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Inflammatory airway disease, nasal discharge and respiratory infections in young British racehorses

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Summary

***Reasons for performing study*: Respiratory disease is important in young Thoroughbred racehorses, but the variation in the rates of occurrence between different ages and training groups has not been characterised.**

***Objectives*: To determine the rates of respiratory disease, particularly inflammatory airway disease (IAD), as well as evidence of infection, and their variation between age and group.**

***Methods*: Horses were examined monthly in 7 British flat training yards over a 3 year period. IAD was defined as increased mucus in the trachea with increased proportions of neutrophils in tracheal wash samples. Frequencies of disease outcomes were estimated from the data.**

***Results*: The prevalence of IAD was 13.8% and the incidence was 8.9 cases/100 horses/month. Rates varied with training and age groups, decreasing in older animals. The prevalence of nasal discharge (ND) was 4.1%. Rates of bacterial isolation were more common than viral infections. The incidence and prevalence of several bacterial species decreased with age.**

***Conclusions*: IAD and ND were common in young racehorses, varying significantly between training groups and decreasing with age, consistent with infection playing a role in aetiology.**

***Potential relevance*: The high prevalence of IAD in 2-year-old horses in Britain suggests that routine endoscopic examination may be helpful in providing early diagnosis and appropriate therapy. The transmission of bacteria and viruses within and between groups of young animals and the role of infection, stable environment and factors inherent to each horse, including their genetic make-up, in the multifactorial aetiology of the disease all merit further study.**

Introduction

Respiratory disease is common in racehorses in training (Jeffcott *et al.* 1982; Burrell *et al.* 1996) and was the second most common cause of lost training days in a study in 5 flat racing stables (Rossdale *et al.* 1985). Although incidence and prevalence are known to vary widely between yards and between years (Morley 1995; Rossdale *et al.* 1985), the extent of this variation and the causes and clinical presentation of the disease have not been so well defined.

Clinical presentation of inflammatory airway disease (IAD) includes mild signs of respiratory disease such as cough and increased secretions in the trachea (Burrell *et al.* 1996; Christley *et al.* 2001; Robinson 2003) and poor performance in racing and training (MacNamara *et al.* 1990; Moore *et al.* 1995; Robinson 1997, 2003). Horses with IAD also have increased proportions of inflammatory cells, particularly neutrophils, in their airway secretions (Robinson 2003). Due to different disease definitions it is difficult to compare some studies directly, but the prevalence of IAD in horses in one training stable in the UK over 2 years was around 33%, whereas nasal discharge (ND) was seen on only 4% of days (Burrell *et al.* 1996). A study of naturally occurring upper respiratory tract disease on a Canadian Thoroughbred racetrack found the incidence of upper respiratory disease (URD) to be 4.9 cases/100 horse months (Morley 1995).

Inflammatory airway disease has a multifactorial aetiology (Burrell *et al.* 1996), including viral (Wood *et al.* 2003), bacterial (Wood *et al.* 1993; Burrell *et al.* 1996; Christley *et al.* 2001; Newton *et al.* 2003a) and environmental components (Burrell *et al.* 1996; Holcombe *et al.* 2001a; McGorum and Pirie 2003). Moreover, recurrence or persistence of IAD within individual racehorses is an important feature of the disease (Moore *et al.* 1997; Wood *et al.* 1997a; Robinson 2003). Studies of the naturally occurring disease must consider this feature.

There is considerable interest in the association between respiratory disease and different bacteria and viruses (Wood *et al.* 1993, 1997b; Burrell *et al.* 1994, 1996; Chapman *et al.* 2000; Christley *et al.* 2001; Hoffman *et al.* 2003; Newton *et al.* 2003b) and rates of isolation of different bacteria and viruses in racehorses have been reported (Allam and Lemcke 1975; Sugiura *et al.* 1988; Burrell *et al.* 1996), although not in a large population of young racehorses in Britain.

We studied naturally occurring respiratory disease and respiratory infections in racehorses of different ages in 7 Thoroughbred (TB) training yards between 1993 and 1996. The study as a whole was designed specifically to test quantitative hypotheses relating to the association between respiratory disease and specific viruses and bacteria, as well as to determine the frequency of disease. We report here estimates of incidence and prevalence and how they varied between age and training groups.

