Rattles in Horses
Effects of stud management on ecology of virulent Rhodococcus equi

A report for the Rural Industries Research and Development Corporation

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Rhodococcus equi (R. equi), commonly referred to as rattles, is an important cause of pneumonia in young foals. Recent findings have established that only some isolates of Rhodococcus equi can cause rattles in foals. However all previous studies on factors affecting the numbers of R. equi in the environment of foals have assumed that all isolates are significant. Limited studies in the laboratory have shown that virulent R. equi grow less rapidly than avirulent isolates under particular conditions, suggesting that the previous studies may not have established the most hazardous conditions on studs for the development of rattles.

As rattles is one of the most important diseases of foals on thoroughbred studs, better information of the conditions on studs which affect levels of virulent R. equi will enable development of measures which reduce disease without incurring additional costs for products such as vaccines or hyperimmune sera. In addition, these measures are likely to increase the benefit derived from such products.

This project aimed to develop novel methods for examining the environment of horse studs for virulent R. equi and then apply these methods to enhancing understanding of the factors increasing the risk of development of rhodococcal pneumonia on some farms.

The application of the novel methods developed for detecting concentrations of virulent R. equi in air and in soil enabled identification of laneways and holding pens as the areas on horse studs exposing foals to the highest risk for development of rhodococcal pneumonia and showed that sandy soils and poor pasture cover could increase the risk.

The method was also applied to sampling the exhaled air of foals and found to have potential as a novel, non-invasive diagnostic test. It also showed that direct transmission of R. equi between foals may be possible and that this may have a significant role in the occurrence of rhodococcal pneumonia on horse studs.

This project was funded from industry revenue which is matched by funds provided by the Australian Government.

This report is an addition to RIRDC’s diverse range of over 1500 research publications. It forms part of our Horses R&D sub-program which aims to to assist in developing the Australian horse industry and enhancing its export potential.

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Executive Summary

Although *Rhodococcus equi* (*R. equi*) is an important cause of pneumonia, commonly referred to as rattles, in young foals there are limited options for control. Current methods of prophylaxis rely upon reducing faecal contamination and dust on studs, to variable effect, but we have little understanding of whether these are the only factors that account for the mortality rates on some studs, and the higher infections rates on some studs.

This project aimed to develop novel methods for assessing the risk associated with particular horse farm environments. These methods were based on microbiological sampling of air and soil on studs, then differentiating virulent *R. equi* from avirulent strains. Once these methods were developed they were applied to examine different horse farms and different places on those farms.

This approach has led to the suggestion that the most dangerous areas on studs for foals are likely to be laneways and holding pens and that control may be aided by minimising the time that foals spend in these environments. In addition areas on farms that had low pasture cover, sandy, dry and acidic soils seemed to be a greater risk.

The method that was developed was also used to examine the exhalation of virulent *R. equi* by foals. This examination suggested that foals may be able to transmit virulent *R. equi* to each other and that some control may be achieved by avoiding close contact between foals, particularly if they are known to be suffering from rhodococcal pneumonia.

In addition, the sampling of exhaled breath from foals may prove to be a sensitive diagnostic technique for the early detection of infected foals.
Introduction

Although *R. equi* is an important cause of pneumonia, commonly referred to as rattles, in young foals there are limited options for control (24). Current methods of prophylaxis rely upon reducing faecal contamination and dust on studs, but we have little understanding of whether these are the only factors which account for the mortality rates on some studs, and the higher infections rates on some studs.

The aim of this project was to specifically examine the factors which influence the population of virulent *R. equi*. There were a number of studies of the ecology of *R. equi* published during the 1980s that established that the presence of horse faeces in soil greatly stimulated the growth of *R. equi* (2, 15) and that further established that the volatile fatty acids in horse faeces were likely to be responsible. Studies during this period also compared the growth characteristics of *R. equi* in different types of soil. In addition studies by Takai and colleagues established that *R. equi* is excreted in largest numbers by foals aged between 4 and 12 weeks (31, 34). However since these studies were performed it has been found that only a small proportion of all *R. equi* in soil are able to cause rattles. Over the last 5 years it has been shown that only *R. equi* carrying a specific plasmid can cause disease in foals. Furthermore preliminary studies have shown that virulent strains grow more slowly than avirulent strains at higher temperatures. These findings strongly suggest that previous studies of the effects of changes in the environment of horse studs may have overlooked the most significant effects because they could not distinguish the small minority of *R. equi* that were responsible for disease in foals.

Although advances in understanding the ecology of the organism have assisted in developing control strategies based on management of faecal contamination and dust levels, it is likely that other measures may further reduce the occurrence of rattles. One key finding in recent studies has been the major contribution foals are likely to make to the *R. equi* population. Only limited studies have been performed to establish what proportion are virulent (31, 34). It may be that virulent *R. equi* are mainly excreted by a more restricted age group of foals. This may allow development of more focussed attempts to reduce faecal contamination. Other ecological influences which may be important include the ambient temperature, the soil pH and the concentration of other nutrients in the soil. One Japanese study has found that foals on a stud with a greater problem with disease excrete much greater proportions of virulent *R. equi* in their faeces than foals on a stud without a prior history of *R. equi* pneumonia (31).

Cost of *R. equi* Infections to Breeding Industry

*R. equi* infection continues to be a major cause of wastage in foals on many Australian Thoroughbred stud farms and has been ranked as among the four most important disease problems of the horse industry (23). Furthermore the prevalence of disease appears to be increasing in all breeds throughout the world. In Australia generally 1-10% of foals are affected and although mortalities are usually maintained below 1% by early, aggressive therapy, on some studs mortalities of 20% or higher occur in some years. If the costs of diagnosis and treatment are included, as well as the potential affect on future performance, it can be appreciated that it is a disease of major economic importance to the horse breeding industry. Although it is difficult to estimate costs across the industry, given the wide variation in morbidity and mortality rates, a conservative estimate of $200/case, with 1% of foals affected, would put the annual cost to the industry at about $2 million. More specific costing of the disease on the industry in Australia will be generated from a currently RIRDC funded project on disease surveillance, and preliminary data from 10 Thoroughbred farms in New South Wales with a prevalence of rattles of 7%, found the cost of rattles treatments was $370/case (J. R. Gilkerson, unpublished data). Thus, the estimated annual cost of this disease to the industry may be somewhere between $2-4 million. In spite of the undoubted significance of this disease, options for control remain limited.
Expected Benefits of Research to Industry

The benefits of this research will flow primarily to the horse breeding industry, although *R. equi* infections are an occasional cause of disease in adult animals. The major benefit likely to result from improved understanding of the environmental factors that influence *R. equi* populations will be the ability to develop management strategies to reduce disease. This may include restricted rotation of foal paddocks, treatment of specific areas on studs to reduce the danger to foals, or strategic implementation of prophylactic measures such as administration of hyperimmune sera to foals born at the most dangerous times during the season.

Environmental Benefits

The major environmental effect of improved control of *R. equi* would be a reduction in antimicrobial drug usage on horse studs. This is likely to lead to reduced development of antimicrobial resistance.

The Disease and its Epidemiology

*R. equi* causes chronic granulomatous pneumonia and abscessation in foals. It is a soil organism which multiplies particularly well under temperate spring and summer conditions in soils contaminated with manure. It is also commonly found in the faeces and intestines of a variety of animals, including horses, and actively multiplies in the intestine of foals up to the age of 12 weeks. Aerosol infection as a result of inhalation of faecally contaminated dust is thought to be the major route of infection in foals (2).

The disease occurs endemically on many studs, and sporadically on others, and there is also variation in the severity of disease (2). However, whatever the disease pattern on individual studs, most horses are exposed to the organism. The reason for variation in patterns of disease is likely to be partially environmental in origin, with infection loads varying between studs depending upon amounts of faecal contamination and airborne dust, as well as conditions for amplification of bacterial numbers in surface soil. However there is also clear evidence of strain variation in virulence (28, 32, 35).

We have little understanding of effect of different environmental conditions on strains of differing virulence, as most studies on the environmental influences on *R. equi* multiplication were performed before the significance of the virulence plasmid (see below) was recognised.

Virulence Associated Plasmids and Antigens

The difference between virulent and avirulent *R. equi* has been linked to expression of particular temperature regulated surface proteins which are encoded on a large plasmid. Although the role of this plasmid in virulence has not been definitively established by experimental infection of horses, it has been shown to be highly correlated with virulence in mice. The virulence associated plasmid was present in 12/12 isolates from clinical cases in foals, compared to 121/1865 (6%) in environmental isolates from stud farms in Japan (29). Furthermore, the prevalence of environmental isolates carrying the plasmid has been found to be higher on a stud with a high prevalence of disease (28% of isolates), than on a stud with no history of disease (4% of isolates). This difference between the environmental isolates of *R. equi* was also reflected in the prevalence of the virulence associated plasmid in isolates from the faeces of foals, but not in those from mares on the two studs. Independent Canadian studies found the virulence associated plasmid in 21/22 isolates from diseased foals and showed that the plasmid in isolates from horses is distinct from that in isolates from other species (35). Our studies have determined that essentially all isolates of *R. equi* from clinical cases of rattles in Australian foals carry the virulence plasmid (12). Thus, it seems reasonable to assume that only *R. equi* carrying the virulence plasmid are relevant when assessing the danger of a particular environment for foals.
Factors Affecting the Ecology of *R. equi*

Studies by Barton and Hughes (3) were unable to demonstrate any influence of soil pH on the ability to isolate *R. equi*, but did find a greater prevalence in sand, sandy loam or loamy sand soils compared to clay, clay loam or sandy clay loam soils. They found that *R. equi* was less often isolated from wet soils, but that once the soils dried, it was able to be isolated. They also found that *R. equi* was more frequently isolated from dung than from rectal contents of horses, and that it was not detectable in rectal contents or dung of horses without access to grazing. However, they showed that *R. equi* concentrations in dung increased 10,000 fold over 7-14 days regardless of whether it was in contact with soil or whether the horses had been grazing. Hughes and Sulaiman (15) subsequently examined the effects of pH and temperature on growth of 7 strains of *R. equi* in vitro. They found that growth was optimal at pH 7.5 and a temperature of 30°C, with reduced growth rates at 20°C and no growth at 10°C. They also established that *R. equi* could use a variety of simple organic acids, which are found in high concentrations in horse faeces, as carbon sources. The growth of one isolate was compared in horse faeces alone, or faeces mixed with various soils. They found that growth was best in faeces alone, and better in faeces mixed with an alkaline soil than with two acidic soils. Growth in the alkaline soil once it had been supplemented with 0.4% sodium acetate was found to be as good as that in faeces. They concluded that neutral and moderately alkaline soils were most likely to favour multiplication of *R. equi*.

