

Rhodococcus equi foal pneumonia: Protective effects of immune plasma in experimentally infected foals

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Summary

The immunoprophylactic capacity of specific immune plasma was evaluated in pony foals infected experimentally with *Rhodococcus equi*. Immune plasma, produced by repeated parenteral administration of viable *R. equi* to adult horses, was harvested and frozen. Group I (six control foals) and Group II (six principal foals) received lactated Ringers solution and immune plasma respectively at three and five days of age. *R. equi* were aerosolised into a caudal lung lobe of all foals at seven days of age. Clinical signs, haematological alterations, immune responses, thoracic radiographs and technetium^{99m} pulmonary perfusion scans were monitored. All foals were destroyed and complete post mortem examinations performed. All foals developed pneumonia as evidenced by clinical, radiographic and perfusion alterations, but the survival rate of principal foals was significantly ($P < 0.01$) greater than that of control foals. Five control foals developed terminal disease, whereas all principal foals recovered. There was no significant ($P > 0.05$) difference in temperature response, or peripheral blood leucocyte, neutrophil or fibrinogen concentrations between groups. ELISA values for *R. equi* antibody were significantly ($P < 0.001$) greater in principal foals following treatment, but there was no significant ($P > 0.05$) difference in IgG or IgM concentrations between groups. Results of the haemolysis inhibition assay indicated that equi factor neutralising antibodies were transferred by immune plasma to the principal foals. Post mortem examinations of five control foals destroyed at approximately three weeks post infection because of terminal disease, revealed severe pyogranulomatous pneumonia. One control and all principal foals were either free of lesions or had resolving lesions and/or minimal scar formation at three months post infection. The results of this investigation document the importance of humoral factors in controlling the disease process, and the capacity of humoral immunoprophylaxis to alter the clinical progression of *R. equi* pneumonia.

Introduction

Rhodococcus (Corynebacterium) equi was initially described by Magnusson (1923) as a causative agent of purulent pneumonia in

foals. The management of *R. equi* infections is often frustrating and unrewarding because specific tests for early diagnosis are not available, therapy is expensive and not always successful, and effective immunoprophylaxis has not been developed.

Previous reports suggested that horses infected or vaccinated with *R. equi* developed little or no antibody (Magnusson 1938; Wilson 1955; Carter and Hylton 1974). Recently however, more sensitive assays have been developed (Seddik and Atia 1982; Prescott, Coshan-Gauthier and Barksdale 1984; Ellenberger, Kaerberle and Roth 1984; Skalka and Svastova 1984/85; Hietala, Ardans and Sansome 1985; Takai, Kawazu and Tsubaki 1985). Studies designed to correlate the presence of *R. equi* antibody with clinical disease have documented the widespread prevalence of antibody to *R. equi* in horses and suggest that specific antibody may be more important to disease resistance than previously considered (Ellenberger *et al* 1984; Hietala *et al* 1985; Takai *et al* 1985). Additional studies have demonstrated the presence and biological effectiveness of opsonic activity in sera from foals with *R. equi* infections and *R. equi* antibody (Martens, Martens, Renshaw and Hietala 1987; Hietala and Ardans, 1987a; Hietala and Ardans 1987b). The pathological findings of advanced *R. equi* lung lesions, as reviewed by Ellenberger and Genetzky (1986), are primarily associated with a cell-mediated immune response. Clinical signs of disease are not usually manifested until foals are aged one to six months. There is however, mounting evidence that the humoral immune response is important in the early stages of infection, and that foals usually become infected at a very young age (Hietala *et al* 1985; Martens *et al*, unpublished data). Consequently, it may be important to specifically activate or augment the foal's immune system within the first few days of life.

The passive transfer of humoral immunity is relied upon to protect foals from many neonatal diseases. This is most commonly achieved by the passive transfer of antibodies via the mare's colostrum; however, it can also be accomplished by parenteral administration of immune plasma, serum or gamma-globulin. There are two brief reports in the literature on the apparent benefits of plasma and serum to treat *R. equi* infections (Magnusson 1938; Henry 1979).