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TABLE 1: Population size, incidence, mean monthly prevalence (95% confidence intervals) and mean duration of inflammatory airway disease (IAD) in different training yards; IAD prevalence varied significantly between training groups and between years (P<0.001)

Trainer	Years	No. horses	No. observations	Incidence (/100 horses/month)	95% CI	Monthly prevalence	% - 95% CI	Mean duration (weeks)
1	1993–1995	15	143	8.1	3.0–13.3	10.5	(5.4–15.5)	6.3
2	1994–1996	28	311	9.1	5.2–12.9	14.8	(10.8–18.8)	8.3
3	1994–1996	25	334	4.2	1.8–6.7	6.0	(3.4–8.5)	6.6
4	1993–1996	31	326	9.6	5.5–13.7	12.0	(8.4–15.5)	6.2
5	1995–1996	29	252	4.9	1.9–7.9	7.1	(3.9–10.3)	6.8
6	1996	15	137	30.3	18.9–41.7	43.1	(34.7–51.5)	10.9
7	1994	20	101	18.5	7.8–29.2	25.7	(17.1–34.4)	8.1
Overall	1993–1996	148	1604	8.9	7.2–10.6	13.9	(12.2–15.6)	7.9

Materials and methods

Data

Horses, ranging from yearlings to 8-year-olds, were studied over a 38 month period from November 1993 to December 1996. Ten to 15 horses in 7 different training stables were selected randomly at the beginning of the study. The trainers were asked to select the horses, at random, to be representative of the age and sex population of horses in the yard. They were asked not to select the horses on the basis of previous respiratory disease history. Replacement horses were selected in the same way. The stables, 5 of which were studied at any one time, were a convenience sample due to the high level of cooperation required from trainers and their staff. Two stables were omitted during the study, one due to trainer death and the other due to ending of cooperation. Some of the horses from the former yard were subsequently included, as the replacing trainer was also recruited onto the study. The study population comprised 148 horses that were observed for a total of 1604 months. The distribution of horses and observations between training yards are shown in Table 1. Much of this work was contained in a PhD thesis (Wood 1998) and the data on concurrent clinical cases have been reported elsewhere (Newton *et al.* 2003b).

Clinical records and examinations

Horses were examined clinically on a monthly basis, including an endoscopic examination of the trachea to the level of the carina immediately after exercise (Burrell 1985; Newton *et al.* 2003b). A tracheal wash sample was collected transendoscopically by instilling 30 ml of sterile phosphate buffered saline into the trachea immediately anterior to the carina through a sterilised polythene catheter and then immediately aspirating it. A serum sample was also collected at this time for viral serology.

Laboratory examinations

Evidence of viral infection (including influenza H7N7 and H3N8, equine herpesvirus-1 [EHV-1] and -4, equine rhinovirus-1 [ERV-1] and -2 and equine adenovirus) was assessed through serological examination of serial blood samples using complement fixation tests for EHV and ERV (Thomson *et al.* 1976) and haemagglutination inhibition tests for influenza and adenovirus. A horse was defined as suffering a viral infection if there was a 4-fold or greater increase in specific titres between 2 samples tested in the same assay. Tracheal wash samples and nasopharyngeal swabs were assessed by standard quantitative cultural methods for presence of bacteria, including mycoplasma (Newton *et al.* 2003b).

Definitions of disease

Four different case definitions are used in this report. These are: 1) increased mucus in the trachea, based on visual assessment of the amount of mucus, scored from 0 to 3 (Burrell 1985); 2) cytological assessment of the degree of neutrophilic inflammation in tracheal aspirates, based on the proportion of neutrophils in the cellular population (Smith *et al.* 2003); 3) the presence of ND at the time of examination; and 4) the presence of IAD. The degree of inflammation of the lower airway was scored on an ordinal scale, from 0 to 3, and IAD was defined as an inflammation score ≥2/3. A score of 3/3 was derived from detecting ≥2/3 amounts of mucus in the trachea, moderate or greater proportions of neutrophils in the tracheal wash and ≥1000 nucleated cells/mm^3 tracheal wash. Each component contributed a maximum of 1 to the score. ND was not recorded in one training yard, which was therefore excluded from relevant analyses.

