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Original article

PRODUCTION OF *RHODOCOCCUS EQUI*-SPECIFIC HORSE POLYCLONAL ANTIBODIES AND EVALUATION OF THEIR THERAPEUTIC EFFICACY AND PROPHYLACTIC POTENTIALS IN A MOUSE MODEL

R. SOLIMAN[1], SH. ABD ELBAKI[1], M. YOUSSEF[2], G. ABDELMALAK[3] & H. ABOUL-ELLA[1]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; [2]Department of Veterinary Hygiene, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; [3]VACSERA Holding Company, Giza, Egypt

Summary

Soliman, R., Sh. Abd Elbaki, M. Youssef, G. Abdelmalak & H. Aboul-Ella, 2024. Production of *Rhodococcus equi*-specific horse polyclonal antibodies and evaluation of their therapeutic efficacy and prophylactic potentials in a mouse model. *Bulg. J. Vet. Med.* (online first).

Rhodococcus equi (*R. equi*), a Gram-positive facultative intracellular pathogen, is the most widely recognised cause of pneumonia in foals. Several antimicrobial drugs are effective against *R. equi in vitro*, however, many of these agents are ineffective *in vivo* because *R. equi* is a facultative intracellular pathogen that can survive and replicate in macrophages. The present study was planned to prepare *R. equi*-specific hyper-immune horse polyclonal antibodies and to evaluate their therapeutic and prophylactic efficacy against *R. equi*-specific infections in mice models. Four horses were immunised with a locally prepared formalin-inactivated *R. equi* vaccine adjuvanted with mineral oil adjuvant. Each horse was immunised with 3 doses of the prepared vaccine (3 mL/dose) at 2-week intervals. One week after the last vaccination dose, serum was collected by plasmapheresis. The titre of *R. equi*-specific antibodies was determined by ELISA and reached up to 10^4 ELISA units/mL two weeks after the primary immunisation and up to 10^5 ELISA units/mL one week after the 2nd immunisation dose. The therapeutic and prophylactic efficacy of the prepared *R. equi*-specific antibodies was evaluated in a mice model, where 87.5% of mice challenged with virulent *R. equi* bacteria (0.2 mL/mice of 4×10^8 CFU/mL) were successfully treated just after the appearance of the clinical signs of the infection by I.V. injection of 0.2 mL of *R. equi*-specific polyclonal antibodies. Also, the prophylactic efficacy of the prepared *R. equi*-specific antibodies for induction of immediate protection was ensured where 100% of passively immunised mice could resist challenges with virulent *R. equi* bacteria. The *R. equi*-specific polyclonal antibodies titre used to evaluate its protective and prophylactic efficacy was 10^5 ELISA units/mL.

Key words: hyper-immune serum from foals, mouse experimental model, *Rhodococcus equi*-specific-pAbs

INTRODUCTION

R. equi is a Gram-positive, non-motile, aerobic, intracellular microorganism. It causes pyogranulomatous bronchopneumonia in foals from 1 to 6 months of age (Sanz, 2023). *R. equi*-infected foals may develop extra-pulmonary illness including septic arthritis, osteomyelitis, ulcerative enterocolitis, mesenteric lymphadenopathy, neonatal diarrhoea, and sudden death (Coleman *et al.*, 2019; Bordin *et al.*, 2021). Immunosuppressed individuals, particularly AIDS patients, are also susceptible to *R. equi* infection (Meijer & Prescott, 2004). This bacterial species is present in soil and horse faeces. Foals are thought to be infected when they ingest or take in soil, dust, or faecal particles harbouring this pathogen (Vázquez-Boland & Meijer, 2019). Inhalation of aerosolised virulent *R. equi* and its intracellular replication inside alveolar macrophages is the basic pathogenetic pathway of *R. equi* pneumonia in foals (Leclere *et al.*, 2011). Virulence of *R. equi* in foals is found to be associated with the presence of 80–90 kb plasmids that encode the 15–17 kDa lipoprotein "virulence-associated protein A" (vapA) (Sanz, 2023). *R. equi* pneumonia has a great impact on the equine industry. It causes financial losses through the cost of therapy and the death of foals (Chaffin *et al.*, 2011). Also, foals recovered from the disease are less likely to race as adults. Treatment with long-term antibiotics does not lead to full recovery (Leclere *et al.*, 2011). Although several antimicrobials are effective against *R. equi in vitro*, these agents are ineffective *in vivo* presumably because the pathogen is a facultative intracellular organism that can survive and replicate in macrophages causing pyogranulomatous lesions (Lin *et al.*, 2019).