The purpose of this study was to determine the effectiveness of parenterally administered *R. equi* immune plasma to prevent or ameliorate the disease process in foals infected experimentally

TABLE 1: Data on control (Group I) and principal (Group II) foals with radiographic, radioisotope perfusion scan, and bacteriological evidence of pulmonary disease after intrabronchial *R. equi* aerosolisation

	Foal	Sex	WT (kg)	Radiographic signs	Perfusion defect*	<i>R. equi</i> culture	Lung affected	Clinical recovery
Control (Group I)	66	F	15.0	Severe	+	+	L	-
	67	M	20.5	Severe	+	+	R	+
	69	F	16.4	Severe	ND	+	R	-
	610	M	20.9	Severe	+	+	L	-
	613	F	19.1	Severe	+	+	L	-
	614	F	16.8	Severe	+	+	L	-
Principal (Group II)	62	M	20.0	Moderate	ND	-	R	+
	63	F	20.5	Moderate	+	-	R	+
	64	M	18.2	Severe	+	-	R	+
	68	F	19.1	Severe	+	+	R	+
	611	M	11.8	Severe	+	-	L	+
	612	F	12.3	Severe	+	+	L	+

*Perfusion defect: reduced Technetium^{99m} distribution in pulmonary capillaries of affected lung; F: female; M: male; R: right; L: left; +: positive; -: negative; ND: not done; Moderate: patchy lung infiltrate with air bronchograms; Severe: solid lung opacity with air bronchograms; a: lung lesion; b: tracheobronchial aspirate

with *R. equi*.

Materials and methods

Twelve clinically healthy newborn pony foals (Table 1), were housed individually with their dams in box stalls with cement partitions. They were divided into two groups containing six animals. Group I (control) was given lactated Ringers solution (LRS) intravenously (iv), 20 ml/kg bodyweight (bwt), at three and five days of age. Group II (principal) was given *R. equi* immune plasma iv, 20 ml/kg bwt at three and five days of age. Three of the principal foals (Nos 62, 64, 611) received plasma from donor 968 and three (Nos 63, 68, 612) from donor 970. A saline suspension of *R. equi* was aerosolised into a caudal lung lobe of all foals at seven days of age.

Physical examinations throughout the investigation included body temperature, observation for distress or discomfort, complete blood counts, thoracic radiographs and pulmonary perfusion scans. Bacterial cultures were obtained at variable times after infection to detect the presence of *R. equi* in tracheobronchial aspirates. Immunological assays were conducted on serum from each foal prior to treatment with LRS or immune plasma, at Day 0, three weeks post infection, and three months post infection or prior to death. Included were luminol-dependent chemiluminescence (LDCL), enzyme-linked immunosorbent assay (ELISA), haemolysis inhibition (HI), and concentrations of IgG and IgM measured by single radial immunodiffusion (Miles Scientific, Illinois).

Foals in the terminal stages of disease, characterised by severe unrelenting dyspnoea, respiratory distress, inability or unwillingness to stand and nurse, or a moribund state, were destroyed using pentobarbital sodium (Sleepaway; Fort Dodge, Lab., Iowa). The investigation was terminated and all surviving foals except Foal 612, were destroyed at three months post infection. Foal 612, which recovered clinically but had a large pulmonary lesion, was monitored for one year. A post mortem examination, including bacteriology and histopathology, was performed on all animals.

Bacterial suspension

Rhodococcus equi (TVMDL 007) was isolated from the lung of a foal with *R. equi* pneumonia. A pure culture was transferred from 10 per cent bovine blood agar and propagated in brain heart infusion broth (BHIB) with 10 per cent calf serum for 24 h at 37°C with rotation (15 rpm). One hundred millilitres of 24hr

broth cultures were transferred to 2 litres of BHIB with 10 per cent calf serum and incubated at 37°C for 48h with rotation. Bacterial suspensions were centrifuged at 500 g for 20 mins, and the pellet washed three times and resuspended in sterile physiological saline solution. Bacterial suspensions were adjusted spectrophotometrically and the concentration of viable bacteria (5.7×10^7 /ml) was determined by standard plate counting techniques. Aliquots of bacterial suspension were stored at -70°C. Immediately prior to use the aliquots were thawed and stored on ice for use in immunisation, challenge and LDCL assays.

Immune plasma

Two clinically healthy mature American Quarterhorse geldings (968 and 970) were hyperimmunised to *R. equi* by repeated inoculations of the bacterial suspension as follows: 1) Day 1 - both animals received 10 ml, iv, 2) Day 14 - both animals received 10 ml subcutaneously (sc), 3) Day 28 - Horse 968 received 5 ml iv and 5 ml sc and Horse 970 received 10 ml iv, 4) Day 56 - both animals received 10 ml iv.

