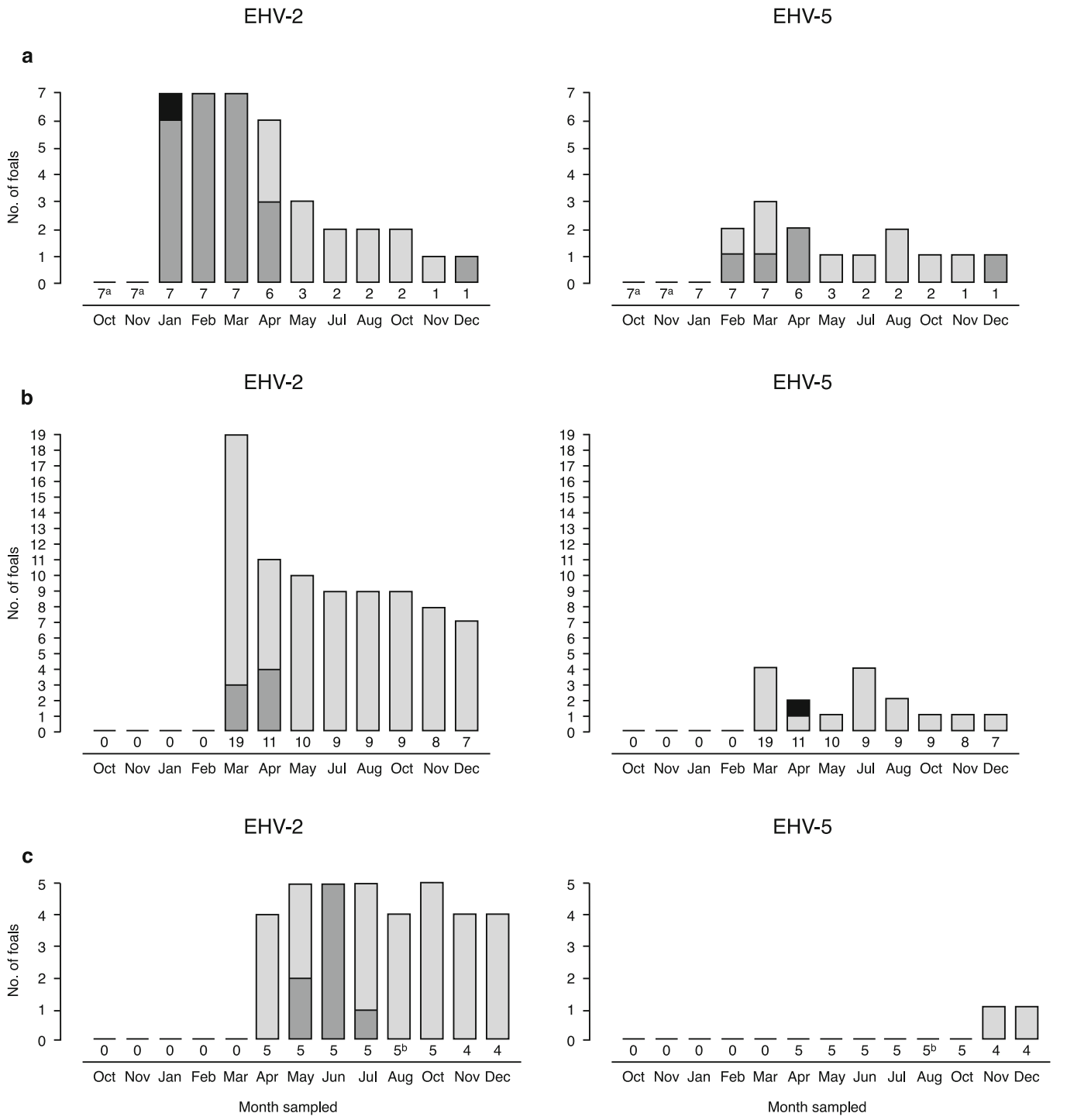

Figure 1. Viruses isolated from nasal swabs (■), peripheral blood leucocytes (PBL) (■) or both nasal swabs and PBL (■) from foals sampled on a monthly basis: (a) Group A; (b) Group B, and; (c) Group C. Numbers above the x axis indicate numbers of foals sampled on each occasion. [a]Virus isolation was attempted from nasal swabs only; [b]Virus isolation was attempted from PBL from four foals only.

were made over a period of 2–4 months from January to April (Group A), March to April (Group B) or May to July (Group C) (Figure 1). EHV-2 was not isolated from subsequent nasal swab samples but continued to be isolated from PBL. EHV-5 was isolated from either PBL or nasal swabs, or both, of 15 foals on 32 occasions. With one exception, all samples that were positive for EHV-5 were also positive for EHV-2. All samples tested for the presence of EHV-1 and EHV-4 using PCR, including 172 nasal swab filtrates, yielded negative results.

