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RESEARCH ARTICLE

Sero-epidemiology of the *Rhodococcus equi* in horses in Eastern Kazakhstan

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Doğu Kazakistan'daki atlarda *Rhodococcus equi*'nin sero-epidemiyolosi

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Öz

Amaç: Bu çalışma Doğu Kazakistan'daki yetişkin atlarda *Rhodococcus equi (R. equi)* sero-prevalansını değerlendirmek için tasarlanmıştır.

Gereç ve Yöntem: Eylül ve Kasım 2021 tarihleri arasında doğu Kazakistan'da bulunan üç bölgede (Zhambyl, Almatı, Doğu Kazakistan) 311 attan serum toplandı ve virülent *R. equi*'ye karşı oluşmuş olan antikorların varlığı enzim bağıntılı immünosorbent test (ELISA) ile tespit edildi.

Bulgular: *R. equi*'nin doğu Kazakistan'da bulunan at popüslayonlarında ki seroprevalansı 2021 yılında %93,6 olarak bulundu. En yüksek seroprevalans Vap A oranı Zhambly Bölgesi'nde (%98,02), en düşük ise Doğu Kazakistan Bölgesi'nde (%88,89) görüldü. Bu bölgeden alınan örnekler toplam örneklerin %69,8'ini oluşturmakta olup, %3,84'ü vapA seronegatiftir. *R. equi*'nin Doğu Kazakistan'da endemik enfeksiyona sebep olduğu tespit edildi..

Öneri: Bu, *R. equi*'nin Kazakistan'da epidemiyolojisi hakkında bilgi sağlayan ilk sero survey çalışmasıdır. Serolojik bulgular klinik vakalar ve tay tavlalarında bakteri izolasyonu ile desteklenmelidir. Doğu Kazakistan'da *R. equi*'ye karşı koruma ve kontrol programları uygulanmalıdır.

Anahtar kelimeler: Kazakistan, *Rhodococcus equi*, sero-prevalans.

Abstract

Aim: This study was designed to evaluate *Rhodococcus equi* (*R. equi*) seroprevalence in adult horses in East Kazakhstan.

Materials and Methods: Sera were collected from 311 horses in 3 regions (Zhambyl, Almaty, East Kazakhstan) across the eastern regions of Kazakhstan between September and November 2021 and the presence of antibodies against virulent *R. equi* was detected by enzyme-linked immunosorbent assay (ELISA).

Results: The equine seroprevalence of *R. equi* was found to be 93.6% in 2021. The highest seroprevalence VapA rate was in the Zhambly Region (98.02 %) and the lowest in the East Kazakhstan Region (88.89%). Samples taken from this region constitute 69.8% of the total samples, and 3.84% are vapA seronegative. *R. equi* appears to be endemic in East Kazakhstan.

Conclusion: This is the first seroprevalence study of *R. equi* in Kazakhstan that provides information on epidemiology. Serological findings should be supported by clinical cases and the isolation of bacteria from foal barns. Based on the findings, protection and control programs against *R. equi* should be implemented in eastern Kazakhstan.

Keywords: Kazakhstan, *Rhodococcus equi*, seroprevalence

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Introduction

R. equi is a Gram-positive, nonmotile, obligate aerobe, intracellular microorganism. *R. equi* causes pyogranulomatous bronchopneumonia in foals aged 1 to 6 months (Takai 1997). Foals may develop extrapulmonary diseases, such as septic arthritis, osteomyelitis, ulcerative enterocolitis, mesenteric lymphadenopathy, neonatal diarrhea and sudden death (Muscatello et al 2007). The disease has been reported with pneumonia, enteritis, abortions and placentitis in the immunocompromised mature horse (Patterson-Kane et al 2002). *R. equi* is additionally considered a zoonosis pathogen of immunosuppressed people (Prescott 1991). *R. equi* is found in the intestinal flora of horses and soil of horse shelters, and adults are resistant to disease (Muscatello 2012). Foals breathe the agent in the dust particles from the environment, and then the agent that undergoes intracellular replication in alveolar macrophages forms pneumonia as the pathogenesis (Cohen et al 2008). The extrachromosomal plasmid synthesizes the 15-17 kDa "virulence-associated protein A" (VapA) antigen is responsible for the virulence of the agent (Willingham-Lane et al 2018). Apart from VapA, other Vaps proteins are responsible for pathogenicity; these are VapC, Vapl, pseudo VapE, and VapF (Takai et al 2000b, Russell et al 2004). The prevalence of the disease on farms may be endemic, sporadic, or absent due to differences in foal population density, farm management, and environmental factors such as climate change (Lönker et al 2020), dust, soil pH, and the number of *R. equi* organisms in the soil (Muscatello et al 2006). It was reported that *R. equi* can be found in significant quantities in the gastrointestinal contents of earthworms, act as an accumulator of *R. equi* in the soil environment, and as a source or reservoir of animal infection (Takai et al 2021). Because it is intracellular and due to reasons such as joint disorders that occur after infection and a decrease in lung capacity. It causes severe economic losses, especially in breeding racehorses (Ainsworth et al 1997). The cost of long-term antibiotic treatment and the death of foals despite joint development disorders and sometimes even all treatment also poses financial risks (Arnold-Lehna et al 2020). Foals are ordinarily hypogammaglobulinemic because of the epitheliochorial placenta. Foals try to close this gap with antibodies in the colostrum in the postpartum period. Foals become infected approximately when maternal antibody concentrations decrease (Giguère and Polkes 2005).