Plasma was harvested 28 days after final inoculation. Six litres of heparinised blood (six USP units/ml whole blood) were collected from each horse in 2 litre Blood-Pack plasma transfer bags (Travenol Laboratories, Illinois) and erythrocytes sedimented by gravity at 4°C for 24 h. Plasma was collected aseptically, and contaminating erythrocytes removed by centrifugation. Purified plasma was tested for bacterial contamination and stored at -20°C.

Bacterial challenge

At seven days of age all foals were restrained with 0.5 mg/kg bwt xylazine (Rompun; Haver Lockhart, Kansas) and 25 to 50 mg/kg bwt guaifenesin, USP 10 per cent (Life Science Products, Missouri). They were positioned in left lateral recumbency and a flexible plastic tube (Tygon; Norton, Ohio) with wire stylet was passed through the mouth and wedged in a distal bronchus. Foals were then positioned in sternal recumbency and 2.5 ml of *R. equi* suspension were aerosolised through the bronchial catheter. The aerosol was generated with 0, (9 litres/min) through an air-jet nebuliser (Cadema Medical Products, New York), which delivers particles 1 to 5 µm in diameter.

Clinical observation and sample collection

Rectal temperatures were obtained daily, between 08:00 h and 10:00 h, from two to four days pre-bacterial challenge until two to four weeks post infection when they either became terminally ill or afebrile. Temperatures above 39.4°C were considered elevated. Complete blood counts and fibrinogen concentrations were determined before *R. equi* exposure (Day 0), weekly for three to five weeks, and at three months or prior to death in non-surviving foals.

Tracheobronchial secretions were obtained for bacterial culture, by transtracheal aspiration (Mansman and Knight 1972), just prior to euthanasia in foals with severe respiratory disease or after the febrile response in surviving foals. Identification of *R. equi* was based on morphological, staining and biochemical characteristics (Barton and Hughes 1980).

Lateral and ventro-dorsal thoracic radiographs were obtained, as described by Martens, Fiske and Renshaw (1982), three weeks post infection and sequentially until the study was terminated. Pulmonary perfusion scans were obtained on all foals, except Nos 62 and 69, sequentially from three weeks post infection until the study was terminated, as previously reported (Shearer, Martens, Hightower and Renshaw 1983). Albumin macroaggregates labelled with technetium^{99m} (Pulmolite; Dupont Medical Products, Massachusetts) were injected into the jugular vein. Approximately 3×10^5 macroaggregate particles containing 2 to 3

mCi were used, and pulmonary capillary distribution was monitored with a scintillation camera (Sigma 410, Ohio Nuclear, Inc., Ohio).

Luminol-dependent chemiluminescence assay

The chemiluminescent response of adult equine polymorphonuclear leucocytes (PMNL) to *R. equi* in the presence of experimental foal sera, was determined as described by Martens *et al* (1987). Samples were assayed in triplicate at 10 min intervals over a period of 2 h and the LDCL response expressed as mean counts per min (cpm).

ELISA assay

Sera were evaluated for *R. equi*-specific antibody by ELISA using capsular antigen (Hietala *et al* 1985), and reported as percentage of positive control serum activity.

Haemolysis inhibition assay

Sera were evaluated for the presence of equi factor neutralising (EFN) antibody by a haemolysis inhibition assay (HI), as previously described (Eugster, Whitford and Angulo 1986). A positive antibody response was characterised by the absence of haemolysis.

Post mortem examination

All organs were examined grossly and representative samples of the major organ systems, including both lungs, and abnormal tissues were fixed in 10 per cent buffered neutral formalin. The fixed tissues were embedded in paraffin, sectioned at 10 μ m and stained with haematoxylin and eosin. Sections of lung and bronchial lymph node were also stained with the Brown and Brenn modification of Gram's stain for bacteria.

Statistical analysis

Student's *t* test was used to analyse variations in haematological values and body temperatures before and after *R. equi* infection. Fisher's exact test was used to analyse variations in incidence of death between control and principal animals. The Statistical Analysis System, using repeated measure analysis of variance (PROC GLM) was used to analyse variations in serological assays before and after treatment with LRS or immune plasma (Anon 1985).

Results

The incidence of death in control animals was significantly ($P < 0.01$) greater than in principal animals (Table 1). All principal foals developed pneumonic lesions, but recovered and were healthy three months post infection when all but Foal 612 were destroyed (Table 1). Foal 612 was clinically healthy when it was destroyed and subjected to post mortem examination one year post infection. The systemic response to disease was more severe and of longer duration in control animals. Eighty-three per cent (5 of 6) of the control animals were destroyed and necropsied approximately three weeks (range 17 to 25 days) post infection because they were in the terminal stages of disease. Control Foal 67 developed pneumonia but recovered spontaneously (Table 1).