Serology

The results of serological testing are presented in Figures 2–4. All foals were positive for SN antibodies to EHV-1 or EHV-4 on at least one sampling occasion and all but one were positive for EHV-1-specific antibodies measured by ELISA on at least one sampling occasion. Serological evidence of recent EHV-1 infection was present at least once during the period of study in 18/23 (78%) foals from which at least two blood samples were collected. Serological evidence of recent EHV-1 infection was first detected between May and August in all 3 groups. Foals B8, B13 and B19 showed serological evidence of EHV-1 or EHV-4 infections twice during the period of study, 5–7 months apart (Figure 3). Three foals (A3, B1 and B19) showed serological evidence of recent EHV-1 infection without a corresponding 4-fold rise in the EHV-1 SN titres (Figures 2 and 3). Foals A1 and A4 showed a rise in ELISA titres without reaching the 60% cut-off value (from 7 or

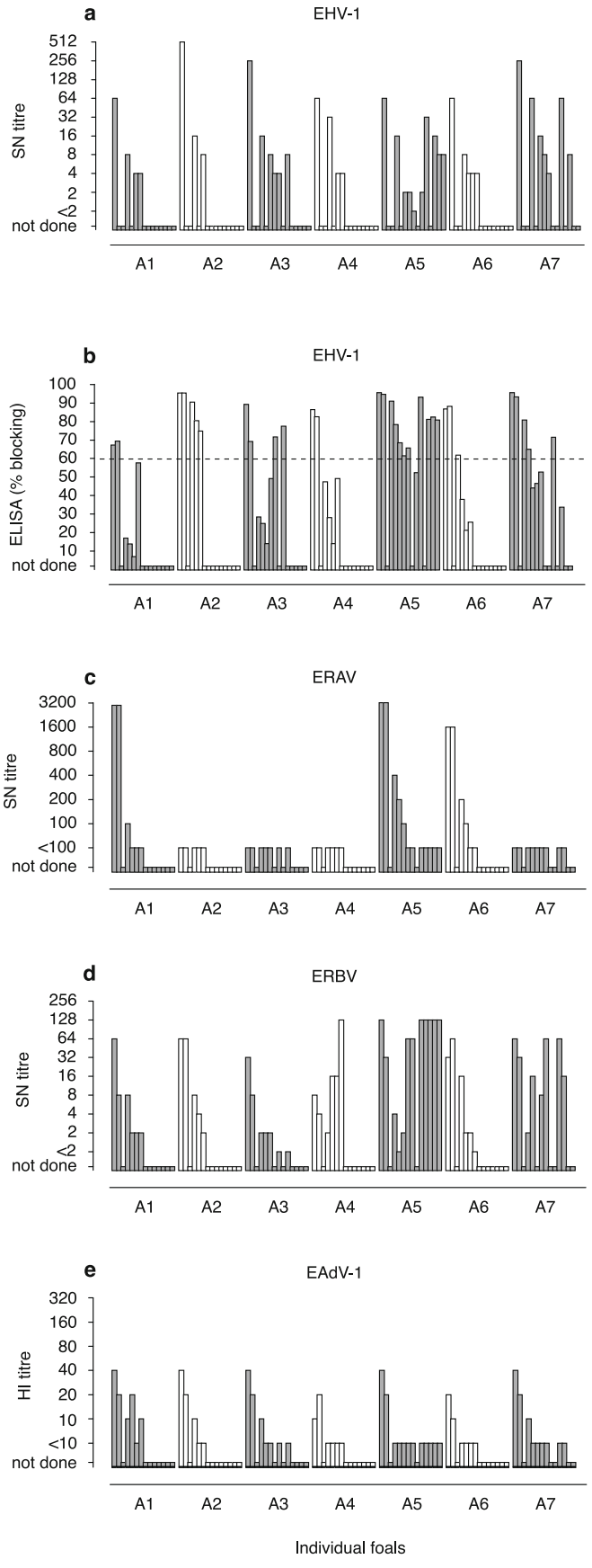

Figure 2. Results of serological testing for the presence of (a) serum neutralising (SN) and (b) ELISA antibodies to equine herpesvirus 1 (EHV-1); (c) SN antibodies to equine rhinitis-A virus (ERAV) and; (d) equine rhinitis-B virus (ERBV); and (e) haemagglutination inhibition (HI) antibodies to equine adenovirus 1 (EAdV-1) in foals in Group A. Titres of individual foals (A1 through A7) are shown, expressed as reciprocal serum dilutions. Consecutive bars represent consecutive months from October 1995 through to December 1996. The first sampling in October-November is regarded as an October sampling. Specific dates of each sampling are shown in Table 1.

14% to 57 or 49%, respectively) at the last sampling in April. Foal B16 showed a 4-fold rise in the EHV-1 SN titre, while its ELISA titre remained below the 60% cut-off value, possibly indicating EHV-4 infection (Figure 3).

Antibodies to ERAV were only detected in serum samples collected from foals from Group A (Figure 2). In this group, 3/7 foals had high ERAV SN antibody titres at their first sampling, in October–November. The sera of the remaining four foals were negative at a 1:100 dilution and were retested at a 1:10 dilution, at which 3/4 remained negative and 1/4 showed a titre of 1:40. During the period November to March, the titres of Foals A1, A2 and A6 gradually declined, indicating that the antibodies were probably maternally derived. Antibodies to ERAV were not detected in any of the serum samples collected from the foals in April and all the foals sampled remained negative until the last sampling in December.