Due to the epitheliochorial placenta of equines, foals must acquire all maternal antibodies from the ingestion of colostrum (Perkins *et al.*, 2014). Ingestion of colostrum from hyper-immunised mares is associated with protection against *R. equi* in foals, which are normally hypogammaglobinaemic at birth (Sanz *et al.*, 2014). Foals become susceptible to infection or infected when maternal antibody concentrations wane (Sanz *et al.*, 2016). Immunisation of horses has been recommended as a promising confrontation approach by several studies (Leclere *et al.*, 2011; Sanz *et al.*, 2014). However, due to the presence of maternal antibodies and the immaturity of the foal's immune system, vaccination of young foals shows variable results (Kang *et al.*, 2023), yet none of the control strategies to prevent *R. equi* infection has proven to be completely successful. Currently, antibodies are very promising candidates that are widely utilised, and incorporated in a variety and wide range of detection and infectious diseases' diagnostic (Hemeda *et al.,* 2022; Aboul-Ella *et al.,* 2023; Hashem *et al.,* 2023; Soliman *et al.,* 2023), prophylactic (Lachmann, 2012), and therapeutic (Wang *et al.,* 2022) protocols. Traditional hyper-immune plasma (HIP) treatment at present is among the demonstrated methods for the prevention of *R. equi* pneumonia in foals, especially in case of failure of passive transfer of maternal immunity (Kahn *et al.*, 2021).

The present work represents a promising trial to evaluate the therapeutic and prophylactic potential of *R. equi*-specific horse polyvalent antibodies against *R. equi* infection using a mouse model.

MATERIALS AND METHODS

Ethical approval

The currently conducted study is reported following the ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines). The guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Cairo University were completely followed during the procedures involving animal use through the current conducted study with approval code Vet CU 08072023717.

Bacteriological and molecular characterisation of R. equi strain

Reference bacterial strain: R. equi reference strain (ATCC 33701) was used for the preparation of the inactivated *R. equi* vaccine.

Cultivation and preservation of R. equi strain: The following culture media were used for culturing and preservation of *R. equi* strain; Blood agar base medium (OXOID, UK), Brain heart infusion agar medium (OXOID, UK), and Brain heart infusion broth (OXOID, UK).

Media used for biochemical identification of R. equi: It included nitrate reduction medium (HIMEDIA, India), nutrient gelatin (HIMEDIA, India), sugar fermentation (HIMEDIA, India), and urea agar base dehydrated medium (HIMEDIA, India).

Preparation of R. equi antigen mass

R. equi strain was cultured on brain-heart infusion (BHI) broth (OXOID, UK) supplemented with 1% yeast extract (OXOID, UK) at 37°C for 48h. The bacterial pellet was harvested by centrifugation at 6000×g and washed three times with sterile PBS (pH 7.2). The bacterial concentration was adjusted to 5×10^9 CFU/dose in 3 mL. The bacterial mass was then inactivated using formalin 0.5% (v/v). Finally, the inactivated bacterin was homogenised and adjuvanted with mineral oil adjuvant to contain 5×10^9 CFU of *R. equi*/dose (Aguilar & Rodríguez, 2007).