There was no significant ($P > 0.05$) difference in temperature response between control and principal groups before or after *R. equi* challenge. Eighty-three per cent (5 of 6) of control foals developed a fever ($>39.4^{\circ}\text{C}$) post infection; whereas only 50 per cent (3 of 6) of principal foals became febrile. Control foals had a mean peak temperature of 40.4°C (range 39.1 to 41.3°C) and principal foals had a mean peak temperature of 39.5°C (range 38.7 to 40.4°C). The mean duration of fever in the five febrile

control foals was 5.2 days (range 4 to 8 days); whereas the mean duration of fever in the three febrile principal foals was 7.3 days (range 3 to 15 days). The control group had a mean time at onset of fever of 14.8 days post infection (range 13 to 19 days), and peak fever was attained at a mean time of 19.0 days post infection (range 15 to 22 days). The principal group had a mean time at onset of fever of 16.0 days post infection (range 13 to 19 days), and peak fever was attained at a mean time of 19.7 days post infection (range 16 to 23 days). Five of the six control foals had fevers at the time of euthanasia whereas principal foals and one control foal were monitored throughout the course of disease and their temperatures returned to normal values.

There was no significant ($P > 0.05$) difference in mean peak WBC or PMNL counts between control and principal groups before or after *R. equi* infection. Control foals consistently had lower peak WBC and PMNL in peripheral blood. The control group had a mean peak WBC count of $17.5 \times 10^3/\mu\text{l}$ (range 11.3 to $36.0 \times 10^3/\mu\text{l}$) and a mean peak PMNL count of $12.7 \times 10^3/\mu\text{l}$ (range 6.5 to $28.0 \times 10^3/\mu\text{l}$); whereas the principal group had a mean peak WBC count of $26.5 \times 10^3/\mu\text{l}$ (range 18.9 to $37.2 \times 10^3/\mu\text{l}$) and mean peak PMNL count of $19.6 \times 10^3/\mu\text{l}$ (range 11.2 to $32.0 \times 10^3/\mu\text{l}$). Peak WBC and PMNL counts were attained two to four weeks post infection in both groups. No immature PMNL were observed in peripheral blood from any of the experimental animals.

All experimental animals, except control Foal 66, developed hyperfibrinogenaemia after infection. The mean peak serum fibrinogen concentrations in control and principal groups were 7 g/litre (range 2 to 11 g/litre) and 6.2 g/litre (range 5 to 8 g/litre), respectively. There was no significant ($P > 0.05$) difference between groups, and no direct correlation between fibrinogen concentrations and severity of disease.

Radiographs

All foals had evidence of consolidation in the caudal aspect of one lung at three weeks post infection (Table 1). Foals 62 and 63 had moderate lesions characterised by patchy infiltration with air bronchograms (Fig 1) and the remainder had severe lesions characterised by solid opacities with air bronchograms (Fig 2). These aberrations decreased appreciably or disappeared in all surviving foals; however, a major defect remained in principal Foal 612. Foal 611 had a severe lesion involving the distal third of one lung at three weeks post infection. At five weeks post infection. Two weeks later the lesion was markedly improved, and at three months post infection there was no radiographic

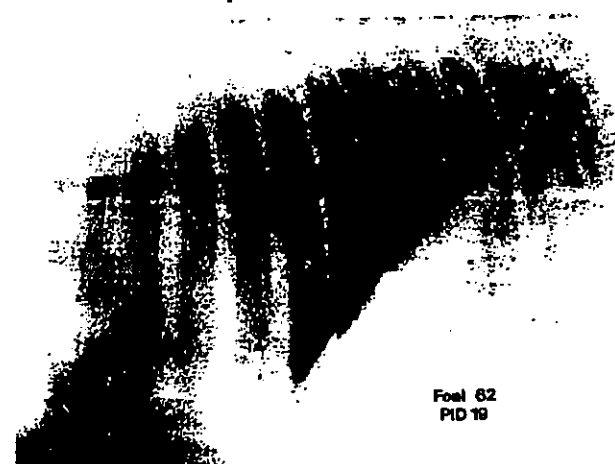


Fig 1: Lateral thoracic radiograph of principal (Group II) Foal 62 on post infection day (PID) 19. Note the diffuse patchy infiltration present throughout the caudal aspect of the lung. This is characteristic of morphological changes present in 33 per cent of immune plasma treated foals after infection with *R. equi*.

equi. This is the first report of consistently effective *R. equi* experimental infection being used to evaluate prophylaxis or therapy and the first report of immunotherapeutic protection against *R. equi* pneumonia.