These studies provide good evidence that *R. equi* growth is likely to be influenced by the concentration of horse faeces in the soil and by soil pH, as well as ambient temperature. However, these studies preceded the demonstration of the exclusive role of *R. equi* strains carrying the virulence plasmid in disease in foals. Thus, while they provide an indication of the significance of environmental conditions in growth of *R. equi* in the environment of foals, their direct relevance to virulent strains is difficult to assess. The potential for differences in the ecology of virulent strains is highlighted by the study of Takai et al (33) which established that *R. equi* carrying the virulence plasmid grow slower at 38°C than their avirulent derivatives which have been cured of the plasmid, but that both virulent and avirulent strains grow at the same rate at 30°C.

It has been demonstrated on Japanese farms that the proportion of virulent *R. equi* in soil on farms with endemic rattles is higher than in soil on farms where the disease is sporadic (31), however the environmental conditions which might explain this difference have not been investigated. Earlier Japanese studies found that concentrations of *R. equi* in soil increased dramatically in April and remained high throughout the breeding season (30).

*R. equi* does appear to be able to grow to high concentrations in the intestinal tract of foals in contrast to adult horses. A limited study of the proportion of virulent isolates among *R. equi* isolated from foal faeces has shown that there appears to be a sharp increase in the number of virulent isolates over the first 4 weeks of life, and then a decline over the next 8 weeks (34). Unfortunately, parallel studies on the proportion of virulent isolates in soil were not conducted throughout the foaling season. Studies of airborne *R. equi* were also performed by exposing agar plates in the environment for a set period of time and this approach does not precisely quantify the concentration of *R. equi* in air and is not reliable enough for developing correlations with differing environmental conditions.

These studies have also been conducted using labour intensive techniques that have limited the number of isolates that could be screened for virulence. Thus, while previous studies have revealed some important factors that may contribute to the ecology of virulent *R. equi*, particularly the potential significance of foals in contamination of the environment, it is not possible to establish whether this was definitively correlated with increased airborne virulent *R. equi*. In addition, the different environment of Australian stud farms (and their greater size compared to the Japanese farms which have been studied) may alter the relative significance of the different factors.
Objectives

- Improve understanding of the ecology of virulent strains of *Rhodococcus equi*.
- Identify factors on horse studs that may result in increased amounts of virulent *R. equi* in the air inhaled by foals.
- Develop management strategies for reducing the airborne population of *R. equi*, thus reducing both the amount and severity of rattles and leading to improved responses to treatment.

Methodology

The aims of this project have been to develop a method of rapidly determining the concentration of virulent and avirulent *R. equi* in environmental samples and differentiating virulent and avirulent isolates, then to apply this method to samples collected from horse studs.

Development of a method for quantifying virulent *R. equi*

To develop a method of quantifying the concentration of *R. equi* in air samples a hand held microbiological air sampling machine was evaluated. This machine delivers a measured quantity of air onto an agar plate that can then be incubated. Two different *R. equi* selective agar media were assessed. These were trypticase soy agar based medium containing nalidixic acid, novobiocin, cycloheximide and potassium tellurite (NANAT) and Mueller-Hinton based agar medium containing ceftazidime, cycloheximide and novobiocin (modified CAZ-NB) (37, 38). These different media were initially assessed using known isolates of virulent *R. equi* and laboratory strains of *Corynebacterium pseudotuberculosis* and *Nocardia asteroides* and subsequently using air and soil samples collected from Thoroughbred breeding farms.

A colony blotting technique was developed that enabled the DNA of the bacteria isolated on selective media to be directly transferred onto a nylon membrane, which could then be hybridised with radiolabelled probes derived from either the 16S ribosomal RNA gene or the *vapA* gene of *R. equi*. The specificity of these probes were assessed on environmental samples and the conditions used for hybridisation adjusted to ensure that they were specific for *R. equi* and did not detect environmental bacteria that could grow on the selective media. These probes could then identify all colonies of *R. equi* and those of virulent *R. equi*, respectively.

Epidemiological Studies of Rhodococcal Pneumonia on Thoroughbred Breeding Farms

Participating farms completed a questionnaire at the end of both the 2000 and 2001 breeding seasons, providing information about farm characteristics, stock and land, and preventative health management, as well as on cases of rhodococcal pneumonia during the breeding season.

The associations between farm characteristics, management variables (size of farm, foal numbers, stocking rate, land, feed and health management), and morbidity and mortality due to *R. equi* pneumonia were examined.

Ecological Studies on Australian Thoroughbred Breeding Farms

Once the technique for quantifying *R. equi* in environmental samples was validated, it was applied to samples collected on Thoroughbred studs in Victoria and New South Wales. Samples were collected from Thoroughbred breeding farms in the 2000 and 2001 horse breeding seasons. In the 2000 season, samples were collected from 6 farms in Victoria. In the 2001 season, samples were collected from 10 farms in Victoria and 12 farms in NSW. *R. equi* pneumonia was endemic on some of the farms while other farms had no recent history of *R. equi* pneumonia prior to the study and did not report a single case of disease caused by *R. equi* during the study period. The six Victorian farms sampled in 2000 were also sampled in 2001.
During the 2000 breeding season, monthly air and soil samples were collected from four mare-foal paddocks selected at random on each Victorian farm, between September 2000 and February 2001 inclusive. Samples were collected around feeding stations, where horses congregated to be fed and there was less pasture cover and dustier conditions, which are considered to be important in infection by many investigators. Air samples were also collected monthly from holding pens and lanes, starting in October. During the 2001 season, two weekly air and soil samples were collected from 4 mare-foal paddocks, a holding pen and a lane on Victorian farm throughout the season. On farms in NSW, air and soil samples were collected from 4 mare-foal paddocks, a holding pen and a lane on each of the 12 farms during November and December 2001.

The number of foals in each of the sampled paddocks was recorded at the time of sampling and the total number of foals on the farm and their ages at the time of each sample collection, and details on cases of *R. equi* pneumonia were obtained.

At the time of air sample collection the pasture height was measured. Mean temperature, humidity and wind speed for the period of the day when samples were collected were also obtained. Statistical analysis of data was performed to evaluate the significance of associations between *R. equi* concentration and the prevalence of disease caused by *R. equi*, and the correlation between the concentrations of *R. equi* in soil and air samples.

A random effects negative binomial model was used to assess the influence of different environmental factors on the concentration of *R. equi* in air or soil, and the concentration of virulent *R. equi* in air or soil.

**Detection of virulent *R. equi* in expired air samples from foals**

The method used for quantification of *R. equi* in air samples was also assessed on exhaled air from foals. Exhaled air samples were collected from 55 foals on 8 Thoroughbred farms in Victoria and NSW during the 2000 and 2001 breeding seasons.

Forty five of the 55 foals were being treated for *R. equi* pneumonia when sampled. Exhaled air samples from foals were collected by holding the air sampling device up to the muzzle of a manually restrained foal. Either 100 or 250 litre (L) samples were collected. The procedure took between 1 and 2 minutes, depending on the volume of air sampled.

Breath sampling was performed on 11 foals in conjunction with routine ultrasonographic thoracic examination. The concentrations of virulent *R. equi* in faecal samples from 9 of these foals were determined using a method similar to that used for soil samples. Whole blood and serum samples were collected from 10 of these foals at the time of breath sampling. Total white cell and fibrinogen concentrations were determined for each sample.

An ELISA was used to detect IgG against VapA in serum samples. All the VapA ELISAs were performed at the Institute of Medical and Veterinary Science Infectious Disease Laboratories by T. Phumoonna under the supervision of Dr. M W. Heuzenroeder and Prof. M. D. Barton.

The concentrations of virulent *R. equi* in environmental and exhaled air samples were compared to those in environmental air samples.
Assessing the Effects of Management Changes on Exposure to Virulent *R. equi*

The effect of watering of holding pens and laneways on the amount of airborne virulent *R. equi* and its effect on disease prevalence.

Air samples and soil samples were collected periodically over the 2001 and 2002 foaling seasons from the holding pens used by resident mares and foals on an *R. equi* affected farm. Holding pens used by resident mares and foals were subjected to intensive daily irrigation prior to use of the pens during October, November and December in the 2002 season and the data obtained in the 2002 season were compared to that obtained in the previous season, when the pens were not irrigated. Soil moisture was measured daily or every second day using a hand held penetrometer and was tested monthly in the laboratory. The incidence and prevalence of *R. equi* pneumonia in foals from resident mares and non-resident mares in the two seasons were recorded. Weather and rainfall data were also collated from the Bureau of Meteorology. The relative disease risk and epidemic curves of *R. equi* pneumonia in resident foals and non-resident foals were compared and these data were evaluated with respect to the environmental and bacteriological data.

Assessment of the Contribution of Subclinically Infected Foals to Exposure to Airborne Virulent *R. equi*

Airborne concentrations of virulent *R. equi* were determined in holding pens prior to the entry of foals, during their entry into the pens, and amongst the mob of foals after they had been held in the pen for some time. The concentrations at these three times were compared to assess whether faecally contaminated dust raised during the movement of foals, or the exhalation of virulent *R. equi* by subclinically infected foals, were more important contributors to exposure in holding pens.
Detailed Results

Development of a method for quantifying virulent *R. equi*

Colony blotting and DNA hybridisation using a vapA probe differentiated the virulent *R. equi* from avirulent isolates. Both virulent and avirulent isolates were detected with an rrnA gene probe. Neither the vapA probe nor the rrnA probe hybridised to other actinomycete species tested. Of the 3 actinomycetes tested, only *N. asteroides* were able to grow on the selective media.

