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Rhodococcus-equi specific hyperimmune plasma decreased rhodococcal pneumonia severity in newborn foals after experimental infection

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Rhodococcus equi is the most common cause of pneumonia in young foals. As a vaccine is not available, farms with an endemic problem rely in the use of *R. equi* specific hyperimmune plasma (HIP), although the efficacy of this practice remains uncertain. The objective of this study was to evaluate the ability of a commercially available HIP to prevent clinical rhodococcal pneumonia in neonatal foals after experimental challenge. Nine foals were given intravenous HIP after birth while 9 remained as controls. Within the first week of life, all foals received 10^3cfu/foal of pathogenic *R. equi* intratracheally as previously described (Sanz et al 2013). Thereafter, foals were monitored for 8 weeks and samples were collected during that period as described before (Sanz et al 2013). VapA-specific IgG and IgG subclasses in serum and in bronchoalveolar lavage fluid (BALF) were evaluated using ELISA (Sanz et al 2014). One foal in the HIP group and 4 in the control group developed clinical pneumonia; however, the power of the study was too low to detect a statistical significant effect of treatment. HIP foals had significantly lower weekly ultrasonographic scores ($p<0.05$, Figure 1), lower white blood cell counts ($p=0.03$), platelet counts ($p=0.01$) and fibrinogen concentration ($p=0.01$) than controls. Serum VapA-specific IgG, IgGa and IgGb were significantly higher in HIP foals and IgGa and IgG(T) significantly increased ($p<0.001$) over time only in control foals. VapA-specific IgG ($p=0.02$) and IgGb ($p=0.04$) were significantly higher in BALF of HIP foals. In this study, HIP administration decreased severity of pneumonia, which reduced the need for antimicrobial treatment. Antibodies present in HIP transferred to BALF of foals shortly after HIP administration. VapA-specific IgG(T), which increases with *R. equi* infection, was only elevated in control foals. In conclusion, while infection after challenge was not prevented by *R. equi*-specific hyperimmune plasma (HIP) administration, severity of clinical pneumonia decreased.

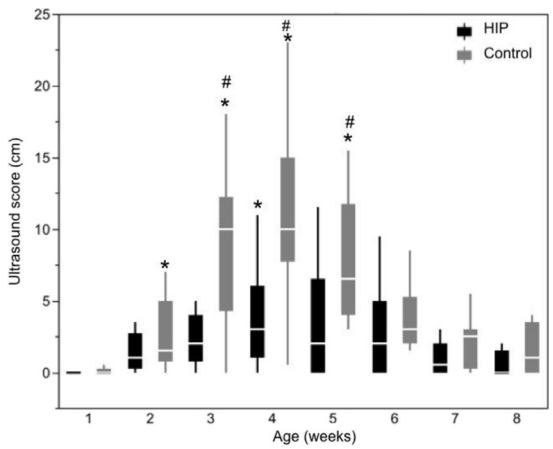

Figure 1. Thoracic ultrasound score (diameter, cm) of neonatal foals experimentally challenged with *R. equi* after administration of HIP (black) or nothing (gray). The horizontal line within the box represents the median and the ends of the box the 75th and 25th quantiles respectively. The asterisk (*) indicates that values significantly differ ($p<0.05$) from the pre-challenge (w1) value for each group. The hash (#) indicates that values significantly ($p<0.05$) differ between groups at a given time point.

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Variation of anti-*Rhodococcus equi* VapA specific IgGs among eleven different lots of one commercially available Rhodococcus equi specific hyperimmune plasma product

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Rhodococcus equi, a gram-positive facultative intracellular pathogen, is the most common cause of pneumonia in foals between 3 weeks and 5 months of age. To date, there is no vaccine available, and mass treatment of foals with antibiotics may incur in serious long-term effects and is strongly discouraged. Therefore, on farms with enzootic rhodococcal pneumonia, prophylaxis measures rely greatly on the administration of *R. equi* specific hyperimmune plasma (HIP). The efficacy of *R. equi* HIP in controlling disease due to either natural or induced infection has been historically controversial. Variability in plasma products may have played an important role. Recently, four commercial *R equi* HIP products were evaluated for their concentrations of Vap-A specific immunoglobulin G (IgG) and IgG subclasses. Marked variation was observed between different products, and different lots from the same product, which may have important clinical and financial consequences. In order to further investigate the amount of variation in a commercially available plasma product, and its relation with specific *R. equi* IgG in the recipient foals, a foal population from a local farm that routinely receives *R. equi* specific HIP due to enzootic *R. equi* pneumonia, was selected. Exclusion criteria for the foals were documented failure of passive transfer, and clinical disease other than pneumonia. All newborn foals received 1 liter of intravenous *R. equi* HIP[1] within 24 hours after being born. HIP samples (n =)were collected at administration time. Serum samples were collected from all healthy foals (n =) and their respective dams (n =) within 24 and 48 hours after HIP administration. All samples were archived in -20C° for batch analysis with previously validated ELISA for equine anti-VapA total IgG and IgG(a), IgG(b), and IgG(t). Eleven different HIP lots