SN antibodies to ERBV were evident in 19/23 (83%) foals on at least one sampling occasion and 15/23 foals showed evidence of seroconversion to ERBV during the study period. All foals in Group A had SN antibodies to ERBV at the first sampling in October–November (Figure 2). Initial titres ranged from 1:8–1:128. ERBV antibody titres gradually declined during the following months, suggesting that the antibodies detected were probably maternally derived. Subsequently, between February and April, 3/7 foals in Group A showed a rise in ERBV antibody titres, possibly indicating recent infection. Most of the foals from Groups B and C were negative for ERBV antibodies at the first sampling in March–April. During the following months, 9/11 (82%) foals from Group B seroconverted to ERBV, titres ranging from 1:4–1:128 (Figure 3). In Group C, 3/5 foals showed a rise in titre to ERBV from <1:2 to 1:4 (Figure 4). None of the foals from this group, however, developed an ERBV titre greater than 1:4 during the study period, except for Foal C5 that had a titre of 1:16 in April and May.

Haemagglutination inhibition (HI) antibody titres to EAdV-1 ≥1:10 were evident in 21/23 (91%) foals on at least one sampling occasion and 16/23 foals showed serological evidence of recent EAdV-1 infection. (Figures 2–4). None of the 67 serum samples tested were positive for antibodies to EAV, reovirus 3 or PIV3. There was no clear association between infection with any of the viruses isolated or tested for and the presence of overt clinical signs of respiratory disease.

Discussion

This study provides serological and/or virological evidence of EHV-1, EHV-2, EHV-5, EAdV-1 and ERBV infection in all three groups of foals studied. The prevalences of EHV-1 SN antibodies (100%) and ERBV SN antibodies (83%) were higher than those reported in an earlier study conducted in New Zealand, in which 67% of five 11-month-old foals were positive for SN antibodies to EHV-1 and 41% were positive for SN antibodies to ERBV (Jolly et al 1986). The higher prevalences evident in our study probably reflect the fact that we sampled foals over a period of several months, whereas the prevalences reported by Jolly et al (1986) were calculated from single serum samples. All foals were positive for EHV-2, as determined by virus isolation, at least once during the current study period. Similar results have been reported by others (Fu et al 1986; Murray et al 1996). Other viruses, including ERAV, reovirus 3 and PIV3, were not detected among the foals included in this study, which corresponds with previous findings (Jolly et al 1986).