Preparation of R. equi-specific polyclonal antibodies (pAbs) in horses

Immunised animals: Four adult horses (400–450 kg body mass) maintained in a special animal house at the Egyptian Company for Production of Vaccines, Sera, and Drugs (VACSERA), animal farm, Helwan City, were used to produce *R. equi*-specific hyper-immune serum. These horses were routinely vaccinated against the most common equine infectious diseases.

Immunisation protocol: The locally prepared formalin-inactivated *R. equi* vaccine adjuvanted with mineral oil was used to immunise the 4 horses for preparation of the *R. equi*-specific hyper-immune serum. The immunisation protocol included subcutaneous injection of 3 doses of the prepared vaccine (3 mL each) at 2-week intervals. One week after the last vaccine dose, serum was collected by plasmapheresis and the titre of *R. equi*-specific antibodies was measured using enzyme-linked immunosorbent assay (ELISA) (Hanly *et al.*, 1995).

Separation and purification of the R. equi-specific pAbs: Briefly, the separation, and purification of immunoglobulins from the immunised horses included diluting the collected plasma with 2 volumes of saline and adjusting the pH to 3.3 using 2N HCl. Pepsin enzyme (pepsin A, EC 3.4.23.1, SIGMA Chemical, USA) was added at a final concentration of 1 g/L. The mixture was incubated on gentle stirring at 30 °C with an initial pH of 3.3 for 1 h, the pH was readjusted to 3.6 using

sodium hydroxide 2N (NaOH, HIMEDIA, India), the stirring continued for a further 30 min, and finally the pH was adjusted to 4.2 with 2N NaOH with continuous stirring for another for 30 min. Ammonium sulfate: $(NH_4)_2SO_4$ (HIMEDIA, India) and toluene (HIMEDIA, India) were added at a final concentration of 15% (w/v) and 1 mL/mL, respectively. The mixture was processed for 1 h at 56 °C under continuous stirring, then cooled below 40 °C and filtered through k300 depth filter sheets (SEITZ, Germany). The pH of the filtrate was increased to 7.2 with 2N (NaOH). $(NH_4)_2SO_4$ was added to a final concentration of 33% and the mixture was stirred overnight at 10 °C. The precipitate was collected, gauze filtered, and dissolved in physiological saline to the initial protein content. The pH was adjusted to 5.8 ± 0.1 using 1.76 N glacial acetic acid (SIGMA-ALDRICH, USA). Drop-wise addition of caprylic acid (SIGMA-ALDRICH, USA) was done to a final concentration of 0.5% and the mixture was maintained under vigorous stirring for 1hr at 18 °C. After centrifugation at 1550 xg for 30 min the mixture was sterilised by filtration through 0.22 mm filter sheets (WHATMAN type 1) (Bergmann-Leitner *et al.*, 2008).

Titration of the R. equi-specific horse pAbs using ELISA

ELISA plates were coated with 25 µL/well *R. equi* antigen. The plates were incubated for two hours at 37 °C. The plates were then washed with phosphate-buffered saline (PBS, HIMEDIA, India), pH 7.2 to remove unbound antigen molecules. A blocking solution containing 1% bovine serum albumin (BSA, SERVA, Germany) was added (50 µL/well) to block uncoated sites in the wells of the microtitre plates (Hietala *et al.*, 1985). The plates were incubated for one hour at 37 °C and then washed with PBS, pH 7.2. One row of the used microtitre plate was used for titration of each tested sample. Twenty-five µL of tested serum samples were added to the first well of the row. Double-fold serial dilution was made for each sample using PBS, pH 7.2. After 1 h incubation at 37 °C, the plates were washed 3 times using PBS, pH 7.2. Fifty µL of the anti-horse immunoglobulin antibody-horse radish peroxidase (HRP, SIGMA-ALDRICH, USA) conjugate 1:200,000 (SIGMA-ALDRICH, USA) was added to each well. After 1 h of incubation at 37 °C, the plates were washed 3 times using PBS, pH 7.2, 100 µL of the substrate (TMB, SIGMA-ALDRICH, USA) was added to wells and the plates were incubated at 37 °C for 20 min. Finally, 50 µL/well of the stop solution was added and the OD was measured at 450 nm. Serum from newly born foals was tested in parallel as a negative control. All samples and controls were applied in duplicates.