The capacity of this technique to identify and differentiate *R. equi* was further assessed on environmental samples from horse farms. Environmental air samples from a holding pen yielded some bacterial colonies that did not have typical *R. equi* morphology, but hybridised with the rrnA probe under the conditions initially used. Sequencing of the rrnA genes of these cross reactive bacteria established that they were *Corynebacterium ammoniagenes, Bacillus fumarioli* and *Rhodococcus rhodochrous*, all of which are either soil or faecal inhabitants.

When hybridisation of the rrnA probe to blotted colonies of the atypical isolates was performed at 68°C, with a final wash in 0.1 x SSC, 0.1 % SDS, at 68°C, the probe bound to the atypical isolates. When hybridisation was performed at 80°C and the final washing step was performed in 0.05 x SSC, 0.1% SDS, at 80°C, the binding to the atypical isolates, but not that to *R. equi*, was eliminated.

Thus a highly sensitive and specific method for quantifying and differentiating virulent and avirulent *R. equi* was developed using a portable air monitoring system (M Air T, Millipore, Saint-Quentin-Yveline, Cedex, France) and selective media for isolation of *R. equi*. This method enabled large volumes of air to be quantitatively sampled and all the bacteria cultured assessed for their significance.

There was variation in the ability of different isolates of *R. equi* to grow on the two selective media. Most isolates had higher yields on NANAT than on mCAZ-NB, but one isolate had almost 5 fold higher yield on mCAZ-NB than on NANAT. The growth of all isolates of *R. equi* was inhibited to some extent on the selective media.

There was no significant difference in the proportion soil samples from which *R. equi* could be cultured on NANAT medium (48/60) or on modified CAZ-NB medium (52/60). Similarly there was no significant difference in the proportion soil samples from which virulent *R. equi* could be cultured on NANAT medium (31/60) or on modified CAZ-NB medium (38/60). There appeared to be a limited association between the isolation of *R. equi* and virulent *R. equi* from paired samples on the two selective media. The results were in agreement for 46/60 samples for the culture of *R. equi* and for 31/60 samples for the culture of virulent *R. equi*.

There were significantly more bacterial colonies isolated from soil samples on NANAT media (P=0.001). There was no significant difference in the number of *R. equi* cultured from soil samples on each of the two media (P=0.10). However, there were significantly more colonies of virulent *R. equi* obtained from these samples when they were cultured on modified CAZ-NB (P=0.03).

Epidemiological Studies of Rhodococcal Pneumonia on Thoroughbred Breeding Farms

All farms involved in the 2000 season survey reported cases of *R. equi* pneumonia during that foaling season. In the 2001 season, 7/10 farms in Victoria and 11/12 NSW farms reported cases of *R. equi* pneumonia. Farms with a prevalence of *R. equi* pneumonia less than 3% were grouped as farms with a low prevalence of *R. equi* pneumonia and those with a prevalence of greater than 9% were grouped as farms with a high prevalence of *R. equi* pneumonia.
Cases of *R. equi* pneumonia were diagnosed using combinations of clinical signs, ultrasonographic examinations of the lungs, haematological examinations and cultures from tracheal lavages. Eight farms reported mortalities due to *R. equi*. Overall the mortality rate associated with *R. equi* pneumonia was 0.4% (15/4221), with a case fatality rate of 5.1% (15/296).

In the 2001 season farms with a high prevalence of *R. equi* pneumonia were more likely to report *R. equi* associated mortalities than other farms (4/5 vs. 4/17; P=0.04). Farms with a low prevalence of *R. equi* pneumonia (<3%) were not significantly less likely to report mortality due to *R. equi* pneumonia than other farms (8/9 vs. 6/13; P=0.07). The mortality rate on farms with a high disease prevalence (8/1465 - 0.5%) was approximately five times that seen on the farms with a low disease prevalence (1/973 - 0.1%). However, the case fatality rate on farms with a low disease prevalence (1/12 - 8.3%) was not significantly different from that seen on farms with a higher prevalence of disease (14/282 - 4.9%). Two farms reported mortalities in the 2000 season but not in the 2001 season and on both of these farms the disease prevalence was lower in 2001 (<3%) than in 2000 (>3%).

The total area dedicated to horse husbandry on the Thoroughbred farms surveyed in Victoria during the 2000 and 2001 seasons ranged from 36 to 144 hectares (Ha) per farm. The maximal number of foals on these farms ranged from 42 to 238 during the breeding season. The total area dedicated to horse husbandry on the Thoroughbred farms surveyed in NSW during the 2001 season ranged from 49 to 182 Ha per farm. The maximal number of foals on these farms ranged from 47 to 488 during the breeding season.

On those farms surveyed in consecutive seasons, no change was observed in the total area dedicated to horse husbandry, whilst maximal foal numbers decreased on four farms, and increased on two.

Farms were divided into groups based on the total area devoted to horse husbandry, maximal stocking rate (foals/Ha) and on the maximal foal numbers. Farms were classified as having small (<70 foals), medium (70-210 foals) or large (>210 foals) numbers of foals, as being small (<60 Ha), medium (60-110 Ha) or large (>110 Ha) and as having a low (<1.1 foals/Ha), medium (1.1-1.9 foals/Ha) or high (>1.9 foals/Ha) stocking rate.

A low prevalence of *R. equi* pneumonia was associated with a small foal population in the 2001 season. Five of the 6 farms with a small foal population reported a disease prevalence below 3%, whilst only 4/16 farms with medium or large foal populations reported a low disease prevalence (P=0.02). Similarly the occurrence of mortalities due to *R. equi* pneumonia was associated with medium or large foal populations. None of the 6 farms with a small foal population reported deaths from *R. equi* pneumonia, whilst 8/16 farms with medium or large foal populations reported deaths from *R. equi* pneumonia (P=0.04). No farm surveyed in consecutive seasons moved into or out of the small foal population category. On the four farms that decreased their maximal foal numbers in 2001, two reported reduced disease prevalence. The two farms that increased their maximum foal numbers, both from the medium to the large foal population category, also reported reduced disease prevalence. Of the two farms that reported mortality due to *R. equi* in the 2000 season, but not in the 2001 season, one reduced its maximal foal numbers, while the other increased its maximum foal numbers.

No significant associations were seen between the farm area used for horse husbandry or maximal stocking rate and morbidity or mortality due to *R. equi* pneumonia.

Grouping of mare-foal pairs on paddocks was generally determined by the reproductive status of the mare and the age of the foal. All farms had a general strategy of grouping mares and foals according to age up until the mare’s first oestrus after foaling. Once the mare had been mated and observed to be pregnant by ultrasonographic examination at 45 days after service, the mares and foals were less intensively handled and were moved to larger paddocks away from the main handling yards on the property. Those mares not owned by the farm, but that had foaled and been served by stallions on the farm (transient mares), were returned to the owner’s property some time after pregnancy diagnosis at 45 days gestation. During the early stages of gestation (<45 days) the mares were checked periodically (routinely at 14 and 28 days after service) by ultrasonographic examination to assess the progression of the pregnancy.
All but 3 farms stocked foals primarily according to the mare’s reproductive status after her foal heat. These farms could have foals over a wide age range in the same paddock. Three farms stocked foals in tight, age-specific groups, regardless of the mare’s reproductive status. On these farms mares and foals were kept in groups of up to ten mare-foal pairs.

All but one farm had both home mares (owned by the farm) and transient mares (owned by other parties) foaling and being served on the farm. Two farms had geographically separate areas for transient mares and foals, while on the remaining farms the transient mares shared the same paddocks as the home mares. All farms had specific yards for sick or injured foals and for foals with poor conformation, and some had stables for these foals.

Mares and foals on all farms were fed a mixture of roughage and concentrates. All but one farm included pelleted concentrate feed in the mixture. In the 2001 season creep feed was used on 6 farms. Creep feed was placed in small, partially confined feeding areas in the paddocks, away from the main feeding bins. Creep feed consisted of increasing quantities of concentrates and/or pelleted supplements. One farm only gave creep feed to foals that were growing slowly.

The number of feeding bins in paddocks varied with the number of mare–foal pairs kept in the paddock. Bins were generally not evenly distributed and were mostly placed in one quarter of the paddock. Large metal feeding stations were used on three farms. Unlike the bins, which allowed a mare-foal pair to feed away from the rest of the mob, the feeding stations created a communal feeding environment, with all horses feeding from a central location. Two farms only used bins in small yards for younger, sick or injured foals, and placed feed on the ground in larger paddocks. Two used bins and feeding stations on larger paddocks and only bins in small paddocks. Most farms rotated the position of bins when the ground became worn or muddy. Farms that used feeding stations usually had one per paddock and they were either permanent constructions or were rarely moved. In the 2001 season six farms rotated bins on a regular basis, regardless of paddock condition, at a frequency ranging from every 5 weeks on two farms to daily on two farms.

Faecal material in paddocks was picked up, harrowed or left. In the main, faeces was only picked up in the small yards used for foaling, which held foals under 14 days of age, and the small yards used for sick or injured foals, and the holding pens. One farm harrowed the small yards daily. Faeces were removed daily on most farms and twice daily on some farms. Harrowing was not performed on five farms in the 2001 season. All other farms harrowed paddocks or yards at least once during the foaling season.

Mare and foal paddocks were irrigated on all but one NSW farm, but only 5 Victorian farms irrigated paddocks in the 2001 season. One farm only irrigated foaling yards. Irrigation was performed daily on this farm. Irrigation frequency on other farms ranged from every 2 to every 12 weeks during the foaling season, depending on the prevailing weather conditions. One farm irrigated each paddock for 3 days each between December and February. Four farms irrigated on a regular basis, irrespective of climatic conditions, with the frequency of irrigation ranging from every 2 to every 6 weeks. One farm increased the irrigation frequency in the 2001 season to every 2 weeks, from every 3 weeks in the 2000 season. Other farms increased or decreased the frequency of irrigation depending on the weather conditions.

The irrigation and harrowing of large paddocks necessitated the movement of groups of horses to adjacent paddocks.