Statistical methods

Incidence rates and prevalences were estimated using standard approaches (Schlesselman 1982; Rothman and Greenland 1997). Confidence limits around prevalence proportions were estimated using the normal approximation. We estimated approximate duration (D) of disease and infection through the use of the formula:

$$D = \frac{P}{(1 - P) \times I}$$

where P = overall prevalence and I = overall incidence rate.

Test sensitivities, specificities and predictive values and their confidence limits for clustered data were estimated using mixed effects logistic regression analyses (Dohoo *et al.* 2003). Data analysis was carried out using SAS-STAT (v8.02)[1]. Variation in probability of disease between age groups and training yards was assessed through the Kruskal Wallis test. Age-specific rates of infection and disease were only presented for horses age 2–4 years, due to small sample sizes for older horses (a feature of flat training in the UK) and because incomplete annual data existed for yearlings, although their rates of disease and those for horses age 5 years are compared for illustrative purposes.

TABLE 2: Estimates of overall and age specific incidence rates, prevalences and mean durations for disease and different infections

	All ages combined			Age 2 years			Age 3 years			Age 4 years		
Variable	I (95% CI)	P (95% CI)	D	I (95% CI)	P (95% CI)	D	I (95% CI)	P (95% CI)	D	I (95% CI)	P (95% CI)	D
IAD	8.9 (7.2–10.6)	14 (12.2–15.6)	7.9	8.9 (6.8–10.9)	19.5 (16.7–22.3)	8.1	4.3 (2.6–6.0)	8.8 (6.5–11.1)	7.5	2.0 (0.0–4.3)	4.0 (0.8–7.2)	6.8
≥Moderate (2/3) mucus*	6.0 (4.6–7.3)	10.9 (9.4–12.4)	8.9	6.2 (4.4–7.9)	15.8 (13.2–18.3)	9.4	2.5 (1.2–3.8)	6.2 (4.2–8.1)	9.0	2.0 (0.0–4.3)	3.3 (0.2–6.2)	5.6
≥Moderate proportions of neutrophils†	40.5 (37–44)	48 (45.8–50.7)	10.0	42.5 (37–48)	50.6 (47.1–54.2)	10.5	36.4 (31–42)	44.2 (40.1–48.3)	9.4	43.1 (30–56)	50.0 (41.9–58.0)	10.1
ND	2.7 (1.6–3.7)	4.1 (3.0–5.2)	–	2.5 (1.1–3.8)	4.1 (2.6–5.7)	–	2.3 (0.7–3.9)	2.4 (0.9–3.8)	–	2.3 (0.0–5.4)	4.4 (0.6–8.2)	–
S. zooepidemicus	23.0 (20–26)	30.4 (28.2–32.7)	8.2	29.2 (25–34)	37.7 (34.3–41.2)	9.0	20.3 (16–25)	25.9 (22.3–29.5)	7.5	17.8 (10–25)	16.0 (10.0–21.9)	4.6
Actinobacillus /*Pasteurella* spp.	23.6 (21–26)	27.3 (25.1–29.5)	6.9	25.1 (21–29)	28.4 (25.2–31.5)	6.9	22.7 (18–27)	27.6 (24.0–31.3)	7.3	20.9 (12–30)	25.3 (18.3–32.4)	7.0
S. pneumoniae	4.9 (3.7–5.6)	6.7 (5.5–7.9)	6.3	7.5 (5.3–9.7)	10.0 (7.9–12.0)	6.4	3.4 (1.7–5.0)	4.6 (2.9–6.3)	6.2	0.9 (0.0–2.5)	1.3 (0.0–3.2)	6.7
Acinetobacter spp.	9.7 (8.0–11.3)	10.0 (8.5–11.5)	5.0	10.4 (8–13)	10.5 (8.4–12.7)	4.9	9.7 (6.9–12.5)	10.6 (8.0–13.1)	5.3	6.1 (1.7–10.5)	5.3 (1.7–9.0)	4.0
Coagulase -ve *Staphylococcus* spp.	40.1 (37–44)	42.9 (40.5–45.3)	8.1	36 (31–41)	40.4 (36.9–43.9)	8.2	44 (39–50)	45.2 (41.1–49.4)	8.1	46 (33–59)	48 (39.9–56.1)	8.8
Nonhaemolytic *Streptococcus* spp.	49.4 (45–54)	57.6 (55.1–60.0)	11.9	53 (46–59)	59.7 (56.2–63.1)	12.2	50 (43–57)	58.1 (54.0–62.2)	12.2	47 (34–59)	47.3 (39.3–55.4)	8.3
Serratia spp.	0.9 (0.4–1.4)	1.3 (0.8–1.9)	6.6	0.8 (0.1–1.5)	1.3 (0.5–2.1)	7.2	0.6 (0.0–1.4)	0.9 (0.1–1.7)	6.1	1.7 (0.0–4.1)	2.7 (0.1–5.3)	6.9
M.felis	1.5 (0.8–2.0)	1.6 (0.9–2.2)	4.6	1.3 (0.4–2.3)	1.9 (0.9–2.9)	6.3	2.2 (0.8–3.6)	1.7 (0.6–2.8)	3.4	1.0 (0.0–2.8)	0.7 (0–2.1)	3.2
M.equirhinis	10.0 (8.1–11.9)	12.3 (10.6–13.9)	6.1	10.8 (8–14)	14.0 (11.4–16.6)	6.5	9.7 (6.7–12.7)	11.4 (8.7–14.1)	5.8	9.3 (3.5–15.1)	9.4 (4.5–14.3)	4.9
Slower growing glucose fermenter	1.0 (0.4–1.6)	1.6 (0.9–2.2)	7.1	1.5 (0.5–2.6)	2.0 (1.0–3.0)	5.8	0.7 (0.0–1.5)	1.1 (0.2–2.1)	6.6	0 (–)	0 (–)	–
'D87 like isolates'	1.4 (0.7–2.0)	1.3 (0.7–1.9)	4.2	1.1 (0.2–2.1)	1.3 (0.4–2.1)	5.5	1.4 (0.3–2.6)	1.3 (0.3–2.3)	4.0	1.9 (0.0–4.6)	1.4 (0.0–3.4)	3.2
Other *Mycoplasma* spp.	0.5 (0.1–1.0)	0.5 (0.1–0.8)	4.1	0.4 (0–0.9)	0.4 (0–0.9)	4.6	0.7 (0.0–1.5)	0.6 (0–1.2)	3.7	0 (–)	0 (–)	–