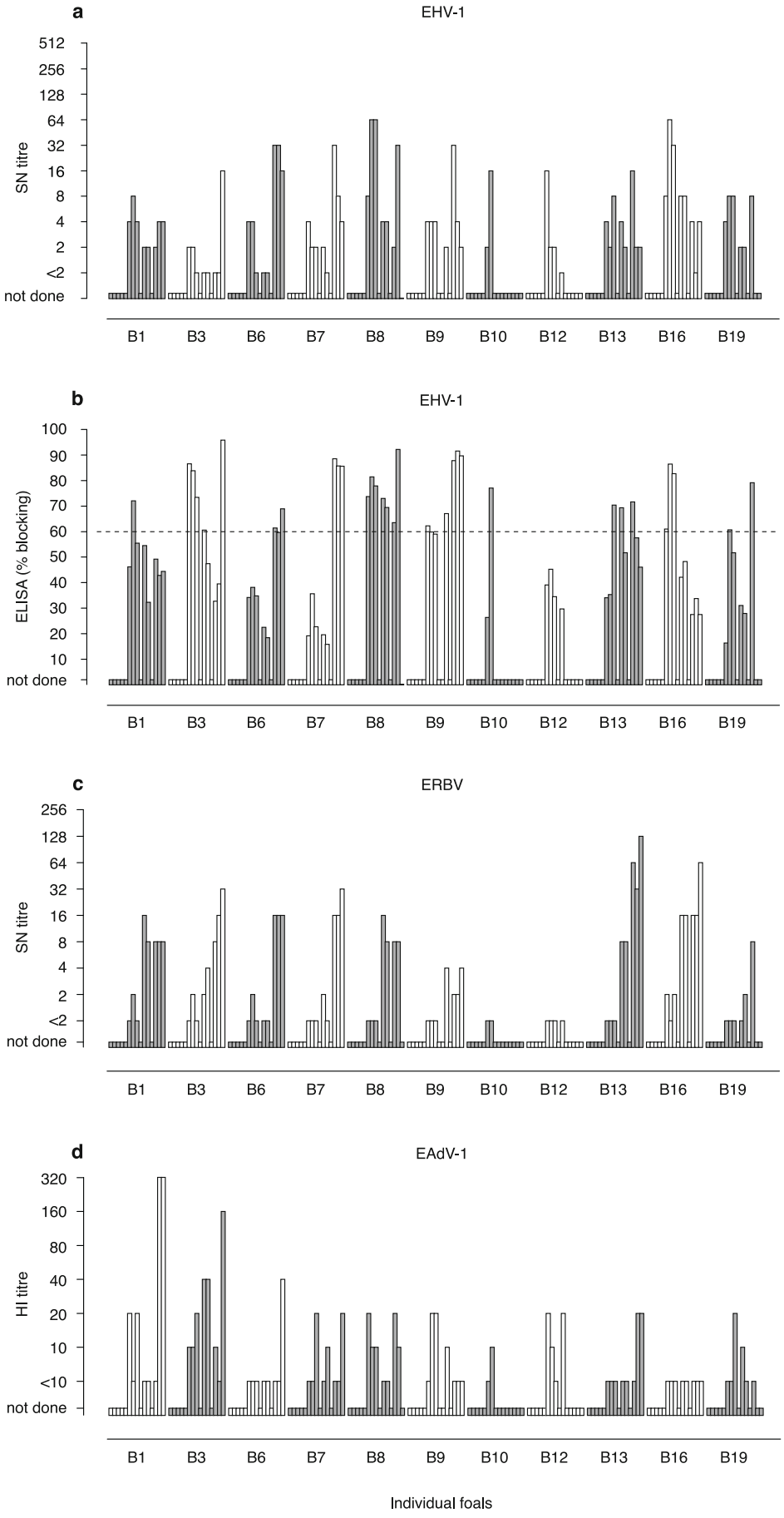

Figure 3. Results of serological testing for the presence of (a) serum neutralising (SN) and (b) ELISA antibodies to equine herpesvirus 1 (EHV-1); (c) SN antibodies to equine rhinitis-B virus (ERBV) and; (d) haemagglutination inhibition (HI) antibodies to equine adenovirus 1 (EAdV-1) in foals in Group B. Titres of individual foals (B1 through B19) are shown, expressed as reciprocal serum dilutions. Consecutive bars represent consecutive months from October 1995 through to December 1996. The first sampling in March-April is regarded as a March sampling. Specific dates of each sampling are shown in Table 1.