Herbicides were not used on the paddocks of nine farms in the 2001 season (4 from Victoria, 5 from NSW). Either herbicides or fertilisers were applied to paddocks on all but five farms (2 from Victoria, 3 from NSW) in the 2001 season. The most commonly used fertiliser was superphosphate, which was applied by 12 farms in the 2001 season. Lime was only used on 2 Victorian farms, whilst potash was used on one farm in Victoria and a nitrogen/sulphur mixture (Grassboosta, Incitec Pivot, Southbank, Victoria) was used on one farm in NSW. Three of the farms surveyed in consecutive seasons changed their herbicide and/or fertiliser usage between seasons. All of these farms applied lime to paddocks in the 2000 season, but did not do so in the following season.
There was no significant association between the different approaches to land management, the feeding of mares and foals, and the prevalence of *R. equi* pneumonia or the occurrence of mortalities due to *R. equi* pneumonia in the 2001 season.

Foals were vaccinated on all farms. The age of the foals when first inoculated ranged from 2 to 6 months. Seven farms vaccinated foals at a specific time of the year, regardless of their age. All farms vaccinated against *Clostridium tetani* (tetanus) and all but three farms vaccinated against *Streptococcus equi* subspecies *equi* (strangles). Seven farms vaccinated foals against equine herpesviruses 1 and 4 (EHV 1 and EHV 4). One introduced the vaccination of foals against EHV 1 and EHV 4 in the 2001 season. Nine farms vaccinated foals against *Salmonella enterica* Typhimurium (salmonellosis). Six of these were located in NSW.

Mares were vaccinated on all farms. Seventeen farms vaccinated mares against EHV 1 and EHV 4 and all but 3 of these farms vaccinated each mare 4-6 weeks before her due foaling date. All but one farm vaccinated mares 4-6 weeks prior to foaling vaccinated against tetanus. All but three farms vaccinated mares 4-6 weeks prior to foaling vaccinated against strangles. Vaccination of mares against salmonellosis was performed 4-8 weeks prior to foaling on seven farms. Three farms vaccinated pregnant mares against rotavirus. All of these farms were located in NSW. The vaccination histories of transient mares coming onto the farm were reliably obtained on six farms while one required strangles vaccination of mares prior to arrival. Nine farms vaccinated some mares without a vaccination history on arrival.

All farms administered anthelmintics to their foals. The age at initial treatment ranged from 7 days to 8 weeks. Seven farms administered anthelmintics to foals at 4 weeks of age or younger. Six farms, all in NSW, rotated between 2 or more anthelmintics in a season. Seven farms used a single anthelmintic agent in a season. Of these, 4 farms in the 2001 season and 3/6 Victorian farms in the 2000 season used ivermectin as the single agent. Only one of the six farms participating in consecutive seasons changed the anthelmintic used between seasons.

Mares were treated with anthelmintics on all farms. The anthelmintics used in home mares were the same as those used in foals. The period between treatments varied from 6 to 8 weeks. Ten farms treated mares within 48 hours of foaling, while two treated mares at or around the time of the foal heat. Pregnant mares on six farms did not receive anthelmintic treatment within one month of their due foaling date. None of the 6 farms that did not administer anthelmintics to mares within one month of their due foaling date reported mortalities due to *R. equi* and all had a prevalence of *R. equi* pneumonia of less than 9%. The anthelmintic treatment histories of transient mares were reliably obtained on five farms. For these mares anthelmintic treatment continued for the duration of their stay on the farm using the anthelmintics previously used on them. Mares with an unknown anthelmintic treatment history arriving on all but two other farms were treated on arrival or within the first month after arrival.

Eight farms used prophylactic antibiotics on all neonatal foals. Neomycin, penicillin and/or oxytetracycline were used. The course of treatment ranged from a single dose within 24 hours of birth to a three day course of administration twice a day. Two farms administered antibiotics to neonatal foals with diarrhoea, a serum IgG concentration less than 800 mg/L or with a complicated birth. Two administered probiotics (Protexin, International Animal Health Pty Ltd, Huntingwood, NSW) to foals, one to foals suffering from diarrhoea in 2001 only, whilst the other gave probiotics to all foals for the first 3-5 days of life.

Hyperimmune serum was administered to all foals on one farm at 48 hours and 14 days of age, but the volume administered was not specified. Six other farms administered hyperimmune serum to foals with poor passive transfer of maternal antibody (serum IgG concentration less than 800 mg/L in the first 24 hours of life).

Farms that vaccinated foals against EHV 1 and EHV 4 were significantly more likely to have a low prevalence of *R. equi* pneumonia (P=0.009). Six of the seven farms that vaccinated foals against EHV
1 and EHV 4 reported a prevalence of *R. equi* pneumonia of less than 3%, whilst only 3/14 farms that did not vaccinate foals against EHV 1 and EHV 4 reported a prevalence of less than 3%. Three farms that vaccinated foals against EHV 1 and EHV 4 were among the six farms (50%) that had a small foal population (maximum < 70). Farms that vaccinated foals against strangles, EHV 1 and EHV 4 were also significantly more likely to have a low prevalence of *R. equi* pneumonia (P=0.03), but no significant association was found between farms that vaccinated foals against strangles and the prevalence of *R. equi* pneumonia.

Farms that administered anthelmintics to mares in the last month of pregnancy were more likely to report *R. equi* associated mortalities (P=0.03) than farms that did not administer anthelmintics to mare within one month of foaling. Eight of the 15 farms that treated mares with anthelmintic within one month of their due foaling date reported deaths due to *R. equi* pneumonia, whilst none of the 6 farms that did not administer anthelmintics to mares within one month of their foaling date reported deaths due to *R. equi* pneumonia.

All farms in the 2001 survey were asked to provide information about the prevalence of diarrhoea, respiratory disease not associated with *R. equi*, conformational problems and traumatic injuries in foals during the season. Nineteen farms reported the occurrence of diarrhoea. The prevalence of diarrhoea in foals on affected farms ranged from 1.9 to 50%. Eight farms reported the prevalence of diarrhoea in foals to be greater than 15% and on five of these farms the prevalence was 30% or greater. Respiratory disease not associated with *R. equi* was reported on 15 farms. The prevalence of respiratory disease in foals on the affected farms ranged from 0.5 to 30.1%. Six farms reported a prevalence greater than 3% and two reported a prevalence greater than 15%. On both of these farms foals had upper respiratory tract disease with primary clinical signs of nasal discharge and no evidence of lung pathology on auscultation or ultrasonographic examination. On one the majority of the cases of upper respiratory tract disease in foals were associated with the isolation of *Streptococcus equi* subspecies *zooepidemicus* and/or *Streptococcus equisimilis*. No attempt was made to isolate microorganisms associated with respiratory disease on the other of these two farms.

All but one farm reported the occurrence of conformational problems, mainly of the limbs, that required some form of correction. The prevalence of foals with conformational problems ranged from 4.5% to 69.5%. Nine farms reported conformational problems in greater than 15% of foals and on three farms the prevalence of conformational problems was 30% or greater. Six of the nine farms with a prevalence of conformational problems of over 15% also reported mortalities associated with *R. equi* pneumonia.

All but one farm reported the occurrence of traumatic injuries requiring intervention. The prevalence of traumatic injuries ranged from 0.8 to 24.7%. Eight reported traumatic injuries in 10% or more of their foals and two reported a prevalence over 15%.

A significant association was observed between the prevalence of conformational problems and mortalities due to *R. equi* pneumonia. Farms with a high prevalence of conformational problems (>15%) were more likely to report *R. equi* associated mortalities (P=0.03) than farms with a lower prevalence of conformational problems. Six of the nine farms that reported a prevalence of conformational problems of over 15% also reported mortalities due to *R. equi* pneumonia, while only 2/12 farms with less than 15% of their foals affected by conformational problems had mortalities due to *R. equi* pneumonia.
Ecological Studies on Australian Thoroughbred Breeding Farms

In the 2000 season the age at diagnosis was provided for 37 cases of *R. equi* pneumonia. All but three of these cases were seen in foals older than 30 days of age, the youngest being 25 days old. In the 2001 season on Victorian farms the age at diagnosis was provided for 41 cases of *R. equi* pneumonia. All but six of these cases were seen in foals older than 30 days of age, the youngest being 22 days old. Most cases (28/41 - 68.3%) were seen in foals between 31 and 60 days of age. Of the 39 foals that recovered fully from *R. equi* pneumonia, all but eight were treated with antimicrobials for less than 21 days, with one case treated for more than 30 days. The two deaths reported occurred within the first week of antimicrobial therapy. In the 2001 season the age at diagnosis on NSW farms was provided for 191 cases of *R. equi* pneumonia. Most cases (128/191 - 67%) were seen in foals between 31 and 60 days of age. The youngest foal diagnosed with *R. equi* pneumonia was 16 days of age, whilst the oldest was 158 days of age. Of the 9 deaths observed as a consequence of *R. equi* pneumonia, the age of eight at the time of diagnosis was reported and all but one was between 31 and 60 days of age. The duration of treatment was reported for 195/202 cases, with nine dying during treatment. Most cases (164/186 - 88.2%) were treated for less than 21 days. Seven of the nine deaths occurred within the first 10 days of treatment.

The concentration of *R. equi* in air samples ranged from 0 to 124 colony forming units (cfu)/1000 L and in soil samples from 0 to 136 cfu/mg. The concentration of virulent *R. equi* in air samples ranged from 0 to 72 cfu/1000 L and in soil samples from 0 to 28 cfu/mg.

The correlation between the concentrations of *R. equi* in the paired soil and air samples was weakly positive (R_s=0.11). Similarly, the correlation between the concentrations of virulent *R. equi* in the paired soil and air samples was weakly positive (R_s=0.12).

A greater proportion of soil samples than air samples yielded both *R. equi* and virulent *R. equi*. *R. equi* were 2.8 times more likely to be recovered from soil samples than from air samples (P<0.001), but virulent *R. equi* were only 1.5 times more likely to be recovered from soil samples than from air samples (P=0.02).

The means and medians of the geometric mean concentrations of virulent *R. equi* in soil and air were similar, but the mean and median of the geometric mean concentrations of total *R. equi* were greater in soil samples than in the air samples. This was reflected in the lower proportion of virulent *R. equi* in soil than in air samples.