were sampled. There was a marked intra and inter-lot variation of all anti-VapA specific IgG concentrations (Table 1). When anti-VapA specific IgG concentrations were compared between the dam's serum, *R. equi* HIP, and foal's serum, either weak positive or negative linear correlations were observed ($-0.15 < r < 0.20$), with the exception of IgG(t), which showed a moderate positive linear correlation between *R. equi* specific HIP, and foal's serum ($r = 0.71$). Our results revealed an even greater variation in anti-VapA specific IgG concentrations within and among the different lots of the selected *R. equi* HIP product, than previously reported. This finding may corroborate in explaining historical disparities regarding *R. equi* specific HIP efficacy in preventing *R. equi* pneumonia in foals. The lack of a positive correlation between the anti-VapA specific IgG concentrations between the dams, HIP and the recipient foals is not fully understood. However, possible explanations include suboptimal time sampling of the dams, low anti-VapA specific IgG concentrations in adult horses, and unknown antibody kinetics of the anti-VapA specific IgGs in the recipient foal. Recently, it has been reported that after intravenous administration of *R. equi* HIP, the concentrations of anti-VapA specific IgGs in the bronchoalveolar fluid increases, suggesting compartmentalization of these antibodies outside the systemic circulation. The moderate positive correlation observed between the concentrations of IgG(t) between the *R. equi* HIP and the recipient foals was likely due to its high concentration in the HIP product. Serial measurements of the foal's serum concentrations of anti-VapA specific IgGs is warranted in order to evaluate their relationship with clinical disease, and therefore the efficacy of this commercially available *R. equi* HIP in preventing naturally acquired pneumonia in foals.

Table 1
Intra- and inter-lot coefficient of variation (CV) of anti-VapA specific IgG concentrations in different lots of *R. equi* HIP[1].

	CV(%)			
Lot #	IgG total	IgGa	IgGb	IgGt
1	49.39	51.74	54.50	32.79
2	45.02	57.73	32.89	28.15
3	58.52	63.38	55.71	39.15
4	57.81	65.87	59.07	58.03
5*	-	-	-	-
6	39.18	55.54	46.33	47.53
7	60.43	61.40	59.22	58.09
8	70.83	91.88	79.37	31.83
9	51.96	56.79	71.16	52.33
10*	-	-	-	-
11*	-	-	-	-
All lots	60.88	62.70	87.63	90.97

* Only one HIP bag was sampled.

Reference

[1] ImmunoGlo, MgBiologics, Aimes, Iowa, USA.

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Antimicrobial susceptibility of Australian virulent *Rhodococcus equi* isolates collected between 1991 and 2014

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Bronchopneumonia caused by *Rhodococcus equi* is an important and often debilitating disease of young horses throughout the world. Advances in diagnostic methods for the early detection of lung lesions, such as ultrasonographic imaging, have been associated with an increase in the use of antimicrobial agents for the treatment of this disease. Concurrent with this increased usage, reports of antimicrobial resistance in *R. equi* have become more common in recent years. The aim of this study was to determine the level of resistance to three commonly prescribed antimicrobial agents in 97 virulent *Rhodococcus equi* isolates collected from infected foals between 1991 and 2014. The second objective was to determine the utility of a novel assay for determining the minimum inhibitory concentration (MIC) of *R. equi* isolates with a single agent, or when rifampicin and erythromycin were combined. Three cohorts of virulent *Rhodococcus equi* isolates were included in the study. Cohort 1 contained 29 isolates collected from clinical cases between 1991 and 1998 and Cohort 2 consisted of 12 recent isolates collected between 2011 and 2014. Fifty six isolates from clinically affected foals undergoing antimicrobial therapy in 2006 - 2007 on an endemically infected farm formed Cohort 3. The MICs for rifampicin, erythromycin and neomycin were determined for all of the isolates using a novel microtitre assay. Rifampicin resistance was detected in 3 of the 12 isolates in Cohort 2. The MIC was 64 μg/mL for two of the isolates and 16 μg/mL for the third, well above the threshold of 8 μg/mL that defines resistance to this agent. All isolates collected prior to 2013 had MICs less than 0.125 μg/mL (limit of detection). None of the isolates were resistant to either neomycin or erythromycin, with MIC values ranging from 0.25 - 2 μg/mL for neomycin and 0.125 - 1 μg/mL for erythromycin. When rifampicin and erythromycin were tested in combination, the MIC value for the isolates resistant to rifampicin were lower, compared to exposure to rifampicin alone. This is the first report of rifampicin resistance in virulent *R. equi* isolated from Australian foals. Sanger sequencing of a portion of the RNA polymerase β subunit was conducted to further characterise the resistant isolates from Cohort 2 [1], [2]. The current therapeutic success of the macrolide - rifampicin combination regimen is thought to rely on the synergistic action of these two agents *in vivo*. It is not clear from these results whether the rifampicin resistance we have detected has any significant impact on synergy *in vivo*.

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Virulent *Rhodococcus equi* isolates from foals in Poland – pVapA characteristics and plasmid new variant, 85-kb type V

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