Development of R. equi infection in a mouse model

Animals: Six- to eight-week-old female BALB/c mice were obtained from the Animal Facility of the Egyptian Company for Production of Vaccines, Sera, and Drugs (VACSERA). This mouse strain was chosen due to previous preliminary experiments with other mouse strains showing considerably increased *R. equi* lung clearance which would adversely affect the *R. equi* infection establishment (González-Iglesias *et al.*, 2014).

Mouse infection and determination of bacterial burdens in organs: Fresh *R. equi* cell suspensions were used for the infections. To ensure purity, frozen stocks of the test bacteria were cultivated on BHI agar, and an *R. equi* colony was used to

seed a pre-inoculum in 10 mL BHI broth. Pre-inoculum was diluted 1/10 in fresh BHI and incubated at 37 °C with 200 rpm rotary shaking until 1.0 optical density at 600 nm (corresponding to 4×10^8 *R. equi* CFU/mL) after overnight incubation at 30 °C. Twice centrifuged bacteria in PBS for 10 min at 4 °C were resuspended to the desired CFU concentration. In each experiment, the actual infection dosages (and inoculum purity) were calculated using plate counting. For intranasal infections, mice were fixed in a 45° supine position, and 20 μL of the bacterial suspension was injected into the external nares using a micropipette with a plastic 30 μL filter tip. It has been previously demonstrated that the intranasal instillation technique and volume employed enabled an appropriate dispersion of drugs into the lower respiratory tract of mice (Southam *et al.*, 2002). One hundred μL of bacterial suspension was injected into the lateral tail vein for infections. Mice were sacrificed, their organs were aseptically removed and were mechanically dispersed in 2 mL PBS by pressing through nylon gauze at the designated time points following the challenge. Colonies were counted after 48 h of incubation at 37 °C of serially diluted tissue homogenates on BHI agar.

Evaluation of the antiserum therapeutic efficacy and prophylactic potential in a mouse model

Therapeutic efficacy was evaluated as follows (Takai *et al.*, 1991) with 12 mice. The first 4 mice were kept as unchallenged treated, negative controls, being injected only with the prepared *R. equi* antiserum. Mice of the second group (8 mice) were challenged with virulent *R. equi* bacteria (0.2 mL/mice containing 4×10^8 CFU/mL). Once the clinical signs of infection developed the diseased mice were treated I.V. with 0.5 mL of the prepared *R. equi* specific antibodies (titre = 10^5 ELISA unit/mL). The protective potentials were evaluated with eight mice: the first 4 mice were kept as untreated controls, and the other 4 mice were injected I.V. with 0.5 mL of the prepared *R. equi*-specific antibodies. Then all mice in both groups were challenged with 0.2 mL/mice of 4×10^8 CFU/mL virulent *R. equi* bacteria. The 8 mice were kept under observation and results were recorded.

RESULTS

Measuring the bacterial burdens in mouse lungs, liver, and spleen after acute systemic I.V. infection is the most widely used method to assess *R. equi* virulence or immune protection. *R. equi* counts in the lungs were 3.3×10^6 CFU. This indicated that a significant part of the intranasal inoculum reached the lower respiratory tract. Bacterial burdens in mice were nearly $1.6–2.5 \times 10^2$ CFU in the liver and the spleen: much lower than those found in the lungs.