The associations between the prevalence and severity of *R. equi* pneumonia and the geometric mean environmental burden of *R. equi* and virulent *R. equi* were examined using Fisher’s exact test and the Mann-Whitney test. Farms on which 35% or more of airborne *R. equi* were virulent were significantly less likely to have a low prevalence of *R. equi* pneumonia than farms on which less than 35% of airborne *R. equi* were virulent (P=0.03). Farms on which 35% or more of airborne *R. equi* were virulent were significantly more likely (P=0.001) to have a high prevalence of *R. equi* pneumonia than farms on which less than 35% of airborne *R. equi* were virulent. A greater proportion of farms on which 35% or more of airborne *R. equi* were virulent were virulent reported mortalities associated with *R. equi* pneumonia (5/7 compared with 6/21), but this difference was not significant (P=0.06). A significantly greater proportion of the farms (10/22) on which the geometric mean airborne concentration of virulent *R. equi* was <1 cfu/1000 L reported a low prevalence (less than 3%) of *R. equi* pneumonia, than the farms (0/6) with a higher geometric mean airborne concentration of virulent *R. equi* (P=0.049). A greater proportion of farms on which the geometric mean airborne concentration of virulent *R. equi* was ≥1 cfu/1000 L had a high prevalence of *R. equi* pneumonia than farms on which the geometric mean airborne concentration of virulent *R. equi* was <1 cfu/1000 L (3/6 compared with 3/22), but this difference was not significant (P=0.09).
Differences in concentration of virulent *R. equi* in the soil were not associated with differences in prevalence or severity of *R. equi* pneumonia. A greater proportion of farms with a geometric mean concentration of virulent *R. equi* of ≥1 cfu/mg in soil had a higher prevalence of *R. equi* pneumonia (4/9 compared with 2/19), but this difference was not significant (P=0.06).

The median prevalence of *R. equi* pneumonia on farms with a geometric mean airborne concentration of virulent *R. equi* ≥1 cfu/1000 L was significantly greater (P=0.008) than on farms with airborne concentration of virulent *R. equi* <1 cfu/1000 L. Similarly, the median prevalence of disease due to *R. equi* on farms on which ≥35% of airborne *R. equi* were virulent was significantly greater (P=0.001) than on farms on which a lower proportion of airborne *R. equi* were virulent.

The geometric mean concentrations of virulent *R. equi* were higher in soil and air from the pens and lanes than in the soil and air from the paddocks. The geometric mean concentrations of virulent *R. equi* were greater in the soil and air samples collected from the pens than those collected from the lanes. The proportions of *R. equi* that were virulent in the air and soil from the pens and lanes were generally higher than in the air and soil from the paddocks, with the exception of Victorian farms in the 2001 season, where the proportions of airborne *R. equi* that were virulent were similar in the pens and lanes to the paddocks. The differences in proportions of *R. equi* that were virulent between the pens/lanes and paddocks were greater in air samples than in the soil samples.

There was considerable variation in the concentrations of *R. equi* and virulent *R. equi*, and the proportion of *R. equi* that were virulent, on farms throughout the foaling season. During the 2000 season the concentration of virulent *R. equi* on all 6 farms increased sharply in November. The geometric mean concentration of virulent *R. equi* in air and soil increased approximately three fold from October to November. The geometric mean concentrations of virulent *R. equi* did not fall to the concentrations seen in October for the remainder of the season. The proportion of *R. equi* in soil that were virulent increased steadily from September to January, although the proportion of airborne *R. equi* that were virulent peaked in December.

In the samples collected from farms in Victoria during the 2001 season the geometric mean concentration of virulent *R. equi* in the air was greatest in the period from the middle of December to the end of January, whilst the geometric mean concentration of virulent *R. equi* in soil varied throughout the season. The proportion of *R. equi* that were virulent in the air or soil did not fall below 10% on Victorian farms during the 2001 season. The proportion of airborne *R. equi* that were virulent did not peak until January, whilst the proportion of *R. equi* that were virulent in the soil was greatest in October. The concentration of virulent *R. equi* and the proportion of *R. equi* that were virulent were lowest during November.

In samples collected from farms in NSW during the 2001 season the geometric mean concentrations of *R. equi* and virulent *R. equi*, and the proportion of *R. equi* that were virulent, in soil and air varied little between the two sampling times (November and December). The geometric mean airborne concentration of virulent *R. equi* on NSW farms was ≥1 cfu/1000 L in both November and December. This was higher than the concentrations seen on Victorian farms at comparable times, where the highest geometric mean airborne concentration of virulent *R. equi* was 0.8 cfu/1000 L.

The holding pens and lanes had drier soil than the paddocks on most farms. The sandy soil in the pens and lanes and the lack of grass cover in these areas probably contributed to drier soil conditions there. Paddocks with sandy soils were drier than those with clay soils. Locations with sandy soils generally had higher airborne concentrations of virulent *R. equi* and higher proportions of airborne *R. equi* that were virulent than locations with clay soils. The higher geometric mean concentrations of airborne virulent *R. equi* in sandy areas were most evident amongst farms in Victoria (75% and 167% higher in the 2000 and 2001 seasons, respectively). On NSW farms the locations with sandy soils had a 30% higher geometric mean concentration of airborne virulent *R. equi*. The proportions of airborne *R. equi* that were virulent were between 21% and 61% higher in locations with sandy soil. The differences between locations with different soil types in the geometric mean concentrations of virulent *R. equi* and in the proportions of *R. equi* that were virulent were less pronounced in soil samples.
In the 2000 season the median group size (number of foals/paddock) reduced from 4-5 foals between October and December to ≤3 foals in January-February. A similar reduction in the median group size in January was observed in the 2001 season on Victorian farms. The soil moisture and pasture height were lowest in December and January in both seasons on farms in Victoria. The soil moisture was higher in the 2001 season on Victorian farms. The median pH of soil samples was slightly lower in the 2001 season on Victorian farms.

The average ambient temperatures at the time of sampling in the 2000 season were approximately 10°C higher in November-February than in September-October. In the 2001 season the median average ambient temperature at the time of sampling was within a 10°C range throughout the sampling period. The median average ambient temperature on farms at the commencement of sampling in the middle of October was less than 1°C different from the median average ambient temperature when samples were taken at the end of November. The median average ambient temperature at the time of sampling on NSW farms in December was approximately 12°C higher than in November. Median group size were similar in both sampling periods, but the median pasture height and soil moisture were approximately 20% lower and 20% higher, respectively, in December than in November. Generally high average ambient temperatures at the time of sampling were associated with lower humidity. Median wind speeds were within a 10 km/h range throughout the sampling periods over the two seasons in Victoria and varied little between sampling periods in NSW.

In the 2000 season, most of the 6 farms had the maximal number of foals less than 10 weeks of age and less than 16 weeks of age in November. In the 2001 season, most of the 10 farms in Victoria had the maximal number of foals less than 16 weeks of age in November. The proportions of foals between the ages of 4 and 12 weeks were high on most Victorian farms in the October-January period of the 2001 season, and then dropped rapidly by the end of January. In NSW all farms had the maximal number of foals less than 16 weeks of age by the start of December. The number of foals aged between 4 and 12 weeks peaked during the sampling period (end of November to the middle of December) on most NSW farms. The number of foals between 4 and 12 weeks of age peaked on all but one of the NSW farms in December.

In univariable analyses the airborne concentration of \( R.\ equi \) was significantly associated (P<0.05) with state/year, date, location within the farm, soil texture, soil moisture, soil pH and temperature. The greatest deviations of the count ratios (CR) from 1.0 were for associations between airborne concentrations of \( R.\ equi \) and different categories of state/year, date and location within the farm. The CR for concentrations of airborne \( R.\ equi \) were 43% lower on Victorian farms in the 2000 season and 32% lower in the 2001 season than on NSW farms in the 2001 season. The CR for airborne \( R.\ equi \) concentrations were 67% greater in the middle of the season (November-December) and 34% greater late in the season (January-February) than early in the season (September-October) and the CR for airborne concentrations of \( R.\ equi \) in pens and lanes was 62% greater than in the paddocks. Other variables associated (P<0.25) with the airborne concentration of \( R.\ equi \) included pasture height and group size.

The airborne concentration of virulent \( R.\ equi \) was significantly associated (P<0.05) with state/year, date, location within the farm, soil texture, soil moisture, pasture height and temperature. The greatest deviations of the CR from 1.0 were for associations between airborne concentrations of virulent \( R.\ equi \) and different categories of state/year, date and pasture height. The CR for airborne concentrations of virulent \( R.\ equi \) were 70% lower on Victorian farms in 2000 and 54% lower on Victorian farms in 2001 than on NSW farms in the 2001 season. The CR for airborne concentrations of virulent \( R.\ equi \) were 151% greater in the middle of the season and 129% greater late in the season than early in the season. The CR for airborne concentrations of virulent \( R.\ equi \) on paddocks with a low pasture height (≤10 cm) was 106% greater than on paddocks with a greater pasture height. Other variables associated (P<0.25) with the airborne concentration of virulent \( R.\ equi \) included soil pH and humidity.

The concentration of \( R.\ equi \) in soil was significantly associated (P<0.05) with date, temperature, humidity and group size. The greatest deviation of the CR from 1.0 was for the association between concentration of \( R.\ equi \) in soil and different categories of temperature. The CR for \( R.\ equi \) in soil samples collected when there was a high average ambient temperature (>25°C) was 38% greater than
for those taken when there was a lower average ambient temperature (≤25°C). Other variables associated (P<0.25) with the concentration of *R. equi* in soil included state/year, soil moisture and pasture height.

The concentration of virulent *R. equi* in soil was significantly associated (P<0.05) with state/year and location within the farm. The CRs for the concentrations of virulent *R. equi* in the soil on Victorian farms in the 2000 and 2001 seasons were 55% and 19% lower, respectively, than those for the concentration of virulent *R. equi* in the soil on the NSW farms in the 2001 season. The CR for the concentration of virulent *R. equi* in soil samples taken from pens and lanes was 27% greater than that for the concentration of virulent *R. equi* in soil from paddocks. Other variables associated (P<0.25) with the concentration of virulent *R. equi* in soil included date, soil texture, pasture height, temperature and humidity.

No significant (P<0.05) associations were found between any variable and the proportion of *R. equi* that were virulent in air or in soil samples.

Some weak associations (P<0.25) were seen between soil texture and soil moisture and the proportion of airborne *R. equi* that were virulent. The greatest odds ratio (OR) was for the association with soil moisture, with the proportion of *R. equi* that were virulent 1.77 times greater in drier soil samples (≤10% H₂O) than in more moist soil samples (>10% H₂O). A weak association (P<0.25) was also seen between soil pH and the proportion of *R. equi* in soil that were virulent. The proportion of *R. equi* that were virulent in soil was 1.57 times greater in more acidic soil (pH≤6) than in more alkaline soil (pH>6).