Although the role of humoral and cell-mediated immunity in preventing certain perinatal equine infections is well understood, the nature of acquired or innate resistance to *R. equi* pneumonia is unclear (Ellisalde, Renshaw and Waldberg 1980; Ellenberger and Genetzky 1986; Woolcock, Mutimer and Bowles 1987). Alveolar macrophages (AM) and PMNL, phagocytic effector cells which co-operate with the humoral and CMI systems, are both present in *R. equi* induced pyogranulomatous lung lesions, with AM predominating in the advanced lesion. Considerable experimental evidence (Ellisalde *et al* 1980; Ellenberger and Genetzky 1986; Woolcock *et al* 1987) suggests that the cell-mediated arm of the immune system is primarily responsible for the elimination of *R. equi* and that macrophages are the primary effector cell involved. Recent investigations have focused attention on the interactions between specific humoral factors, phagocytic cells and *R. equi* (Hietala and Ardans 1987a; Martens *et al* 1987; Yager *et al* 1986; Yager, Duder, Prescott and Zink 1987; Martens, Martens and Renshaw 1988). Hietala and Ardans (1987a), demonstrated that AM from foals sensitised to *R. equi* have a greater capacity to kill *R. equi* than those from non-sensitised foals, and that AM from sensitised foals kill significantly more *R. equi* which have been opsonised with specific antibody. In addition, lymphocytic factors, derived from peripheral blood lymphocytes incubated with *R. equi* surface antigens, enhance bactericidal activity of AM from sensitised and non-sensitised foals (Hietala and Ardans 1987a). Other investigators (Martens *et al* 1987) have demonstrated the presence and capacity of specific opsonising antibody to enhance *R. equi* killing by equine PMNL. Pre-sensitisation of the PMNL donor is not necessary to obtain maximal killing, but the PMNL from certain neonatal foals may be functionally immature and unable to effectively eliminate *R. equi* (Martens *et al* 1988). Previous observations, using the experimental infection protocol described in this study and others (Martens *et al* 1982; Martens, Renshaw and Fiske 1983) indicate that foals under two weeks of age are more susceptible to experimental *R. equi* pneumonia. This corroborates information known about other foal diseases and indicates the importance of specifically activating the immune system within the first few days of life. Although passive transfer of humoral immunity is relied upon to protect foals from many neonatal diseases (McGuire, Crawford, Hallowell and Macomber 1977) there are no reports of its prophylactic use against *R. equi*. There are, however, two anecdotal reports about the beneficial effect of serum or plasma for the treatment of *R. equi* foal pneumonia (Magnusson 1938; Henry 1979).

Although passive immunisation can be accomplished by administration of colostrum, gamma globulin, plasma or serum from sensitised donors, plasma and serum contain the largest array of potential protectants. In addition to specific opsonising antibody (Martens *et al* 1987; Hietala and Ardans 1987a; Hietala and Ardans 1987b) and neutralising antibody (Prescott *et al* 1984; Skalka and Svastova 1984/85; Eugster *et al* 1986), plasma and serum may contain non-specific factors such as fibronectin, complement components, interferons, lymphokines, monokines and others.

Plasma donors were immunised with live *R. equi* to ensure exposure to all capsular, somatic and exosubstance antigens. Donor animals did not manifest adverse systemic responses to iv or sc inoculations, but pyogenic responses did develop at the sites of sc injection.

All experimental animals developed pneumonia following intrabronchial aerosolisation of *R. equi*; however, the clinical course of disease was dramatically different between groups. Five of the control foals were destroyed at approximately three weeks

post infection because they were in the terminal stage of disease, whereas control Foal 67 recovered spontaneously. All of the principal foals were clinically healthy when the study was terminated.

There was no significant difference in mean peak temperature or duration of fever between groups. These data, however, may be somewhat misleading because 17 per cent of control foals and 50 per cent of principal foals did not develop a fever. Additionally, five of six control foals were febrile when they died, and temperature of principal animals returned to normal values. Blood WBC and PMNL counts were consistently lower in control foals, and the peak responses of both groups occurred two to four weeks post infection. Control foals' leucocytes may have been consumed by advancing lesions or their production may have been suppressed by toxic factors. Principal foals' leucocytic responses may have been enhanced by factors in the immune plasma, or toxins neutralised by plasma components.