I = Incidence (/100 horses/month); P = Prevalence; D = Mean duration (weeks); *amounts of mucus visible in trachea; †in tracheal wash samples.

Results

Mucus in the trachea

The overall prevalence of moderate or greater (score ≥2/3) amounts of mucus in the trachea, assessed monthly, was 10.9% (95% confidence interval (95% CI) 9.4–12.4%) and overall incidence was 6.0 cases/100 horses/month (95% CI 4.6–7.3%; Table 2). The mean duration of moderate or greater amounts of mucus in the trachea was 9 weeks. There was significant variation in the prevalences between horses (P<0.001), training yards (P<0.001; data not shown) and with age of the horse, where it decreased with increasing age (P<0.001; Fig 1). The incidence rate of increased mucus in the trachea decreased from age 2 and 3 to 4 years, and duration and prevalence also decreased with age (Table 2).

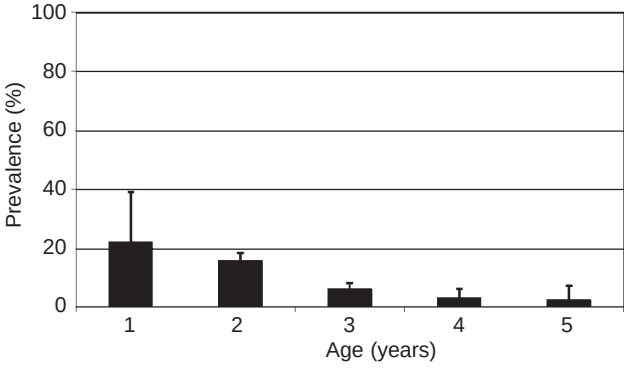

Fig 1: Age-specific prevalence (± 95% confidence limits) of moderately increased (score ≥2/3) mucus in the trachea.

Proportions of neutrophils in tracheal wash samples

The overall prevalence of moderate or greater proportions of neutrophils was 48.2% (95% CI 45.8–50.7%) and the overall incidence was 40.5 cases/100 horses/month (95% CI 36.8–44.3%; Table 2). Prevalence varied significantly between training yards (P<0.001; data not shown), although not with respect to age of the horse (P = 0.14; Fig 2). The incidence rates, prevalence and mean duration of moderate or greater proportions of neutrophils varied little between different age groups (Table 2).