Although several epidemics and endemic *R. equi* infections have been reported in all countries bordering Asia, including China, Japan, Korea, Bangladesh, Malasia and some Asian countries, limited information exists on the endemic status of the disease in Kazakishtan (Takai et al 2001, Takai et al 2003, Liu et al 2014). This study aimed to determine by analysis of horse sera with ELISA the endemic status of *R. equi* in the east region of Kazakhstan.

Material and Methods

Sample collection and study area

Kazakhstan, which has a vast land area of 2.725.000 km2, is located part of Asia and the northern hemisphere and has approximately 2.3 million horses. The samples were collected from horses in 3 regions (Zhambyl, Almaty, East Kazakhstan) across the eastern regions of Kazakhstan between September and November 2021. Blood samples were taken from 311 healthy horses with a high horse population in various regions (From 7 different farms-156 samples were from the Almaty region, 3 farms-101 samples from the Zhambyl region, 24 different farms-50 samples from the East Kazakhstan region). Samples were divided into four age groups (I, II, III, and IV, consisting of 1-5, 6-10, 11-15, and >15 years old, respectively). Blood samples (5–8 ml) were collected from horses' jugular vein into a sterile sera tube using needles. Sera were obtained from clotted blood samples by centrifugation (3000 rpm for 10 min) and stored at -20 °C until used in a serological test. For serological tests, frozen sera samples (in the cold chain with -20 °C) were sent to Selcuk University, Faculty of Veterinary Medicine, Microbiology department Konya, Turkey. Positive control sera required for evaluation was obtained from horse sera hyperimmunized with inactive *R. equi* antigen. Negative control sera were obtained from horses that had not previously encountered antigen and were confirmed by indirect ELISA (Erganiş et al 2014).

Bacterial strain and antigen preparation for enzyme linked immunosorbent assay (ELISA)

In this study, *R. equi* whole cell lysate and concentrated VapA protein were prepared as antigens for homemade ELISA. *R. equi* strain (SUVF stock No. 185) was passaged blood agar. A single colony from the isolated bacteria was passaged into 25 ml of BHI broth. The enriched culture was passaged into 250 ml and 1 liter of BHI broth. The culture was centrifuged at 9000 rpm for 5 minutes and the bacterial pellets were collected in sterile bottles. The obtained *R. equi* bacteria pellet was frozen at -80 degrees and thawed 10 times. Sonication was performed at 25 msn x 10 pulse during each thawe process (Takai et al 1985). Pure VapA antigen was concentrated by filtration through 50 kDa and 10 kDa cassette filters. Whole cell *R. equi* antigen and VapA antigen concentrated and lysed were measured for protein according to the BCA Protein Assay Kit procedure (71285-M, Sigma) (Erganiş et al 2015). After dilution with 0.01 M phosphate-buffered saline (PBS) pH 7.2 at 5 µg/ml in each well, they were distributed on flat bottom 96 well ELISA plates (Kartell S.p.a, Italy) and incubated overnight at +4°C. Washed 5 times with 0.01 M PBS 0.05% Tween 20, pH 7.2 (PBS/T) to remove unbound antigens. Then, it was incubated with 200 µl of blocking solution (5% skim milk powder in PBS) for 2 hours

at room temperature, washed 5 times with PBS/T and dried thoroughly (Erganiş et al 2015).