To evaluate the relationships and potential interactions between binary categorical environmental and stocking predictor data used in the regression analyses, Spearman’s correlation coefficients were calculated. There was no significant collinearity (Rs>0.8) between any of the predictor variables tested. However, a strong positive relationship was seen between temperature and humidity (Rs>0.5) when assessing the binary relationship between high temperature (>25°C) and low humidity (≤70%). Other variables with positive relationships (0.3<Rs<0.4) were sandy soils and pen/lane locations, low soil moisture (≤10% H₂O) and sandy soil, and low soil moisture (≤10% H₂O) and pen/lane locations.

The concentration of *R. equi* and virulent *R. equi* in both air and soil had multiple variables associated with them (P<0.25) in the univariable analyses, so these were used in the multivariable analyses.

Variables that were significantly associated with the airborne concentrations of virulent *R. equi* on both paddocks and pens and lanes were the state/year, date, location within the farm and soil moisture. The greatest deviations of the CR from 1.0 were for the associations between airborne concentrations of virulent *R. equi* and state/year, date and location within farm. The airborne concentration of virulent *R. equi* was 62% lower on Victorian farms in the 2000 season and 54% lower in the 2001 season than on NSW farms in 2001. The airborne concentration of virulent *R. equi* was 75% greater in the middle of the season and 112% greater late in the season than early in the season, and in the pens and lanes the airborne concentration of virulent *R. equi* was 91% greater than in the paddocks.
The state/year, pasture height, soil moisture and average ambient temperature were significantly associated with the airborne concentrations of virulent *R. equi* in paddocks. The airborne concentration of virulent *R. equi* was 63% greater in paddocks with a low pasture height (≤10 cm) than in paddocks with a greater pasture height, 52% greater in paddocks with low soil moisture (≤10% H₂O) than in those with higher soil moisture, and 64% greater in paddocks when the average ambient temperature at sampling was high (>25°C) than when the average ambient temperature was lower (≤25°C). The paddocks on Victorian farms in the 2000 and 2001 seasons had a 74% and 46% lower concentration of airborne virulent *R. equi*, respectively, than those on NSW farms in 2001.

Soil moisture, humidity and date were significantly associated with the concentrations of *R. equi* in soil on both paddocks and pens and lanes. Dry soils (≤10%) had a 23% lower concentration of *R. equi* than moist soils (>10%), whilst a low average air humidity at sampling (≤70%) was associated with 31% higher concentrations of *R. equi* in soil than in samples collected in more humid conditions. Soil samples collected in the middle and late parts of the season had higher concentrations of *R. equi* than those collected early in the season.

The foal group size on a paddock and air humidity were significantly associated with the concentrations of *R. equi* in soil on paddocks. Soil samples collected when the average air humidity was low (≤70%) had concentrations of *R. equi* 30% higher than soil samples collected in more humid conditions, whilst the concentration of *R. equi* in soil samples collected from paddocks with a greater foal group size (containing >7 foals/paddock) was 23% lower than in samples from paddocks with fewer foals (≤7).

State/year was the only variable significantly associated with the concentration of virulent *R. equi* in soil on both paddocks and pens and lanes. The concentration of virulent *R. equi* was 54% lower in soil samples from Victorian farms in the 2000 season than in those from NSW farms in the 2001 season.

No variables were significantly associated with the concentration of virulent *R. equi* in soil from paddocks alone.

**Detection of virulent *R. equi* in expired air samples from foals**

The air sampling method was also used to assess breath samples collected from 55 foals, 48 of which had been diagnosed previously with rhodococcal pneumonia. Of the 48 clinical cases, 31 foals (64.6%) had detectable levels of virulent *R. equi* in their breath samples. Of the 7 clinically normal foals, 6 were exhaling virulent *R. equi*. This suggests that the clinically normal foals may have been exposed to virulent *R. equi* and may have been subclinically infected. The concentration of virulent *R. equi* in foal breath was 5 - 9 fold greater than that in environmental samples taken from lanes and holding pens. These observations suggest that foal breath can be a significant source of virulent *R. equi* and that direct foal to foal transmission of aerosolised virulent *R. equi* may be a significant factor in the epidemiology of this disease.

Eleven foals between 4 and 6 weeks of age were recruited from an endemically affected stud during routine thoracic ultrasonographic examination. Breath samples were collected, as well as faecal samples for culture and blood samples for haematological examination and serological analysis. Serology was performed using a VapA epitope specific ELISA. Virulent *R. equi* was detected in the breath of 10 of 11 foals sampled. All foals tested (n=10) had detectable antibody against virulent *R. equi*, with the highest concentration seen in a foal being treated for *R. equi* pneumonia. Virulent *R. equi* was detected in the faeces of six foals, with the highest concentration in faeces from the foal being treated for *R. equi* pneumonia. Four foals had lesions typical of rhodococcal pneumonia detectable by ultrasonography. Three of these foals had leucocytosis or hyperfibrinogenae mia.
Assessing the Effects of Management Changes on Exposure to Virulent *R. equi*

The prevalence of *R. equi* pneumonia was compared in foals of resident and non-resident mares on a single farm. The relative risk (RR) of *R. equi* pneumonia in foals of resident mares was significantly higher than those of non-resident mares in the 2001 season (RR=1.5, 95% confidence interval (CI) = 1.0-2.2, P=0.45). In the 2002 season, when irrigation of holding pens was introduced in the area used for handling foals of resident mares, there was no difference in the relative risk of *R. equi* pneumonia between the two groups (RR=0.8, CI=0.6-1.3, P=0.43). In 2001, virulent *R. equi* were detected in 2/2 air samples collected in the holding pens used for handling foals of resident mares, while in 2002 no virulent *R. equi* were detected in air samples collected in irrigated holding pens (0/6) (P=0.036). Of the airborne *R. equi* detected in the holding pens used for handling foals of resident mares in 2001, 10/18 were virulent, whilst none of the 5 airborne *R. equi* detected in the irrigated holding pens in 2002 were virulent (P=0.046).

Mean soil moisture was 2-4 fold higher in the holding pens in 2002 compared to the corresponding period in 2001. The plot of the weekly incidence of *R. equi* pneumonia among the foals of resident mares in the 2002 season showed a low and declining incidence of *R. equi* pneumonia from October onwards, in contrast to peaks observed in November and December both groups of foals in 2001 and in 2002 for the foals of non-resident mares.

Assessment of the Contribution of Subclinically Infected Foals to Exposure to Airborne Virulent *R. equi*

Air samples were collected periodically during the 2002 foaling season on four *R. equi* affected farms. Samples were collected from the holding pens and lanes before horses had moved into them and while foals were mustered into pens and moved down lanes. The concentrations of virulent *R. equi* in these samples were compared to those taken at the muzzle level of foals within a resting mob held within a pen.

Virulent *R. equi* were detected more frequently at muzzle level in a resting mob of horses (20/24-83%) than in pens and laneways before horses moved into them or while horses were moving into them (33/96-34%). The median airborne concentration of virulent *R. equi* was significantly higher (P<0.001) at the muzzle level of foals (median = 4.0 cfu/1000 L, interquartile range (IQR) = 1.3-15.8 cfu/1000 L) than in pens and laneways before horses moved into them or while horses were moving into them (median = 0.0 cfu/1000 L, IQR 0.0-1.0 cfu/1000 L). The proportion of airborne *R. equi* that were virulent was also significantly higher (P<0.001) at the muzzle level of foals (165/371-44%) than in pens and laneways before horses moved into them or while horses were moving into them (98/368-27%).
Discussion of Results

This project has developed methods that allow a portable air sampler to be used for collection of a defined volume of air and deposition of airborne bacteria onto selective agar medium that facilitates *R. equi* growth. The plates can then be incubated to allow colony growth and these colonies blotted onto nylon membranes. The blotted bacterial cells can be lysed to release their DNA and the DNA fixed to the membrane. The DNA fixed to the membranes can then be probed with radiolabelled DNA probes that detect *R. equi* genes and the virulence plasmid. The total number of *R. equi* and the proportion of virulent isolates can then be determined for each sample.

Few studies have investigated the associations between farm characteristics and management practices and the impact of *R. equi* pneumonia. Farms with high prevalence of *R. equi* pneumonia reported a high rate of mortality due to *R. equi*. This may reflect an increased likelihood of mortality when *R. equi* pneumonia is very prevalent, or it may reflect the size of the dose of virulent *R. equi* a foal receives when it is exposed.

In previous studies in Texas, farms on which ≥60 acres were used for horses, that kept >160 horses and/or >17 foals, and had foal densities of >0.25 foals/acre were more likely to have foals develop *R. equi* pneumonia than other farms (4, 8). While there was no association in our study between farm area or maximal stocking rate and the prevalence of disease or occurrence of mortalities due to *R. equi* pneumonia, there were associations between the maximal number of foals on farms and the prevalence of *R. equi* pneumonia and the occurrence of mortalities due to *R. equi*. Farms with a small foal population were more likely to have a low prevalence of *R. equi* pneumonia and not to have mortalities due to *R. equi* than farms with medium to large foal populations. As the case fatality rates were no greater on the farms with a high disease prevalence than those with a low disease prevalence, the likelihood of mortality does not appear to be simply a consequence of the number of affected foals on the farm. The association between a small foal population and a low prevalence of *R. equi* pneumonia could reflect the communicable nature of the disease, as emphasised by our observations that infected foals could be a significant source of infection. Farms with a larger foal population may also have a greater environmental burden of virulent *R. equi* due to increased contamination of soil with *R. equi* in foal faeces (34) and an increased amount of equine faeces, which is utilised by *R. equi* for proliferation in the environment (15), than smaller farms.

The lack of association between the farm area used for horses or the maximal stocking rate and the prevalence of *R. equi* pneumonia and mortality due to *R. equi* may be a consequence of the generally large size and high stocking rates of farms involved in our study compared to the Texan study. All farms in our study used >24 hectares (or 60 acres) for horses and had >0.6 foals/Ha (or 0.25 foals/acre) and would have been defined as large farms with high stocking rates in the Texan studies (4, 8).