Inflammatory airway disease

The overall prevalence of IAD was 13.9% (95% CI 12.2–15.6%) and the overall incidence was 8.9 cases/100 horses/month (95% CI 7.2–10.6; Table 2). Mean duration was 8 weeks.

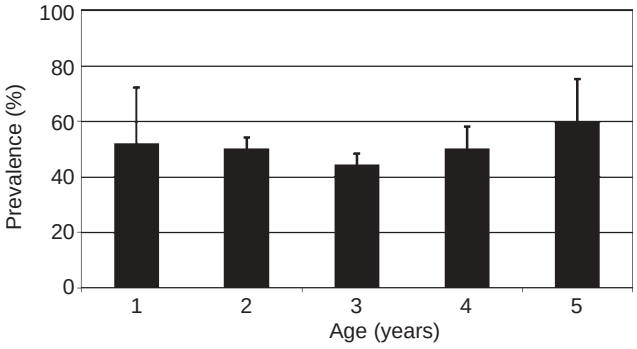

Fig 2: Age-specific prevalence (± 95% confidence limits) of moderate proportions of neutrophils in tracheal wash samples.

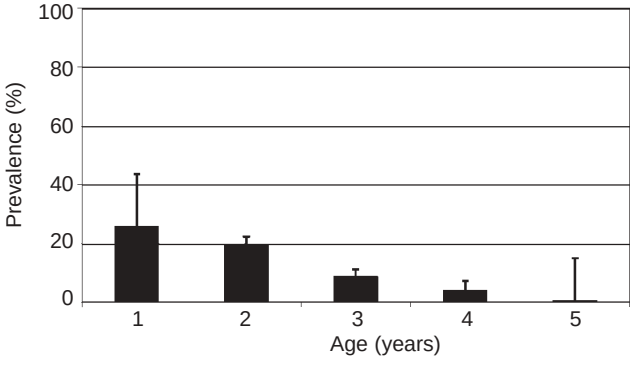

Fig 3: Age-specific prevalence (±95% confidence limits) of inflammatory airway disease.

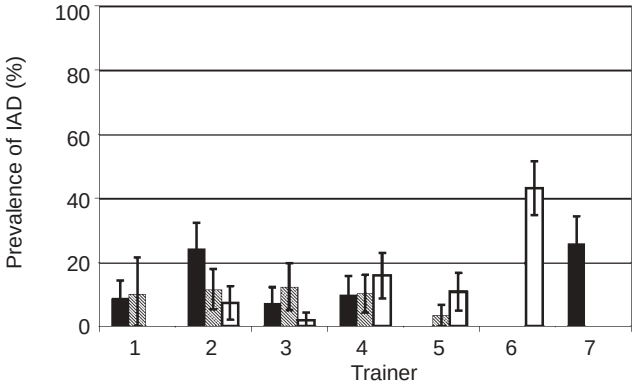

Fig 4: Trainer- and year-specific prevalence (± 95% confidence limits) of inflammatory airway disease. ■ = 1994; ■ = 1995; □ = 1996.

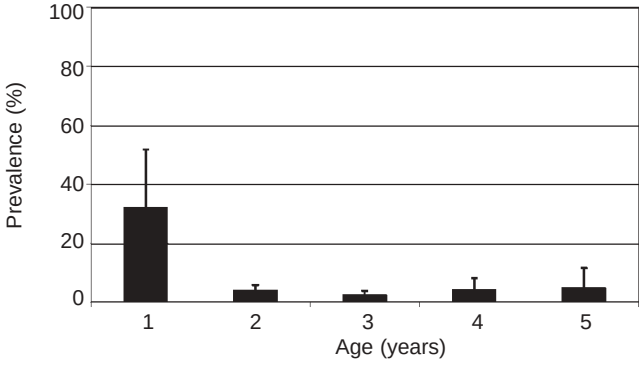

Fig 5: Age-specific prevalence (± 95% confidence limits) of nasal discharge.