The *R. equi*-specific antibody titre was measured in the immunised horses 2 weeks after the first primary vaccination dose and after one week from the second immunisation dose. As shown in Table 1, two weeks after the first primary vaccination dose two horses (1 and 3) showed a titre of *R. equi*-specific antibody of 10^3 ELISA units/mL. The *R. equi*-specific antibody titre reached 10^4 ELISA units/mL one week after the second immunisation dose. In horse (2), the antibody titres reached 10^5 ELISA units/mL one week after the second immunisation dose. Horse (4), however, was a weak responder where an antibody titre of 10^3

ELISA units/mL was recorded after the second immunisation dose.

No deaths were recorded in the mice that were passively immunised with *R. equi*-specific polyclonal horse antibodies when challenged with virulent *R. equi* bacteria. These mice manifested no clinical signs of infection. All control mice challenged with non-prophylactic doses died within 24–48 h.

As shown in Table 2, four out of five *R. equi*-infected mice showed complete recovery from the infection with the disappearance of the clinical signs after the therapeutic intervention with the *R. equi*-specific polyclonal antibodies adminis-

Table 1. Optical density reading of different serum dilutions from horses immunised with *R. equi* inactivated mineral oil adjuvanted vaccine

Immunised horses	Time of serum collection	Optical density reading of different serum dilutions							
		10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8
Non-immunised horse	Negative control	0.156	0.156	0.159	0.165	0.168	0.169	0.178	–
Horse (1)	Primary dose	0.251	0.229	0.241	0.161	0.159	0.156	0.144	0.143
	Secondary dose	0.245	0.249	0.206	0.176	0.163	0.162	0.142	0.143
Horse (2)	Primary dose	0.261	0.231	0.239	0.191	0.163	0.161	0.118	0.152
	Secondary dose	0.247	0.266	0.257	0.221	0.218	0.161	0.160	0.136
Horse (3)	Primary dose	0.214	0.276	0.285	0.160	0.159	0.163	0.158	0.147
	Secondary dose	0.231	0.269	0.245	0.213	0.161	0.155	0.151	0.137
Horse (4)	Primary dose	0.212	0.214	0.162	0.159	0.140	0.143	0.148	0.128
	Secondary dose	0.234	0.224	0.206	0.163	0.162	0.160	0.148	0.141

Table 2. Therapeutic potential of the *R. equi*-specific polyclonal antibodies

	Challenge of mice with virulent *R. equi* (0.2 mL/mice of 10^7 CFU/mL)	Therapeutic i.v. injection of 0.5 mL *R. equi*-specific polyclonal antibodies
Mouse (1)	Showed clinical signs	Recovered
Mouse (2)	Showed clinical signs	Recovered
Mouse (3)	Showed clinical signs	Died
Mouse (4)	Showed clinical signs	Recovered
Mouse (5)	Showed clinical signs	Recovered
Mouse (6)	No clinical signs	Remained healthy
Mouse (7)	No clinical signs	Remained healthy
Mouse (8)	No clinical signs	Remained healthy

tered 48 h after the appearance of the clinical signs of the infection.

DISCUSSION

Using horses for *in vivo* research with *R. equi* is financially and logistically challenging, and an acceptable surrogate *in vivo* model is required to investigate the pathobiology of this illness. Measuring the bacterial burdens in mouse liver and spleen after an acute systemic I.V. infection is the most widely used method to assess *R. equi* virulence or immune protection (Lopez *et al.*, 2008; Miranda-Casoluengo *et al.*, 2012). Surprisingly, the mouse pulmonary challenge has not been applied as often for respiratory pathogens. Comparable findings from different isolates of *R. equi* have only been obtained from two prior research studies (Yager *et al.*, 1991). While these studies did show differences in pulmonary clearance, it was not obvious if the respiratory challenge was adequate to assess the pathogenicity of *R. equi* because the strains were not isogenic and had variable virulence plasmid carriage status. The respiratory challenge's usefulness in *R. equi* experimental vaccination trials was also largely unexplored, except for one investigation (Phumoonna *et al.*, 2008) that used aerosol-infected mice. This information highlights the need to evaluate *R. equi* pathogenicity and vaccine-induced protection experimentally utilising both local (intranasal) and systematic (intravenous) infection models.

