Epidemiological Survey of *Corynebacterium equi* Infections on Five Ontario Horse Farms

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ABSTRACT

Corynebacterium equi **was cultured from manure or soil on five horse-breeding farms in Ontario at monthly intervals on three occasions during the summer of 1982. The organism was widespread. Contamination by *C. equi* of the loafing paddock and pasture areas was significantly greater in a farm established 30 years than in two established for four and six years and there was a significant correlation between the *C. equi* burden in stables, paddocks and pastures and the length of use of the five farms for horses. In all farms, numbers of *C. equi* in pasture soil exceeded numbers in fresh manure, suggesting that environmental multiplication of the organism might occur.**

A farm with an endemic *C. equi* pneumonia problem differed significantly from the other four farms, where disease was not endemic, in the larger number of *C. equi* isolated in the stable area. By contrast the farm with a *C. equi* pasture soil burden significantly heavier than on all other farms had no deaths due to *C. equi* pneumonia. There was a correlation (r = 0.78, p = 0.061) between the number of cases of *C. equi* pneumonia on the farms and numbers of *C. equi* in the area of the stables, but not on the paddocks or pastures.

About two-thirds of randomly chosen isolates from the farms belonged to the three capsular serotypes most commonly found in pneumonic foals.

Key words: *Corynebacterium equi*, epidemiology, Ontario, horse farms.

RÉSUMÉ

Cette expérience consistait à rechercher la présence de *Corynebacterium equi*, dans des échantillons de fumier et de sol prélevés sur cinq fermes d'élevage chevalin de l'Ontario, à trois intervalles mensuels, au cours de l'été de 1982. Elle révéla que ce microbe y était très répandu. La contamination des enclos de repos et des pâturages s'avéra significativement plus élevée, sur une ferme qui comptait 30 ans d'existence que sur deux autres qui n'en comptaient respectivement que quatre et six. Une corrélation significative existait aussi entre la longueur d'utilisation de chacune des cinq fermes d'élevage et l'abondance de *C. equi*, dans les écuries, les enclos de repos et les pâturages. Sur toutes les fermes, le nombre de *C. equi* du sol des pâturages excéda celui du fumier, indice que le microbe peut se multiplier dans l'environnement.

Une de ces fermes d'élevage, aux prises avec un problème enzootique de pneumonie imputable à *C. equi*, différait sensiblement des quatre autres exemptes de ce problème, par le nombre plus élevé de *C. equi* qu'on isola du voisinage de l'écurie. Par ailleurs, la ferme dont le sol des pâturages recelait un nombre sensiblement plus élevé de *C. equi* que celui des quatre autres, ne connut aucune mortalité attribuable à la pneumonie causée par ce microbe. Il existait aussi une corrélation (r = 0,78; p = 0,061) entre le nombre de tels cas de pneumonie et celui des *C. equi* isolés du voisinage des écuries, mais non des enclos de repos et des pâturages.

Environ 66% des souches de *C. equi*, isolées sur ces cinq fermes d'élevage et choisies au hasard, appartenaient aux trois sérotypes capsulaires recouvrés le plus souvent des poulains atteints de pneumonie.

Mots clés: *Corynebacterium equi*, épizootiologie, Ontario, fermes d'élevage chevalin.

INTRODUCTION

Corynebacterium equi is an important cause of pneumonia in foals (1). It is characteristic of the disease that it occurs endemically on some horse farms, sporadically on others and is not recognized on most (2). Many healthy horses and other herbivores carry *C. equi* in their intestine (3-5) and most horses produce a cellular immune response to such clinically inapparent infection (6). It is postulated that pneumonic disease occurs only on those farms where foals are challenged by large numbers of *C. equi* by the respiratory rather than the intestinal routes (6). The recent development of selective methods of isolating *C. equi* from the intestine or soil (7) gave us the opportunity to examine some epidemiological aspects of the infection on horse-breeding farms. The purpose of the work described here was to determine 1) whether there was a relationship between the burden of bacterial infection on a farm and the history or incidence of *C. equi* pneumonia on the farm, 2) to assess the infection levels in different sites on farms and 3) to determine the distribution of *C. equi* serotypes on horse farms.

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MATERIALS AND METHODS

FARMS

Five horse breeding farms in Southern Ontario were used in the study. Details on the time during which the farms had been used for horse breeding, the history of *C. equi* problems on the farm, the number of foals reared in 1982 and the number of confirmed and suspected cases of *C. equi* pneumonia in 1982 are shown in Table I. Farm 1 was a horse farm where the disease had been a problem for several years, clinically affecting at least 10% of foals born, farms 2, 4 and 5 had experienced occasional cases of *C. equi* pneumonia over several years, and farm 3 had no history of the disease. Soil from a golf course on which no domestic animals had grazed for about 20 years was also cultured.