Of the 223 examinations where IAD was diagnosed, moderate or greater amounts of mucus were seen in 158 (70.9%) and moderate or greater proportions of neutrophils were detected in 216 (96.9%). The sensitivity of detecting IAD through observing moderate or greater amounts of mucus in the trachea, having taken into account the repeated observations, was 76.2% (95% CI 63.6–85.5%) and the specificity 98.9% (95% CI 96.5–99.7%). IAD was present for 158 of 175 observations where mucus score was ≥2/3 and the positive predictive value was 91.1% (95% CI 78.4–96.6%). IAD was diagnosed in only 37/355 (10.4%) of examinations when slightly increased amounts of mucus (score = 1/3) were observed in the trachea. The sensitivity and specificity for IAD of observing at least slightly increased amounts of mucus in the trachea were 94.8% (95% CI 83.5–98.5%) and 76.5% (95% CI 72.9–79.8%), respectively. The positive predictive value for the same was 33.1% (95% CI 17.2–54.0%).

Both the incidence rate and prevalence of IAD decreased significantly in horses (P<0.001; Fig 3) from age 2 to 4 years (Table 2). Mean duration of IAD decreased slightly with age. There was significant variation in the prevalence of IAD between training yards (P<0.001; Table 1) and this prevalence also varied markedly between years, within each yard (Fig 4). There was a >500% difference in overall prevalence between lowest and highest yards (Table 1), with strong correlation between prevalence and incidence. Mean duration of disease did not vary to the same extent (170% difference), but was higher when both prevalence and incidence were high.

Age-specific cumulative annual prevalences were also estimated from horses where at least 8 observations were recorded during a 12 month period. The annual prevalence in 2-year-old horses was 78% (95% CI 67–89%), in 3-year-old horses was 50% (95% CI 33–67%) and in 4-year-old was 20% (95% CI 0–50%).

Nasal discharge

The prevalence of ND was 4.1% (95% CI 3.0–5.2%) and did not vary significantly between training yards, although it did vary significantly with age (P<0.0001), being much greater in yearlings than in any other age group (Fig 5). The overall incidence rate was 2.7 cases/100 horses/month (95% CI 1.6–3.7). Very few nasal discharge incidents had a duration of more than one month and it was therefore adjudged that these data were not suitable for estimation of duration.

Bacterial and viral infections

Prevalence and incidence rates for the isolation of the more common bacteria detected from tracheal wash samples are shown in Table 2. Other bacteria were occasionally isolated from the trachea, including *Corynebacterium* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila*. Mycoplasmas were generally less commonly isolated from the trachea than most species of bacteria. Not all species of mycoplasma have been serologically identified, but morphological and biochemical characteristics allowed grouping of the isolates; 2 such groups were common enough to group separately (Table 2). Of the tracheal wash samples, 22.4% were bacteriologically sterile.

TABLE 3: Overall and age-specific incidence rates (/100 horses /month) of viral infections

	All ages	Age 2 years	Age 3 years	Age 4 years
Influenza A/equi-2	0.7 (0.3–1.1)	0.9 (0.2–1.6)	0.7 (0.0–1.3)	0 (–)
Equine herpesvirus (1&4)	4.0 (3.0–4.9)	4.4 (2.9–5.8)	3.9 (2.2–5.5)	3.3 (0.4–6.2)
Equine rhinovirus-1	1.9 (1.3–2.6)	2.9 (1.8–4.1)	0.7 (0.0–1.4)	1.3 (0.0–3.2)
Equine rhinovirus-2	0.7 (0.3–1.2)	1.2 (0.4–1.9)	0 (–)	0.7 (0.0–2.0)
Equine adenovirus	1.4 (0.8–1.9)	1.8 (0.9–2.2)	1.1 (0.2–1.9)	1.3 (0.0–3.2)

The incidence of the different bacterial and mycoplasma infections varied widely (Table 2) and there was also some variation in mean duration of infection between the different species. There was a reduction in incidence and prevalence of some species of bacteria with age, including *S. zooepidemicus*, *S. pneumoniae* and *Mycoplasma equirhinis*, but not with others (e.g. coagulase-negative *Staphylococcus* spp. and nonhaemolytic *Streptococcus* spp.). There was very little evidence of any reduction in mean duration of infection of most bacterial species with age.

The most common viral infection was EHV-1/4 (Table 3). No horses had serological evidence of repeated infection with any virus. There was significant aggregation in time for EHV and influenza, as would be expected for such infectious agents (data not shown).