Despite serological evidence of infection with other viruses, only EHV-2 and EHV-5 were isolated from the nasal swabs and PBL samples collected in our study. It is possible that viruses other than EHV-2 and EHV-5 were not present in the samples collected. The success of virus isolation depends to a large extent on the timing of sampling in relation to infection, and many infections are only diagnosed retrospectively from serology results. The fact that none of the nasal swab filtrates tested directly for the presence of EHV-1 or EHV-4 DNA using PCR methods yielded positive results supports the view that neither EHV-1 nor EHV-4 were present in the samples collected.

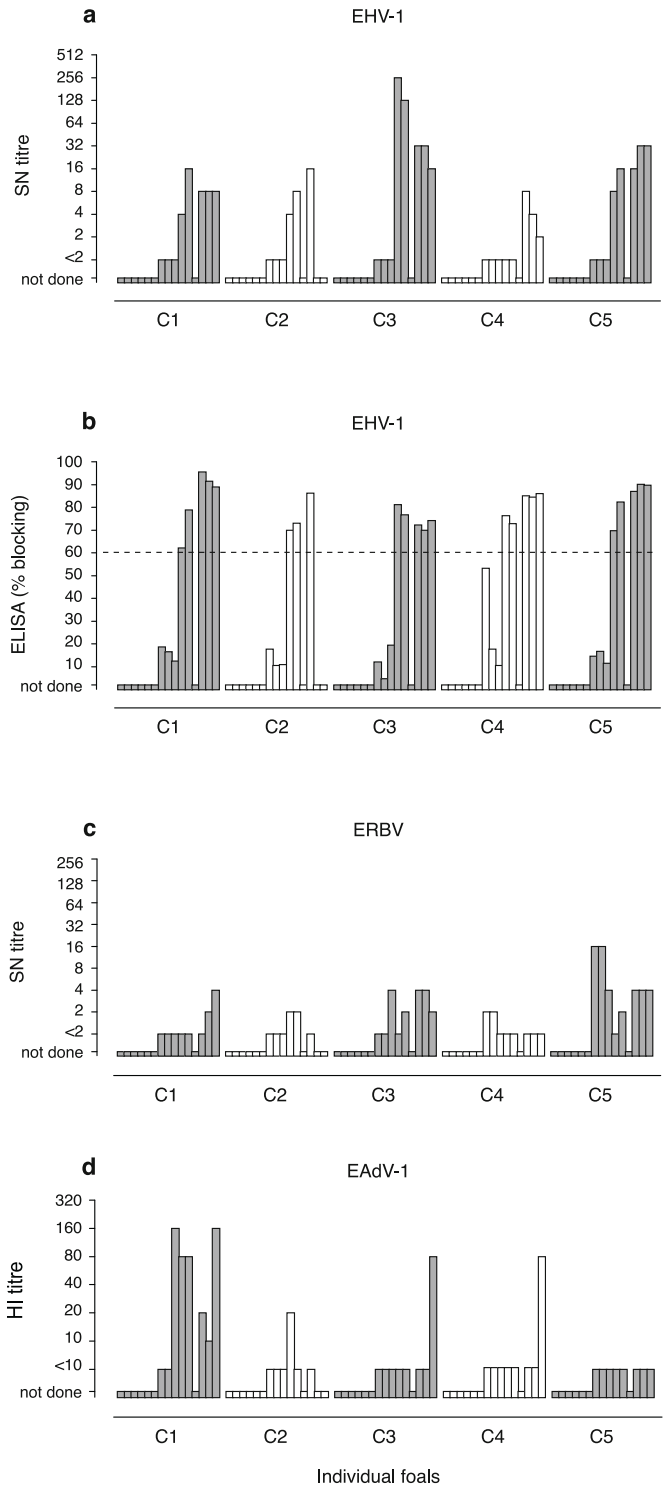

Figure 4. Results of serological testing for the presence of (a) serum neutralising (SN) and (b) ELISA antibodies to equine herpesvirus 1 (EHV-1); (c) SN antibodies to equine rhinitis-B virus (ERBV); and (d) haemagglutination inhibition (HI) antibodies to equine adenovirus 1 (EAdV-1) in foals in Group C. Titres of individual foals (C1 through C5) are shown, expressed as reciprocal serum dilutions. Consecutive bars represent consecutive months from October 1995 through to December 1996. Specific dates of each sampling are shown in Table 1.