Although the precise cause of foal immunity to *R. equi* pneumonia is still unknown, it is likely dependent on both the antibody and cell-mediated components of the immune system. The partially protective impact of passively transferred anti-*R. equi* hyperimmune horse plasma provides the greatest evidence for the role of antibodies in protection against *R. equi*. Due to *R. equi*'s facultative intracellular nature, cell-mediated immune systems are believed to play a key role in infection resistance. Mice infections provide a substantial portion of the information regarding cell-mediated immunity to *R. equi* infections. Mice lacking NK cells or the complement component C5 do not have impaired lung clearance of pathogenic *R. equi* (Yager *et al.*, 1991). On the other hand, mice must have functioning T cells to eradicate pathogenic *R. equi* (Madarame *et al.*, 1997). Nevertheless, mice devoid of functional T lymphocytes can eliminate plasmid-cured variants from their lungs within a week of infection, indicating that innate defensive mechanisms are primarily responsible for the clearance of avirulent plasmid-negative strains in mice, rather than the need for functional lymphocytes (Madarame *et al.*, 1997). Adult horses are usually less susceptible to *R. equi* infections than foals. Immune adult horses have been utilised as a pertinent model to gain a better understanding of the reactions required for immunologic protection. The formation of *R. equi*-specific cytotoxic T lymphocytes (CTL), IFN-γ production, and lymphoproliferative responses to *R. equi* antigens are linked to the clearance of virulent *R. equi* in immunological adult horses (Patton *et al.*, 2004). Remarkably, the *R. equi*-specific CTLs are major histocompatibility complex (MHC) class I-unrestricted and seem to be able to identify distinct bacterial lipids from the cell wall (Harris *et al.*, 2010). As has been well documented in *M. tuberculosis*, the current theory is that these lipid antigens may be given to T lymphocytes via the CD1 pathway. Research is ongoing to determine how these

results in adult horses and mice apply to the foal (Pargass *et al.*, 2009).

Traditional HIP therapy is currently the only proven method for the prevention of *R. equi* in foals, especially those exhibiting passive antibody transfer failure (Sanz *et al.*, 2014). Administration of the HIP before infection is important, because of evidence that many foals become infected early in life. It is commonly recommended that foals receive a transfusion of at least 1 L of HIP no later than 2–4 weeks of age (Takai *et al.*, 2022). The onset of clinical signs of pneumonia was significantly delayed after the administration of HIP (Zimmermann and Curtis, 2019). Transfusion of HIP carries some risk to foals, both in terms of trauma that may occur during handling and adverse reactions to transfusions. The process is also time-consuming, labor-intensive, and expensive. In the present work, *R. equi*-specific antibodies have been prepared in horses. The antibodies were separated, and purified and their concentration was determined. The obtained results agreed with Dawson et al 2010, who observed a significant increase in IgG antibodies against the *R. equi* after vaccination (Dawson *et al.*, 2010). The prophylactic and therapeutic efficacy of these *R. equi*-specific antibodies was determined in a mouse model. There were no dead mice after passive immunisation and subsequent challenge with virulent *R. equi* as well as complete recovery and disappearance of the symptoms of *R. equi*-infected mice after treatment with *R. equi*-specific polyclonal antibodies. In contrast, Nordman *et al.* (1992) reported failure of passive immune transfer that was based on whole serum intraperitoneal administration, not intravenous pAbs injection as used in the current work. According to the currently obtained results, hyper-immune plasma containing *R. equi*-specific antibodies proved to have prophylactic and therapeutic efficacy during experimental *R. equi* infection. Several studies reported similar data (Gong *et al.*, 2016). However, other studies reported no protective efficacy of this form of hyper-immune plasma (Kahn *et al.*, 2023). Foals are the most susceptible to virulent organisms when maternal antibodies wane (von Bargen *et al.*, 2019). The passive transfer of antibodies plays an important role in the resistance to a variety of infectious agents. Due to the epitheliochorial placentation of equines, foals must obtain maternally derived antibodies by ingesting colostrum (Perkins *et al.*, 2014). The lowest level of antibody in foals appears from 1 to 6 months of age due to the combined effects of waning maternally derived antibodies and low antibody production (Gong *et al.*, 2016). Therefore, foals are most susceptible to *R. equi* pneumonia during this period.