Discussion

This epidemiological study of respiratory disease in young racehorses in training in yards in England has provided new information on patterns of disease and microbial prevalence and incidence in different groups of animals. The results are consistent with previous work (Burrell *et al.* 1996), but the practical difficulties of obtaining unbiased estimates of parameters such as mean duration of disease or infection episodes require that care be taken in interpretation of results. The confidence limits around the estimates provided should always be considered. The method of selection of horses in the study could have introduced some bias into the estimation of disease rates, but at the time we felt it essential to work closely with trainers in order to ensure compliance and there was never any indication from extensive parallel routine investigations undertaken by the trainers' own staff that the horses we were studying were in any way different to those in the rest of the yard.

The figures of incidence are probably under- rather than overestimated, not so much because of the largely subclinical nature of IAD (Burrell *et al.* 1996), but rather because the adopted monthly sampling regime might have failed to detect a proportion of incidents of duration of less than one month (i.e. one sampling interval). Any such underestimation would have resulted in a corresponding overestimation of disease duration, although in practice any overestimation of duration was not likely to be of more than half the sampling interval. It was therefore of interest that our overall estimate of IAD duration (8 weeks) was very similar to the 7 weeks estimated directly in a previous study of IAD in UK racehorses (Burrell *et al.* 1996). Despite the difficulties in estimating prevalence when sampling is not continuous, we found that the annual cumulative prevalence of IAD in 2-year-old racehorses was nearly 80%, dropping considerably in older animals. Although we used rather stringent inclusion criteria for this (at least 8 months' observation for each horse), the estimates were very similar when the criteria were dropped to 4 months' observation as the minimum.

The study demonstrated clearly that IAD was much more common than nasal discharge. Increased mucus in the trachea, a component of IAD using our case definition, has been associated with poor racing (MacNamara *et al.* 1990) and training (Robinson 1997) performance. In this study, the prevalence of IAD was 13.9%, in contrast to the 33% reported from one yard, but that study used a less stringent case definition (Burrell *et al.* 1996). However, prevalence varied widely between training yards (and years of study) and was >25% in 2 yards in our study. A prevalence of 33% was also reported from North American racehorses (Sweeney *et al.* 1992), although this study used only increased proportions of neutrophils in bronchoalveolar lavage fluid (BALF) as its case definition.

This study has not, in itself, shed light on the significance of moderately increased proportions of neutrophils in tracheal wash samples. It was of note that, in contrast to all other signs of respiratory disease, the prevalence and incidence rates of increased proportions of neutrophils did not reduce with age, although the prevalence did vary significantly between training yards. The finding that the prevalence of increased proportions of neutrophils was high (~50%) in all age groups studied was interesting. Although not our primary consideration in this paper, these increased neutrophils were associated statistically with moderately increased tracheal mucus ($P<0.001$; data not shown), as has been previously reported for IAD (Robinson 2003). However, a large number of horses had increased neutrophils without fitting the stringent case definition of IAD. This appears, at first glance, to be at odds with the statement that there should be <20% neutrophils in healthy horses age 2 and 3 years (Robinson 2003). In reality, the data do not suggest that healthy horses can have increased neutrophils and the finding is probably more a reflection of a case definition that does not allow for a variation in disease severity, which creates a rather artificially exaggerated dichotomy between the states of health and disease. It may be that that developing an airway neutrophilia is an inevitable consequence of stabling (Tremblay *et al.* 1993; Holcombe *et al.* 2001b). More work is required before assigning significance to tracheal neutrophilia in housed racehorses when it occurs in the absence of other clinical changes such as increases in the amount of mucus visible in the trachea. It is these changes occurring together that have been particularly associated with infection (Wood *et al.* 1993; Burrell *et al.* 1996; Chapman *et al.* 2000).

The prevalence of nasal discharge in this study was 4.1%. This figure was similar to the 4% previously reported (Burrell *et al.* 1996). The incidence of 2.7 cases/100 horses/month was lower than the incidence of URD of 4.9 cases/100 horses/month reported in Canada (Morley 1995; Morley *et al.* 2000), but those investigators included more signs than ND in their definition of

URD and they found influenza and *S. equi* infection to be important and common causes of URD, whereas influenza was nearly absent in our well-vaccinated population and *S. equi* was never diagnosed. Investigations carried out by Animal Health Trust staff over the last 20 years have found that IAD is the most important clinical presentation in outbreaks of respiratory disease and loss of performance in racehorses (Wood *et al.* 1994a; J.L.N. Wood *et al*. unpublished data). Every effort to improve our understanding of and ability to diagnose, treat and prevent this syndrome should therefore be made.