There was little variation between the farms in our study in housing and mob management so it was not possible to examine the effects these might have on the prevalence and severity of *R. equi* pneumonia. Most farms kept mare-foal pairs on paddocks in groups based on the reproductive status of the mare post partum. Farms that kept mare-foal pairs in groups based on the age of the foal did not have similar prevalences of *R. equi* pneumonia.

Management and feeding practices that may be thought to influence disease prevalence and severity were investigated. Creep feed, usually high in concentrates, may alter the flora of the gastrointestinal tract of the foal and thus the replication of *R. equi* in the gastrointestinal tract. However, no association was seen between the use of creep feed and the prevalence of *R. equi* pneumonia or the occurrence of mortalities due to *R. equi*. 
Feeding bins and stations promote congregation of mares and foals in paddocks and induce them to feed in areas where grass cover is sparse. Inhalation of \textit{R. equi} from the soil is likely in such areas. However, rotation of feeding bins and stations around the paddock to prevent excessive wear did not have any effect on the prevalence of \textit{R. equi} pneumonia or the occurrence of mortalities due to \textit{R. equi}, which suggests that inhalation of contaminated dust in paddocks may not totally explain the occurrence of \textit{R. equi} pneumonia.

Young foals spent up to the first 2 weeks of their lives in small yards. Sick or injured foals were also kept in similar small yards. Mobs of mares and foals were mustered into holding pens to await farriery and/or veterinary treatment and these yards and pens were often worn and dusty. Soil contaminants may become airborne more easily in these environments and hence the dose of virulent \textit{R. equi} inhaled may be greater than in areas with more grass cover and lower stocking densities. Removal of faeces from these areas may reduce the dose inhaled (2, 7, 11, 24). However, no association was seen in this study between the routine removal of faecal material from small yards and holding pens and the prevalence of \textit{R. equi} pneumonia or the occurrence of mortalities due to \textit{R. equi}.

Irrigation of paddocks and yards and changes in soil pH may influence the prevalence of \textit{R. equi} pneumonia by affecting replication in the soil and aerosolisation of the organism from the soil, affecting the concentration and proportion of virulent \textit{R. equi} inhaled by foals (2, 11, 15). Harrowing, irrigation, herbicidal treatment of pasture and the use of fertilisers had no association with the prevalence of \textit{R. equi} pneumonia or the occurrence of mortalities due to \textit{R. equi}. As the discriminatory power of our study was limited, further studies were performed examining the influence of these factors on the ecology of virulent \textit{R. equi} on farms and are discussed below.

The accepted dogma on most horse breeding farms is that good preventative health practices are important in reducing the risk of \textit{R. equi} pneumonia. This is based on observations that improvements in preventative health practices on single farms between seasons were associated with reductions in morbidity and mortality due to \textit{R. equi} (1, 7, 9). However, extensive studies of the value of these practices have not been conducted. In our study some associations were seen between preventative health practices and the prevalence of \textit{R. equi} pneumonia and the occurrence of mortality due to \textit{R. equi} pneumonia. Associations were seen between the vaccination of foals against EHV 1 and EHV 4 and the prevalence of \textit{R. equi} pneumonia, and between mortalities due to \textit{R. equi} and pre-partum anthelmintic treatment. However, no association was seen between the timing of vaccination of foals or the vaccination of foals against \textit{S. equi} subspecies \textit{equi} and disease due to \textit{R. equi}. Similarly, the Texan studies have found no association between vaccination of foals against \textit{S. equi} subspecies \textit{equi} and the occurrence of \textit{R. equi} pneumonia (5).

Infection with other respiratory pathogens may predispose foals to \textit{R. equi} pneumonia. Thus, immunisation against other respiratory pathogens may reduce this predisposition (7). Vaccination against EHV 2 multiple times within the first two months of life has been shown to reduce the prevalence of \textit{R. equi} pneumonia (22, 36), possibly by preventing EHV 2 suppression of the cell mediated immune response.

The association between EHV 1 and EHV 4 vaccination of foals and a low prevalence of \textit{R. equi} pneumonia is likely to be attributable to some other correlated environmental or management factor. The effect of vaccination initiated at approximately 3 months of age and \textit{R. equi} pneumonia, which usually manifests itself before the foal reaches this age, is not likely to be causal. There was some correlation between farms with small foal populations and farms that vaccinate foals against EHV 1 and EHV 4, which may explain the apparent association between this vaccination strategy and low disease prevalence. The lack of association between vaccination of mares against EHV 1 and EHV 4 and low prevalence of disease also suggests that antibody passively acquired by the foal against EHV 1 and EHV 4 is not influencing the development of \textit{R. equi} pneumonia. A cohort study examining the effects of earlier vaccination of foals against EHV 1 and EHV 4 on a farm with a high prevalence of \textit{R. equi} pneumonia would be required to establish whether vaccination against EHV 1 and EHV 4 influences the risk of a foal developing \textit{R. equi} pneumonia.

As most parasite control programs are initiated within the first two months of a foal’s life it is possible
that effective parasite control could affect the predisposition of a foal to developing *R. equi* pneumonia. However, in this study the lack of association between anthelmintic programs for foals (timing and number of anthelmintics used) and the prevalence of, or the occurrence of, mortalities due to *R. equi* pneumonia inferred that the implementation of parasite control strategies in foals is not an effective strategy for reducing the prevalence or severity of *R. equi* pneumonia on farms. Other recent studies have also failed to find an association between anthelmintic regimens for foals and the occurrence of *R. equi* pneumonia on farms (5).

A common predisposing factor for infectious disease in young foals is failure of passive transfer (FPT) of immunoglobulins. Numerous studies have associated FPT with septicaemia and other infectious disorders in foals (19, 20, 25, 26). Infectious disease and parasitism could compromise the mare’s health in gestation and the foal’s health and development *in utero* and when at foot. Pre-partum immunisation and pre- and post-partum parasite control in mares, so as to reduce the risk of disease and parasitism and maximise the quality of colostrum and milk produced, may well influence the foal’s chance of developing *R. equi* pneumonia and/or surviving infection. No associations were seen between immunisation strategies in mares nor the timing of the first anthelmintic treatment of mares *post partum*, and the prevalence of, or the occurrence of mortalities due to *R. equi* pneumonia. However, farms administering anthelmintics to pregnant mares within one month of their due foaling date were more likely to have mortalities due to *R. equi*. This association may be related to the effect of stresses caused by mustering and administration of a substance late in gestation on the quality of colostrum produced by the mare, and hence on the level of passive immunity against *R. equi* acquired by foals. The association between anthelmintic treatment of mares close to foaling and the occurrence of mortalities due to *R. equi* would benefit from a cohort study to evaluate the potential significance of late-term stresses on mares and the risk of foals subsequently dying from *R. equi* pneumonia. In general, parasite and immunisation programs appeared to have relatively little effect on the prevalence of, or the occurrence of mortalities due to, *R. equi* pneumonia on farms in the 2001 season.

Prophylactic therapy aimed at helping the neonate combat disease, or enhancing its immune system, did not reduce a foal’s predisposition to *R. equi* pneumonia in our study. Our inability to detect an association between the use of prophylactic therapies in foals and the prevalence of, or the occurrence of mortalities due to, *R. equi* pneumonia confirmed the findings of another study (5) that suggested that prophylactic therapy is not an effective strategy for reducing the prevalence or severity of *R. equi* pneumonia on farms.

In our study only one farm used hyperimmune serum routinely on all foals. However, this farm still experienced a high prevalence of *R. equi* pneumonia and mortalities during the 2001 season. Administration of hyperimmune serum has been shown to be an effective prophylactic measure against *R. equi* pneumonia in some studies (13, 17), but has been ineffective in others (5, 6, 10, 16). As the mechanisms by which hyperimmune serum might protect foals from *R. equi* pneumonia are unknown, and optimal timing and dosage regimens for administration are unknown (11, 13, 21), it is not surprising that its efficacy on farms with a high prevalence of *R. equi* pneumonia may be unpredictable.

The prevalences of other diseases affecting foals were examined for associations with the prevalence of, or the occurrence of mortality due to, *R. equi* pneumonia on farms. Foal diarrhoea, respiratory disease not caused by *R. equi* and traumatic injuries were not associated with the prevalence of, or occurrence of mortalities due to, *R. equi* pneumonia. There was a significant association between a high prevalence of conformational problems in foals and the occurrence of mortalities due to *R. equi* pneumonia. No such association was observed between conformation and the prevalence of *R. equi* pneumonia. It is common industry practice to confine foals with limb conformational problems in small yards to facilitate assessment and correction of the fault. These yards are usually devoid of pasture cover, and thus are dry and dusty environments. Extended stays in such areas may result in a greater risk of inhalation of high concentrations of virulent *R. equi* and subsequent severe bronchopneumonia.
Even with the numerical limitations in our study, associations were seen between the prevalence of *R. equi* pneumonia or the occurrence of mortalities due to the disease and several farm characteristics and management strategies. Associations were seen between high disease prevalence and mortalities due to *R. equi* pneumonia, and a small foal population and a low prevalence of, and reduced risk of observed mortalities due to, *R. equi* pneumonia. There was no conclusive evidence that immunisation, parasite control or other health management practices in mares or foals reduced the prevalence or severity of *R. equi* pneumonia.

The effects of other variables on the prevalence and severity of *R. equi* pneumonia on farms, such as irrigation and use of fertilisers, may be better assessed by evaluating their potential to alter the levels of virulent *R. equi* in specific farm environments. Similarly, the association between a high prevalence of foals with limb conformational problems and an increased risk of mortality due to *R. equi* pneumonia may be better assessed by evaluating the levels of virulent *R. equi* in the farm environments in which these foals are kept. These aspects were explored in later stages of the project using the quantitative air sampling method we developed to determine concentrations of virulent *R. equi* at different sites on farms.