Radiographic and perfusion scan evidence of lung disease was most advanced in all foals at approximately three weeks post infection, as previously described by Shearer *et al* (1983). The clinical, morphologic and functional signs of pneumonia progressively increased in all control animals except Foal 67, whereas, they decreased in all principal foals. By three months post infection, pulmonary radiographs and perfusion scans indicated very minimal or no aberrations in control Foal 67 and all principal foals except Foal 612.

R. equi was not consistently cultured from foals which survived. A previous investigation (Martens *et al* 1982) indicated that ante mortem bacterial cultures do not consistently detect the presence of *R. equi* infection. Also, transracheal aspirates in the current study were not obtained from principal foals until after the febrile response had subsided. Although that was not the optimal time to obtain cultures, this sequence was deemed appropriate to prevent mechanical dissemination of bacteria. The clinical progression of Foal 611 was unique and warrants special consideration. As indicated, at three weeks post infection there was radiographic and perfusion scan evidence of severe and extensive lung damage; however, by five weeks post infection there was marked improvement indicating a rapidly resolving lesion (Fig. 3). Presumably, the observed defect was not caused by the degree of consolidation and abscess formation observed in non-surviving foals. The lesions, however, were indistinguishable by radiographic or pulmonary perfusion analysis. Foal 611 was clinically normal by three months post infection, and the only gross lesion was a small linear zone of atelectasis in one lung. This information is of vital importance for clinicians when assessing pulmonary disease and formulating a prognosis.

Foal 612, the most severely affected principal animal, recovered clinically but retained radiographic and perfusion scan evidence of extensive involvement in the caudal aspect of one lung. This animal was retained for one year to ascertain its long term response to infection. Although the growth rate of Foal 612 appeared to be depressed, no overt clinical signs of disease were observed after the initial febrile response. Post mortem examination at one year post infection revealed a resolving granulomatous lesion which did not contain bacteria.

There was no significant difference in serum IgG or IgM concentrations between control and principal groups after treatment with LRS or *R. equi* immune plasma respectively. There was, however, significantly more *R. equi* specific antibody in principal foals after treatment, as measured by LDCL, ELISA and HI. The LDCL assay correlates well with the *in vitro* bactericidal assay to evaluate serum for opsonising *R. equi* antibody (Martens *et al* 1987). Recently, ELISA assays were developed for the detection of *R. equi* antibody (Ellengerger *et al* 1984; Hietala *et al* 1985; Takai *et al* 1985). These investigators have demonstrated increased antibody in experimentally and naturally infected foals, and passive transfer of antibodies via colostrum:

however, it is not known if the measured antibody is protective.

The HI assay measured equi factor neutralising (EFN) antibody (Prescott *et al* 1984; Skalka and Svostova 1985; Eugster *et al* 1986). Equi factors are exosubstances, primarily cholesterol oxidase and phospholipase C, produced by *R. equi* (Prescott *et al* 1984). Previous studies suggested that EFN antibody may be passively transferred via colostrum (Eugster *et al* 1986; Skalka 1987). In the present investigation, only Foal 63 had EFN antibody prior to receiving immune plasma; whereas all principal foals had EFN antibody immediately after plasma transfusion and at three weeks post infection (Table 3). There was little or no EFN antibody in control foals throughout the study. Skalka (1987) determined that some foals do not develop EFN antibody naturally until the second month of age.

As expected, individual variations in serological responses occurred in the current study and were best exemplified by the responses of control Foals 66 and 67. The sera from Foal 66 induced very high LDCL and ELISA responses, but did not have EFN antibody. This foal was destroyed 25 days post infection because of terminal signs of disease. In contrast, sera from Foal 67 induced very low LDCL and ELISA responses, and also failed to neutralise EF. Foal 67 developed moderate signs of clinical disease and spontaneously recovered.

This investigation demonstrates the value of specific antibody; however, the contrasting responses in Foals 66 and 67 challenge the importance of specific antibody alone to clear *R. equi* infections. Foal 67 may have possessed a very efficient and effective mononuclear-phagocytic cell system that rapidly killed *R. equi* and cleared antigens from the body, resulting in low specific antibody production. Biozzi, Mouton, Stiffel and Bouthillier (1984) demonstrated this phenomenon in mice selectively bred for low specific antibody production.

Future studies should be directed toward identifying and characterising the humoral components which imparted protection to principal foals in this study. Additionally, the development, active stimulation and passive transfer of these components should be evaluated further for the prevention and treatment of *R. equi* pneumonia.

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