TABLE I. 1982 *Corynebacterium equi* Epidemiological Study: Details of Five Ontario Horse Farms Used in this Study

Details	Farm Number 1	2	3	4	5
C. equi pneumonia status	Endemic	Sporadic	None	Sporadic	Sporadic
Time farm established	25 years	4 years	6 years	30 years	8 years
Number mares bred	200	400	30	320	480
Foals reared to 4 weeks	125	225	25	85	150
Foals reared to 12 weeks	100	300	25	120	100
Deaths with *C. equi* pneumonia	4	2	0	0	3
Suspected cases of *C. equi* pneumonia (non-fatal)	10	5	0	0	1
Average age *C. equi* cases	4 weeks	12 weeks	—	—	10 weeks

COLLECTIONS OF SAMPLES

Four sites on each farm were cultured for *C. equi*. These were: 1) freshly voided manure, 2) soil from within 15 metres of the stabling (walkways, ditches, roads, flower beds, etc.), 3) the "loafing paddocks" for mares and foals near the stables (usually within 100 metres) and 4) the grass pastures distant from the stables and barn areas. The sites were chosen to assess the degree of infection of horses and of three environmental sites expected to have progressively less contact with horses or their products.

Ten manure samples were collected from randomly chosen mares and ten samples were taken from separate areas in each of the three environmental sites. Soil was scraped from the surface of the ground with a small spoon and placed in separate plastic photographic film containers. Samples were cultured for *C. equi* within 24 hours of collection; they were stored at 4° C after reaching the laboratory, within four hours of collection. Soil sampling was based on arbitrarily selected points made on a map of each farm, designed so that widely separated parts within each area were sampled on each visit. Samples were taken on three or four occasions between June and August 1982; the visits to each farm were about four weeks apart. Farms 1-3 were visited three times and farms 4 and 5 four times. On the first visit only qualitative culturing for *C. equi* was done; results are not included in the quantitative analysis, although isolates were serotyped as described below. Ten soil samples from a golf course were cultured on three occasions at four-week intervals.

ISOLATION AND SEROTYPING OF *CORYNEBACTERIUM EQUI*

A weighed 1.0 g sample of feces or soil was diluted and mixed in 9.0 mL of phosphate buffered saline, pH 7.2. A 0.05 mL aliquot was spread over the surface of NANAT medium (7), a selective medium containing nalidixic acid, novobiocin, actidione and potassium tellurite. The numbers of *C. equi* colonies present were counted after 72 hours incubation at 37° C. The minimum detectable count was 200 colony-forming units. Representative *C. equi* colonies were identified by their morphology, reduction of potassium tellurite (7) by Gram stain and by their production of *equi* factor (8). One representative isolate from each sample was stored at 4° C on a nutrient agar slope for serotyping. Ten isolates were randomly selected from each farm from the stored strains collected at the first three visits and their capsular serotype determined by typing with antisera against the three most common equine serotypes, 1, 2 and 6 (9,10).

ANALYSIS OF RESULTS

Results of quantitative culturing were analyzed by analysis of variance and Student's t-test.

RESULTS

DIFFERENCES IN *C. EQUI* COUNTS BETWEEN VISITS TO THE SAME FARM

No differences in *C. equi* isolation were detected for combined sites on the different sampling visits for farms 1, 2 and 5. The numbers of *C. equi* isolated in August was significantly heavier on farm 3 than the number isolated in July ($p < 0.001$) and the numbers isolated in June on farm 4 were significantly fewer than in both July and August ($p < 0.05$).

ISOLATION OF *C. EQUI* FROM FOUR DIFFERENT SITES ON THE SAME FARM

Results of all visits to each farm were combined for analysis. Analysis of variance showed that there were differences between sites on the same farm; these differences were further investigated using Student's t-test. A comparison of the mean numbers of *C. equi* isolated from the four different sites on each farm is shown in Table II.