Samples and positive negative control sera were diluted 1/800 with PBS/T and incubated overnight at +4°C. After washing five times with PBS/T at the end of the incubation, peroxidase labeled antibody to horse produced in goat (5220-0370, Sera Care) diluted with 1/2500 PBS 5% milk powder was incubated for 2 hours at room temperature. At the end of the incubation, washing was done five times and 100 µl of the substrate containing o-phenylenediamine dihydrochloride (OPD tablets, p8287, Sigma) in phosphate-citrate buffer with sodium perborate was added. Afterwards, it was incubated for 60 minutes and the results were evaluated with the ELISA reader at 450 nm as follows (Erganiş et al 2014).

Statistical analysis

The categorical variables were presented in percentage frequency. And the continuous data were indicated as mean±standard deviation. One-way ANOVA test was used to assess whether there was a significant difference between whole-cell antigen ($p<0.001$) and VapA ($p=0.04$) results between regions, and Levene test variances were detected and supported by the Bonferroni test. Tamhane's T2 test was used to determine the difference between regions in groups with unequal variances according to Levene's test ($p<0.001$). The sensitivity and specificity of the diagnostic methods were calculated using the negative and positive predictive values. The significance level was indicated to be $\alpha = 0.05$. The SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical package program analyzed the data. P values were calculated to compare the test performances and to determine the level of statistical significance between geographic regions and age, groups according to the obtained data.

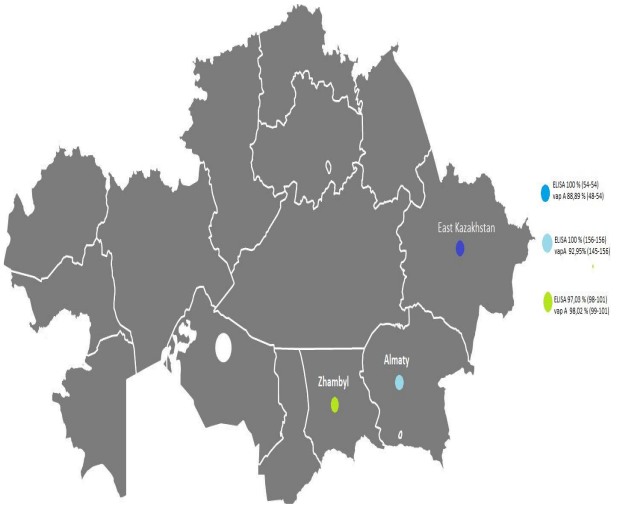

Figure 1. Distribution of the horses from which samples were taken on the map of Kazakhstan.

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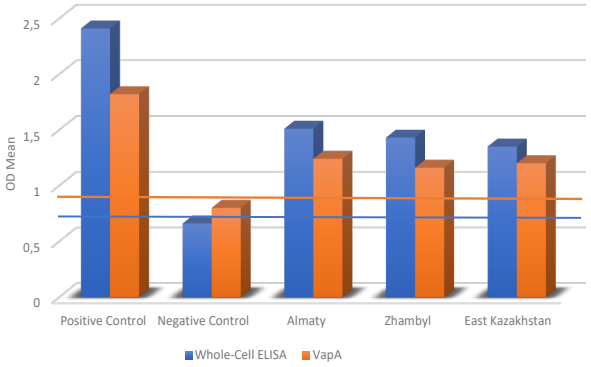

Figure 2. Compared results of groups whole-cell antigen (blue line 0.6815) and VapA (red line 0.8195) ELISA Cut-off value-line determined with 87 % sensitivity and 100 % specificity according to ROC analysis

Results

Of the 311 sera samples, were found to be positive for anti-R equi antibodies using the indirect ELISA test with 308 (99.03%) whole-cell and 292 (93.9%) VapA protein as the antigen. The highest seroprevalence VapA rate was in the Zhambly Region (98.02 %) and the lowest in the East Kazakhstan Region (88.89%) (Figure 1). The cut-off value of positive and negative control sera was 0.8195 for VapA and 0.6815 for Whole-cell antigen in the receiver operating characteristic (ROC) curve analysis performed with 87% sensitivity and 100% specificity, and 100% confidence interval of the OD values obtained by ELISA (Figure 2).

There is no statistical difference between the collected samples in terms of gender in the study (Table 1). In the three regions where the sample was taken, most samples were collected from age group I among all age groups.

In the study, the highest number of samples were taken from 7 different professional farm in the Almaty region, where professional horse shelter is carried out. Samples taken from this region constitute 69.8% of the total samples, and 3.84% are VapA seronegative. Again, the oldest animal in this group is 22 years old, and the seropositivity rate in the IV group is 100%. This has been shown that the adult animals are immune to the agent and that the amount of antibodies increases by encountering the agent in recurrent dry seasons and there is a transmission between old and young. The finding of 3.84% of young seronegative animals proves that this chain of transmission can be broken with resistant foal. Samples were collected from only three professional businesses from the Zhambyl region, and among all areas, the VapA seropositive rate was the highest at 98.02% (Table 2).