Due to age-dependent susceptibility to *R. equi*, foals need to develop anti-*R. equi* immunity shortly after birth (Lopez *et al.*, 2008). *R. equi* lives in macrophages so it resists many antibiotics. Antibiotics-based therapy is prolonged, expensive, and possibly associated with adverse effects (Dawson *et al.*, 2010). Studies investigating the active immunisation of mares have yielded mixed results. Vaccination of mares has not proven protective against *R. equi* pneumonia in foals, despite a significant increase in colostrum-specific antibodies (Kahn *et al.*, 2021). Also, Varga *et al.* (1997) did not observe protection in foals against *R. equi* pneumonia after mare vaccination. Moreover, Giguère *et al.* (2011) reported that immunoglobulin in mares may not be transferred through colostrum. However, other researchers affirmed that passive antibody transfer

from ingested colostrum was associated with protection against *R. equi* in normally hyper-gamma-globulinemic foals at birth (Rocha *et al.*, 2016). Immunisation of pregnant mares with virulent *R. equi* and vapA protein antigen associated with a water-based nanoparticle adjuvant as a candidate vaccine developed a higher serum IgG and opsonic activity, which resulted in passive antibody-mediated protection of foals (Kahn *et al.*, 2021). The protective effect was associated with an increase in the opsonic capacity of leukocytes against virulent *R. equi* in foals from vaccinated mares (Leclere *et al.*, 2011). The first investigation showing the immune-prophylactic capacity of specific hyper-immune plasma in an experimental model of *R. equi* pneumonia in foals was reported by Kahn *et al.* (2021). Other researchers have reported a decrease in foal morbidity and mortality after the administration of *R. equi* hyper-immune plasma (Caston *et al.*, 2008). However, other studies (Kahn *et al.*, 2023), reported no protective effects of *R. equi* hyper-immune plasma. The protective components of HI plasma are not completely known. Antibodies to vap proteins, specifically vapA, appear to be important (Leclere *et al.*, 2011). The opsonic ability of foal serum was found to be a limited factor for phagocytosis from 1 to 6 weeks of age (Lopez *et al.*, 2020). Nevertheless, the phagocytic activity of foal neutrophils was found to improve when mixed with adult serum or plasma (Cohen *et al.*, 2021), which may be related to unknown, non-specific immune factors provided by the hyper-immune plasma and normal adult equine plasma that are absent from colostrum as fibronectin, complement, and cytokines (Hooper-McGrevy *et al.*, 2001). The effectiveness of hyper-immune plasma is affected by factors such as the dose, timing of administration, management conditions, and the number of virulent bacteria in the environment (Anna *et al.*, 2022).

CONCLUSION

The current study results confirmed *Rhodococcus equi*-specific equine polyvalent antibodies' therapeutic efficacy and prophylactic potentials against *R. equi* infection by using mice as surrogate *in vivo* models. Post-infection administration of *Rhodococcus equi*-specific equine polyvalent antibodies may be an effective therapeutic strategy and promising prophylactic confrontation approach for rhodococcosis, especially in foals with failure of passive transfer of immunity.

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Correspondence:

Hassan Aboul-Ella
Department of Microbiology,
Faculty of Veterinary Medicine,
Cairo University, Giza, Egypt, 12211,
e-mail: hasanabo@cu.edu.eg