The amount of contamination of soil with virulent *R. equi* has been regarded as the explanation for differences between farms in the prevalence of *R. equi* pneumonia. It has been suggested that on farms with high concentrations of virulent *R. equi* in soil and on which a high proportion of *R. equi* in soil were virulent, *R. equi* pneumonia is likely to be endemic (27, 31). However, a more recent study has challenged this, finding that the concentration of virulent *R. equi* in soil on farms with endemic *R. equi* pneumonia was not significantly different from that on those farms that did not experience the disease (18). In our study the differences between farms with varying prevalences of *R. equi* pneumonia were more likely to be associated with the proportion of *R. equi* that were virulent and the concentrations of virulent *R. equi* in air than in the soil. This concords with the current understanding of the route and source of infection, the inhalation of contaminated dust, and supports the suggestion that the level of exposure to aerosolised virulent *R. equi* affects the relative prevalence of *R. equi* pneumonia on farms (11, 24).

A number of environmental variables influenced the concentration of both *R. equi* and virulent *R. equi*, and the proportion of *R. equi* that were virulent, in both air and soil. Low humidity, high average ambient temperature and high soil moisture were significantly associated with an increased concentration of *R. equi* in the air or soil in multivariable analyses. However, the significant associations of low soil moisture and low pasture height with elevated concentrations of airborne virulent *R. equi*, but not with elevated concentrations of total *R. equi*, and associations of low soil moisture and acidic soil with elevated proportions of *R. equi* that were virulent in air and soil, respectively, suggest that virulent *R. equi* may have a different ecological niche from avirulent *R. equi*. Environmental conditions such as dry and acidic soils and poor pasture cover appear to favour virulent *R. equi* in the environment.

As the airborne *R. equi* population is influenced by environmental variables (i.e. soil moisture, pasture height, temperature), management strategies that affect these environmental variables are likely to influence disease prevalence on a farm.

The concentrations of virulent *R. equi* were highest in the holding pens, with lesser burdens in the lanes. Both pens and lanes were significantly more heavily contaminated with virulent *R. equi* than the paddocks on most farms. Dry areas with low pasture cover also had significantly higher airborne concentrations of virulent *R. equi*. The dry soils and lack of grass in the holding pens and lanes were probably partially responsible for the greater airborne concentrations in these areas. These findings showed that holding pens and lanes were dangerous areas for foals to occupy for prolonged periods of time. Prolonged exposure to elevated airborne concentrations of virulent *R. equi* is likely to increase the risk of developing *R. equi* pneumonia.

Aerosolisation of virulent *R. equi* appeared to be influenced by the soil texture. The most prominent environmental factor influencing airborne concentrations of virulent *R. equi* and the proportions of
airborne *R. equi* that were virulent was soil moisture and this variable was correlated with soil texture. Sandy soils tended to be drier than clay soils. The soils in holding pens and lanes were sandy on many farms. However, the lack of statistically significant associations between environmental variables and the concentration of virulent *R. equi* in soil suggests that key environmental factors, other than faecal contamination, influencing the proliferation of virulent *R. equi* in the soil may still need to be identified.

The differing strengths of association between predictor variables and the concentration of virulent *R. equi* in air compared to soil may reflect the habitat of virulent *R. equi* in the surface soil. As the soil samples that were analysed were core samples taken down to a depth of 5 cm, they were unlikely to fully reflect the population in the most superficial soil, which was more likely to be represented in air samples. The air samples were also likely to reflect a relatively greater area than the soil samples. Thus the greater relative concentrations of virulent *R. equi* in air, compared to other *R. equi*, might suggest that virulent *R. equi* may predominate in the most superficial soil layers.

Previous studies have noted that the number of foals on farms influences the chance of the farm reporting cases of *R. equi* pneumonia (4). These findings were supported by the association noted above between small foal populations and a lower prevalence of disease on farms. These associations may also be affected by the age dynamics of the foal population. The tightly regulated Thoroughbred breeding season results in a close relationship between the average age of foals and time of the year on most farms, so variations in environmental factors between and within years may affect the prevalence of disease. The majority of cases occurred in the middle of the season, in association with significant increases in the airborne concentrations of virulent *R. equi* and the peak in the number of foals <4 months of age. In the 2001 season Victorian farms experienced milder conditions during October and November than in the previous season, and the incidence of *R. equi* pneumonia increased considerably in December, a month later than in the previous season. Elevated airborne concentrations of virulent *R. equi* and proportions of airborne *R. equi* that were virulent were also noted later in the 2001 season (December-January), but this was not associated with a concurrent rise in prevalence of *R. equi* pneumonia as the number of foals aged between 4 and 12 weeks had begun to fall on most farms.

Lengthy antimicrobial therapy has been traditionally recommended for the successful treatment of *R. equi* pneumonia (14). The majority of cases of *R. equi* pneumonia reported in the 2001 season received less than 20 days of treatment and many received less than 10 days of treatment. This may be a consequence of the increased vigilance of staff and clinicians on Australian Thoroughbred farms and the increasing use of ultrasonographic examination to diagnose cases earlier in the course of disease. The low case fatality rate in NSW during the 2001 season may also be a reflection of the increased awareness of the disease, with early diagnosis and treatment leading to a higher survival rate and more rapid recovery.

This work highlights the likely importance of the airborne *R. equi* population in determining the prevalence of *R. equi* pneumonia. Ecological factors (i.e. soil moisture, sandy soils, poor pasture cover) affecting the concentration of airborne virulent *R. equi* and the proportion of airborne *R. equi* that were virulent in the environment of susceptible foals appear to influence the prevalence of *R. equi* pneumonia and contribute to the severity of disease. Environmental management strategies focusing on reducing the level of aerosol challenge to susceptible foals, together with early diagnosis and treatment of cases, seems to be the best way forward in reducing the impact of *R. equi* pneumonia on the Australian Thoroughbred breeding industry.

We have used the methods developed in these projects to conduct a collaborative study with the Irish Equine Centre, establishing that, under the management conditions in this environment, stables appear to be a major site of risk.
Effect of environmental intervention on *R. equi* pneumonia

Preliminary studies examining the effect of irrigation of holding pens indicated that this may have a significant impact on the concentration of virulent *R. equi* in the air during mustering and that it also appeared to be correlated with a reduced prevalence of disease. These findings will need to be confirmed with further studies to establish their repeatability.

Use of exhaled air sampling in diagnosis of *R. equi* pneumonia

A preliminary study suggested that foal breath sampling was more sensitive than thoracic ultrasonography in detecting foals infected with virulent *R. equi*. However, the clinical significance of breath sample findings needs to be further defined because subclinically infected foals were also detected. We have also conducted complementary studies with researchers on serological tests at the Institute for Medical and Veterinary Sciences in Adelaide to assist them in the assessment of their assays. A combination of breath sampling and serology may allow earlier detection of foals exposed to virulent *R. equi*.

Significance of subclinically infected foals as a source of infection

Further studies conducted in this project has also found high concentrations of virulent *R. equi* in the expired air of clinically and subclinically infected foals, suggesting a direct aerosol route of transmission may occur. The significance of these findings were further explored by sampling the air in holding pens before mustering of foals into the pens, during mustering, and then from among the foals while they rested in the pens. These studies showed that the airborne concentrations of virulent *R. equi* were sixteen fold higher in the regions around the foals’ muzzles than could be accounted for by the raising of faecally contaminated dust during mustering, implicating exhalation of virulent *R. equi* by subclinically infected foals as a major source of infection.

These findings raise major questions about the focus of measures for controlling *R. equi* pneumonia in foals. While faecally contaminated dust is undoubtedly a potential source of infection, transmission from one infected foal to another may well be the most important factor in endemic *R. equi* pneumonia.
Implications

The environmental surveys conducted during this project indicate that the major areas for focussing management strategies to control airborne concentrations of *R. equi* on horse studs were probably the laneways and holding pens. Subsequent preliminary studies have shown that irrigation of these areas may reduce the airborne concentrations of virulent *R. equi*. Further consideration of our findings in the design of management strategies on horse studs may reduce the amount and/or severity of disease.

However a more significant finding is that subclinically infected foals may be a far more important source of infection than faecally contaminated dust. Thus, while dust may be a source of infection, particularly early in the foaling season, it seems likely that most infections later in the season are a result of transmission directly from one foal to another. Thus control of disease might be most effectively achieved by reducing the number of times foals are mustered into large groups, reducing the amount of time they are kept together in close proximity and reducing the size of the groups, thus reducing exposure to subclinically infected foals. A further measure could be to identify and quarantine subclinically infected foals.

Our observations that infected foals exhale high concentrations of virulent *R. equi* have a further implication in early diagnosis. Although early diagnosis has been shown to dramatically influence the success of treatment, currently the options available to effect an early diagnosis are limited. Microbiological methods rely on trans-tracheal wash samples or bronchoalveolar lavage, both of which are time consuming and relatively invasive. Ultrasonic scanning of the thorax is capable of detecting abscesses on the surface of the lung, but is likely to miss cases where the abscesses are deep in the lung parenchyma or very small. Serological testing may be of value, but as yet interpretation of higher antibody titres against *R. equi* is difficult. These studies have shown that infected foals excrete detectable concentrations of virulent *R. equi* in expired air. This may allow breath sampling to be used as an early diagnostic technique.

In addition, the non-invasive breath sampling method developed may be able to be used to assess the level of subclinical *R. equi* infection in foals and possibly to monitor the response to treatment of clinically affected foals so that the appropriate duration of treatment can be established.

In conclusion, the findings from this project suggest that simple control strategies might be focussed on environmental manipulation within specific areas on studs, particularly in the holding pens and laneways. The efficacy of different strategies could be assessed on studs by measuring the airborne concentration of virulent *R. equi* and by determining the number of subclinically affected foals by breath sampling. Control programmes could consider:

1. Different surfaces in laneways and yards.
2. Strategies for control of airborne bacteria in laneways and yards, such as irrigation.
3. Handling strategies for minimising exposure of susceptible foals to high risk areas and to subclinically infected foals.
4. Early diagnosis and prophylaxis measures.
5. Different treatment regimens.
Dissemination/Adoption

The findings of this project have been applied immediately to field situations and ongoing work is aimed at using the techniques developed to establish practical methods for reducing airborne concentrations of virulent *R. equi* on horse studs. The findings have been disseminated to veterinarians and stud managers by direct contact during the conduct of the project and by presentations at industry forums throughout the last 3 years. The results of these studies have been presented at both national and international veterinary conferences and have been submitted for publication in both microbiological and veterinary peer reviewed journals.
Publications

Papers


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Presentations


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