Table 1. Age and gender distribution by regions

			Age Groups							
			I		II		III		IV	
			Count	Row N %	Count	Row N %	Count	Row N %	Count	Row N %
Almaty Region	Sex	Mare	51	73,9	13	18,8	4	5,8	1	1,4
		Stallion	58	66,7	17	19,5	9	10,3	3	3,4
Zhambly Region	Sex	Mare	52	80,0	13	20,0	0	0,0	0	0,0
		Stallion	28	77,8	8	22,2	0	0,0	0	0,0
East kazakhstan Region	Sex	Mare	25	62,5	14	35,0	1	2,5	0	0,0
		Stallion	12	85,7	2	14,3	0	0,0	0	0,0
Total	Sex	Mare	128	73,5	40	22,9	5	2,87	1	0,57
		Stallion	98	71,5	27	19,7	9	6,5	3	2,1

Table 2. Regional comparison of *R. equi* seroprevalence determined by whole-cell antigen and vapA ELISA.

	Whole-Cell ELISA				VapA ELISA			
	Negative		Positive		Negative		Positive	
	N	%	N	%	N	%	N	%
Almaty Region	0	0,0	156	50,6	11	57,9	145	49,7
Zhambly Region	3	100,0	98	31,8	2	10,5	99	33,9
East Kazakhstan Region	0	0,0	54	17,5	6	31,6	48	16,4
Total	3	0,96	308	99,03	19	6,11	292	93,9

Discussion

Historically, the importance of the horse in warrior societies is still maintained in the nomadic tribes of Central Asia (West 2017). Kazakhstan is one of these societies, and horse games such as kokpar and baiga of ancient origin are still popular (Aizhan et al 2015). There are no reported cases of *R. equi* in horses in Kazakhstan. However, *Rhodococcus* spp. species have been isolated from oil-rich Kazakhstan (Akhmetov et al 2022). Neighbors of Kazakhstan, *R. equi* in racehorses in China, cattle and pigs in Russia, *Rhodococcus* spp. in polluted soils in Uzbekistan, no case was reported in Kyrgyzstan (Lazovskaya et al 2010, Liu et al 2014, Lobar Khasanova et al 2022). In addition, in the study conducted in Mongolia, non-pathogenic *R. equi* was isolated from only two of the Przewalski's foal feces examined from various soil and feces samples; however, it had not been studied serological prevalence in Mongolia (Takai et al 2005). Although there have been *R. equi* in immunosuppressive humans in some Asian countries, such as Pakistan and Malaysia, no reported case in horses (Puthucheary et al 2006, Khan et al 2013). In central Asia, information about the detection of *R. equi* in horses is negligible because this is probably related to the characteristics of the traditional lifestyle of nomadic people. In this study, results from samples of healthy horses have a significantly high seroprevalence of 93.6% than in a region where no disease has been reported. The prevalence rates in other *R. equi* serosurvey studies are quite low, for example; in the southeast Turkey, 11.7% in 2011 and the north-west Turkey, 14.8% in 2006, Japan, 11% in 1992, Italy, 13.45% in 2006 (Sanada et al 1992, Cuteri et al 2003, Attili et al 2006, Tel and Arserim 2011). Prevalence rates vary in *R. equi* molecular-epidemiology and culture and isolation studies, for example, 26.6% in Poland in 2016, 11.1% in Turkey in 2021, and 50% in Japan in 2000 (Takai et al 2000a, Witkowski et al 2016, Kirkan et al 2021). Several reasons come to mind as the cause of high seroprevalence in Kazakhstan; The dominance of traditional breeding rules, the ignorance of the disease, the lack of protection and control programs, or the resistance of Kazakh horse breeds to the disease were associated with the presence of immune antibodies without showing symptoms.

The prevalence of *R. equi* infection is typically seasonal, peaking in the late spring and summer months when the number of susceptible foals is high because of birth season, corresponding with an increased risk of aerosol dust

challenge from the environment (Muscatello et al 2007). In addition, the sampling was carried out in the autumn after the dry summer season. Horses are more likely to get *R. equi* with dust this season. Maternal-derived IgGb has a relatively longer half-life than other IgG sub-isotypes and coincides with the 1-3 months age range in which the foal is susceptible to *R. equi* infection (Sheoran et al 2000). In studies conducted, when adult horses encountered *R. equi* and VapA antigen, the highest increase in IgGb and IgGa concentrations was observed among the IgG subclasses (Lopez et al 2002, Jacks et al 2007). This information supports that the high seroprevalence rate obtained in the study partially encountered antigens in all age groups sampled last summer season.