TABLE II. 1982 *Corynebacterium equi* Epidemiological Study: Comparison of Isolation of *C. equi* from Different Sites on the Same Ontario Horse Farm

Site	Farm 1	2	3	4	5
Manure	1.04 ± 1.34^{ab}	0.67 ± 1.19	1.50 ± 1.55	1.25 ± 1.37^{e}	1.91 ± 1.42^{g}
Stable	2.68 ± 1.22^{b}	0.83 ± 1.31	0.98 ± 1.56^{d}	1.81 ± 1.56^{f}	1.97 ± 1.26^{g}
Paddock	2.88 ± 1.29^{b}	0.52 ± 1.07^{c}	2.14 ± 1.35^{d}	3.46 ± 0.42^{ef}	2.54 ± 1.02
Pasture	2.14 ± 1.56^{b}	1.40 ± 1.49^{c}	1.63 ± 1.42	3.59 ± 0.36^{ef}	2.66 ± 1.16^{g}

[a]Mean ± standard deviation, $\log_{10}$ bacterial count/gram of manure or soil
[b-f]Differs ($P < 0.05$) from figure with lowest value (in the same column) having the same superscript
[g]Differs ($P < 0.05$) from figure with highest value (in the same column) having the same superscript

Corynebacterium equi was not isolated from 30 soil samples from a golf course.

ISOLATION OF *C. EQUI* FROM FOUR SITES ON THE FIVE DIFFERENT FARMS

Results of all visits to each farm were combined for analysis. Analysis of variance revealed that there were differences between sites on the different farms. These differences were compared using Student's t-test. A comparison of the mean number of *C. equi* isolated from the four sites on each of the farms is shown in Table III.

There was a correlation between the number of cases of *C. equi* (number of cases/number of foals at 12 weeks) on the different farms and the numbers of *C. equi* in the stable area, but not in other environments ($r = 0.78$, $p = 0.061$). There was also a correlation between the number of *C. equi* isolated (total of stable, paddock and pasture number) and the time the farm had been used for horses ($r = 0.84$, $p < 0.05$).

SEROTYPING OF *C. EQUI* ISOLATES FROM THE FIVE FARMS

Ten randomly selected *C. equi* strains isolated from each of the four different sites on the first three visits to each farm belonged to the serotypes shown in Table IV. About one-third of the isolates could not be typed, but of those which could, the majority were capsular type 1.

DISCUSSION

This study confirms the findings of others that *C. equi* is widespread on horse farms (3, 11). The results show that an increasing *C. equi* burden of infection occurs on a farm the longer that it is used to rear horses. Farm 4, established 30 years, had significantly heavier paddock and pasture contamination than farms 2 and 3, established only for four and six years respectively. The farms were chosen for the study because they included among the largest horse-breeding farms in Ontario, reliable records were available, and three had resident veterinarians. Differences in age of the farms and in history of *C. equi* pneumonias were also known.

The results tend to confirm the suggestion of Barton and Hughes (11) that *C. equi* behaves like *Rhodococcus coprophilus*, an organism which multiplies in soil contaminated by cow manure (12). In all the farms the number of *C. equi* in pasture soil exceeded the number in horse manure; in the three older farms this difference was statistically significant. Barton and Hughes (13) claimed that *C. equi* multiplied 10,000-fold over a three-week period in freshly passed horse manure. Our results show that there is a progressive increase of infection on horse farms with use. The organism was not recovered from a golf course where animals had not grazed for many years. No domestic species other than horses were present on these horse farms so the source of *C. equi* was the horses. The contribution by wild animals, if any, would be negligible. The results do not resolve the argument as to whether *C. equi* should be considered as primarily an intestinal organism of horses and other herbivores (3,4) or of soil (13). The numbers of *C. equi* in the environment may fluctuate with changes in temperature, humidity or other factors. Magnusson (14) suggested that the organism multiplied at Swedish summer temperatures, since bringing forward the foaling date decreased the incidence of foal pneumonia. On two farms in the present study there were significantly fewer *C. equi* isolated in June than in July and August. Whether these differences were because of environmental conditions rather than sampling site differences is hard to determine given the small sample sizes. The results for all visits to each farm were combined to increase the variance.

The farm endemically affected with *C. equi* pneumonia in its foals (farm 1) differed markedly from all other farms in the high level of *C. equi* in the immediate area of the stables. Farm 5, which experienced several deaths due to *C. equi*, had the second highest stable environmental infection. The levels of pasture and paddock contamination seem irrelevant *per se* as far as the incidence of disease is concerned, since no cases of suspected *C. equi* pneumonia occurred in 1982 on farm 4, even though pasture contamination was significantly heavier than on all other farms. The numbers of *C. equi* in the stable environment in farm 4 were lower than on farms 1 and 5. There was correlation between the number of cases of *C. equi* pneumonia and the burden of infection in the stable area, but not in other areas.