There are several possible explanations for failure to isolate viruses other than EHV-2 or EHV-5 even if foals were shedding them at the time of sampling. These include collection of nasal rather than nasopharyngeal swabs, suboptimal processing of samples or cell culture conditions, or interference between herpesviruses present in the samples with the ability of other viruses to grow in vitro. The inhibitory effect of EHV-2 on the propagation of EHV-1 in cell culture has been reported by others (Dutta et al 1986; Welch et al 1992). The cell culture conditions used in our study may not have been optimal for the growth of rhinitis viruses. Despite serological evidence of their presence, isolation of rhinitis viruses has never been reported in New Zealand, suggesting that isolation of these viruses in cell culture may be difficult. However, 19 and 28 ERBV isolates obtained during studies conducted in America (Carman et al 1997) and Switzerland (Steck et al 1978), respectively, were isolated using standard cell culture techniques. It is also possible that rhinitis viruses may have been replicating in the cell culture but not producing any visible cytopathic effect, as has been demonstrated by others (Li et al 1997).

In all three groups of foals, the isolation of EHV-2 and EHV-5 from nasal swabs preceded serological evidence of infection with other respiratory viruses. This is consistent with the notion that infection with EHV-2 and EHV-5 predisposes horses to infection with other viruses. Several recent investigations have provided indirect evidence that the role of EHV-2 and possibly EHV-5 in causing respiratory disease in horses may have been underestimated in the past. A significantly greater rate of EHV-2 isolation from tracheal aspirates of foals showing signs of respiratory disease compared with healthy foals was reported by Murray et al (1996). Borchers et al (1997) showed that PBL samples from 70% of horses showing signs of respiratory disease were positive for EHV-2, whereas samples from only 42% of healthy horses were positive. In a further study, an EHV-2 vaccine was shown to protect foals from pneumonia due to *Rhodococcus equi* infection (Nordengrahn et al 1996). The fact that EHV-2 is able to infect foals in the presence of maternally-derived antibodies (Wilks and Studdert 1974; Pálfi et al 1978; Fu et al 1986; Murray et al 1996) and can be isolated from horses with high anti-EHV-2 antibody titres (Borchers et al 1997), provides further evidence that EHV-2 (and possibly EHV-5) modulates the immune response of its host.

Alternatively, detection of EHV-2 and EHV-5 infections by virus isolation before infections with other viruses were detected serologically could be explained by differences between these diagnostic methods. Serological diagnosis, especially in young animals, is less reliable than virus isolation because residual levels of maternal antibodies can interfere with the animal's ability to mount an active humoral response. Three foals showed serological evidence of recent EHV-1 infection using ELISA, without a corresponding 4-fold rise in SN antibody titres. These infections would not have been detected by a SN test alone. In addition, Foals A1 and A3 showed a significant rise in ELISA titre without reaching the 60% cut-off value used in our study, and their corresponding SN titres remained low. The rise in ELISA titres could represent a serological response to EHV-1 infection in the presence of residual levels of maternal antibodies. Alternatively, it could represent cross-reaction to EHV-4 antibodies and EHV-4 infection in these foals. In Foal B16, the 4-fold rise in EHV-1 SN titre concomitant with a low ELISA titre is suggestive of infec-

tion with EHV-4. In any case, these presumed EHV-1 or EHV-4 infections were not detected by the SN test and, thus, some infections with other viruses may also have gone undetected during the first 4–6 months of life.

Some of the foals sampled showed serological evidence of infection with EHV-1, EHV-4, EAdV-1 or ERBV in April and May (Groups A and B) or in June–July (Group C). The majority of foals from Group A remained healthy, whereas most of the foals from Groups B and C showed signs of respiratory disease at some stage between April and June. Since there was serological and/or virological evidence of the presence of the same viral infections among these three groups of foals, the presence or absence of clinical signs of respiratory disease could reflect some unidentified differences in environmental or husbandry conditions between these groups. It could also result from the fact that only limited numbers of foals were sampled. Another possibility is that the signs of respiratory disease observed in some of the foals, particularly foals from Group C, were due to bacterial rather than viral infections. The data of Hoffman et al (1993) implicated the predominant role of primary bacterial infections in respiratory disease in foals, as these authors did not isolate any viruses from 101 cases of respiratory disease in foals. Also, seroconversion to EHV-1 or EHV-4 was only detected twice among 47 randomly selected, paired serum samples. These results are in contrast to the results of our study. Although the occurrence of bacterial infections in foals in our study was not monitored, our results show clearly that several respiratory viruses were active among them.

Acknowledgements

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