As well as investigating rates of disease, we also recorded rates of different infections, which are rarely recorded in any equine population. As an association between some of these and disease has been reported (Burrell *et al.* 1994, 1996; Chapman *et al.* 2000; Christley *et al.* 2001; Newton *et al.* 2003b; Wood *et al.* 1993), our estimates should not be regarded as those in the healthy population. Although our overall prevalence figures for specific bacteria were somewhat different to those from a study in one stable in Newmarket (Burrell *et al.* 1985), broad conclusions relating to relative prevalence of common species are similar, particularly with respect to *S. zooepidemicus* and *Staphylococcus* spp. Many other published studies used a case-control approach, which makes comparison of prevalences difficult. We are not aware of any other study that has reported incidence rates of equine respiratory infections.

A subset of the data was used to study in detail the association between disease and different species of *Actinobacillus/Pasteurella* spp. (Ward *et al.* 1998). The identity of the species was important and *A. suis* and *A. lignieresii* were more closely associated with lower airway disease than the most common isolate, *A. equuli* (Ward *et al.* 1998). Ward *et al.* (1998) reported that, when the presumptive methods of classifying *Actinobacillus/Pasteurella* spp. isolates, as identified during the study presented here (similar to those used in many diagnostic laboratories), were compared with the more species-specific methods, the presumptive methods were too blunt to identify isolates accurately enough to justify separation in statistical analyses.

We confirmed our earlier findings (Wood *et al.* 1994b) that mycoplasma, particularly *M. equirhinis*, is common in racehorses in Britain. Despite our finding that *M. felis* can be associated with acute respiratory disease (Wood *et al.* 1997b; Newton *et al.* 2003b), we found this species to be relatively uncommon (Table 2).

The results from this study demonstrate clearly a significant reduction in incidence of IAD with age from 2 to 4 years. The prevalence similarly reduced from ages 2–3 to 4 years. Similar results were seen for moderately increased mucus in the trachea. The reduction of rates of IAD with age suggests the development of resistance or immunity and is consistent with IAD in young racehorses having an infectious aetiology. This is in contrast to the prevalence of RAO increasing with age (Dixon *et al.* 1995; Couëtil and Ward 2003). The hypothesis of a largely infectious aetiology is further supported by the reduction in incidence and prevalence of several bacterial infections with age, including *S. zooepidemicus*, *S. pneumoniae* and *M. equirhinis* (these bacteria being associated with IAD (Wood *et al.* 1993, 1994b; Burrell *et al.* 1996; Chapman *et al.* 2000; Christley *et al.* 2001). In contrast, the prevalence and incidence of other bacterial infections, such as nonhaemolytic *Streptococcus* spp. and coagulase-negative *Staphylococcus* spp., did not reduce with age (these bacteria not usually being associated with IAD). It is probable that some bacteria were detected in tracheal wash samples due to contamination from the nasopharynx. However, contamination was not an invariable result of our sample collection, as 22.4% of all tracheal wash samples were bacteriologically sterile. Interpretation of the significance of bacterial isolates from individual horses must take into account the clinical and cytological profile and, critically, the numbers of bacteria present. Other work has addressed the question of whether bacteria grown from tracheal samples collected as in this study are there due to contamination; it was reported that some species appeared preferentially to colonise the trachea and were not just detected in the trachea due to contamination from the upper respiratory tract (Newton *et al.* 2003a).

This study advances the understanding of the epidemiology of respiratory disease in young racehorses in training, in particular establishing prevalence and incidence rates of different presentations of disease and different infections. IAD was probably the most important presentation of respiratory disease, having a mean duration of nearly 8 weeks. The prevalence and incidence rates of inflammatory airway disease showed a decline with age, consistent with an infectious component in the aetiology. Analyses demonstrating the association between infections and other factors of respiratory disease in these studies shed more light on the probable aetiology of respiratory disease in racehorses (Wood *et al*. 2005).

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