A study by Smith and Robinson (15) attributed an outbreak of *C. equi* pneumonia in foals to inhaled dust carrying the organism. Previous studies have shown that ingestion of *C.*

TABLE III. 1982 *Corynebacterium equi* Epidemiological Study: Comparison of Isolation of *C. equi* from Similar Sites on Five Different Ontario Horse Farms

Farm	Site			
	Manure	Stable	Paddock	Pasture
1	1.04 ± 1.33^{ab}	2.68 ± 1.22^{c}	2.80 ± 1.29^{ef}	2.14 ± 1.56^{g}
2	0.67 ± 1.19^{b}	0.83 ± 1.31^{cd}	0.52 ± 1.07^{efi}	1.40 ± 1.49^{gh}
3	1.50 ± 1.55	0.98 ± 1.24^{cd}	2.14 ± 1.35^{ei}	1.63 ± 1.42^{gh}
4	1.25 ± 1.37	1.81 ± 1.56^{c}	3.46 ± 0.45^{e}	3.59 ± 0.36^{g}
5	1.91 ± 1.42^{b}	1.97 ± 1.26^{cd}	2.53 ± 1.02^{ei}	2.66 ± 1.26^{gh}

[a]Mean ± standard deviation, $\log_{10}$ bacterial count/gram of manure or soil
[b-h]Differs ($P < 0.05$) from figure with highest value (in the same column) having the same superscript
[i]Differs ($P < 0.05$) from figure with lowest value (in the same column) having the same superscript

TABLE IV. 1982 *Corynebacterium equi* Epidemiological Study: Capsular Serotypes of *C. equi* Isolates on Five Ontario Horse Farms

Serotype	Farm Number					Total
	1	2	3	4	5	
1	12	6	14	13	11	56
2	3	8	7	7	2	27
6	1	2	1	1	4	9
Untyped	14	14	8	9	13	58
Totals	30	30	30	30	30	150

equi by foals usually results in a self-limiting intestinal infection (16); disease probably occurs when foals are challenged by large numbers of bacteria by the respiratory route. Foals on grass pasture, even where large numbers of *C. equi* may be present under the grass in the soil (as in farm 4) are probably less at risk than foals in the stable environment (as in farm 1), where environmental dust is disturbed by the movement of people, vehicles and horses along walkways and roads.

About two-thirds of the *C. equi* isolated (Table IV) belonged to serotypes commonly found in pneumonic foals (9,10). These serotypes were found on all farms but whether there were differences in serotype predominantly found on any of the farms was not determined because of the small numbers of isolates typed. The distribution of several serotypes on all the farms may influence the choice of antigens for a potential vaccine, if the capsular polysaccharide is shown to be a protective immunogen. Of the one-third of the isolates which were not typed some may be those strains of serotypes 1, 2 and 6 which are poor antigen producers and which would not be identified by the methods used (Prescott, unpublished observations).

The control of disease on a farm where *C. equi* have reached the levels in the stable environment seen in farm 1 is difficult. Magnusson (14) reported complete control of endemic disease on a horse farm by moving mares to foal on a separate farm. It was not stated whether or when foals were brought back to the original farm. The results presented here suggest that such a measure would likely be effective in greatly reducing the level of challenge to the young foals, but the few cases of pneumonia seen on farm 2, the newest and least contaminated of all the farms, suggest that factors other than simply the level of environmental challenge may also be important.

Table I indicates the widespread movement of horses which occurs between farms during the breeding season. Many mares are kept on farms just long enough for breeding and subsequent pregnancy diagnosis, while others are kept longer. Some of the foals with *C. equi* pneumonia may not have acquired the infection on the farm where the diagnosis was made. The majority, however, most likely acquired the infection on the farm of diagnosis. Table I also shows that pneumonia was diagnosed in foals on farm 1 at four weeks of age, whereas on the sporadically affected farms the disease occurred in older foals.

The studies described here were made on a limited number of farms, which differed in size, geography, the types of horse (Standardbred, Thoroughbred), size of individual boxes for mares and foals, ventilation of stables and in other managemental factors. These factors, particularly management, are hard to quantify. Farm 4, the farm with the heaviest infection in paddocks and pasture, had no cases of *C. equi* pneumonia. In our opinion it was the best managed of the farms sampled, had the largest loose boxes for mares and foals, good ventilation and an excellent standard of hygiene. These and perhaps other factors appear to have kept contamination by *C. equi* of the stable environment to relatively low levels. By contrast farm 1 had small loose boxes for mares and foals, which appeared dusty and poorly ventilated. These factors may have contributed on farm 1 to the development of pneumonia in a stable environment heavily seeded with *C. equi*.

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