There is no statistically significant difference between age and infection relationship. However, seropositivity is lower in older animals in aged and northeastern regions than in the other areas. Since the disease is the disease of foals, the study focused more on this age group, and the place of old horses in the herd in the chain of infection was determined, albeit to a lesser extent.

The Zhambyl region, located in the southeast of the area, is considered the reason for the high seropositivity rate in the dusty and dry summers. In addition, ignoring this risk situation for businesses in this region increases the infection rate due to traditional breeding methods and keeping old and young horses together.

Since there is no professional horse breeding in Eastern Kazakhstan, samples were taken from 24 different small farms. It has been determined that seropositivity decreases in horse barns where old and young horses are not housed together. In addition, since the region is located in a mountainous and cold region close to the Russian border, the transmission may be less than in the other areas.

Although this method is found to be costly, since the antibody levels of mares receiving hyperimmune plasma are not an example, various studies have reported that disease incidence is reduced in farms hyperimmunized by vaccination (Dawson et al 2010). The serology difference according to the regions is stated in studies conducted in Turkey (Attili et al 2006, Tel and Arserim 2011). Turkey has been aware of *R. equi* infection in the racehorse industry for years and is trying to reduce the prevalence by continuing various vaccines and postpartum hyperimmune serum applications to foals (Erganiş et al 2014, Erganiş et al 2015). Considering these conditions, the picture that will emerge when the disease is ignored, as in every epidemic, is a high seroprevalence.

Conclusion

According to ELISA results with whole-cell antigen, the seropositivity rate in eastern Kazakhstan provinces is 99.03%. This table shows that, whether pathogen or not, horses in Kazakhstan encountered *Rhodoccocus* spp. antigens at some point in their life, and the agent was present in the country. In addition, the seropositivity of the VapA antigen shows that the pathogen *R. equi* causes endemic infection in the eastern Kazakhstan region in the country. The study focused on eastern Kazakhstan; however, different results are likely to be obtained according to the differences in horse breeding across the country. In addition, it is aimed to reveal the serological findings in detail by determining the strains carrying pathogen VapA with future culture studies. Being aware of the disease, veterinarians and professional horse breeders in this region should pay attention to the relationship between foal birth timing in the dry season, vital maternal antibody supplementation against clinically symptomatic foal assets, and, if necessary, the addition of *R. equi* to their vaccination programs, within the framework of protection and control programs.

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This study has not been presented as a presentation in congress or published.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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References

Ainsworth DM, Yeagar A, Eicker SW, Erb H, et al. 1997. Athletic performance of horses previously infected with R. equi pneumonia as foal. P Annu Conv Am Equin, 43, 81-82.

Aizhan T, Tursun G, Aliya A, Moldir Y, et al., 2015. Psychological aspects of physical culture and agonistics in Kazakh culture. Procedia Soc Behav Sci, 205, 524-528.

Akhmetov LI, Puntus IF, Narmanova RA, Appazov NO, et al., 2022. Recent Advances in Creating Biopreparations to

Fight Oil Spills in Soil Ecosystems in Sharply Continental Climate of Republic of Kazakhstan. Processes, 10(3), 549.

Arnold-Lehna D, Venner M, Berghaus LJ, Berghaus R, et al., 2020. Changing policy to treat foals with Rhodococcus equi pneumonia in the later course of disease decreases antimicrobial usage without increasing mortality rate. Equine Vet J, 52(4), 531-537.

Attili A, Kennerman E, Takai S, Or M, et al., 2006. Seroepidemiological survey of Rhodoccocus equi infection in asymptomatic horses from Bursa, Izmir and Istanbul provinces, Turkey. Comp Immunol Microbiol Infect Dis, 29(5-6), 323-333.

Cohen ND, Carter CN, Scott HM, Chaffin MK, et al., 2008. Association of soil concentrations of Rhodococcus equi and incidence of pneumonia attributable to Rhodococcus equi in foals on farms in central Kentucky. Am J Vet Res, 69(3), 385-395.

Cuteri V, Takai S, Moscati L, Battistacci L, et al., 2003. A serological survey of Rhodococcus equi infection in foals in central Italy: Comparison of two antigens using an ELISA test. Comp Immunol Microbiol Infect Dis 26(1), 17-23.

Dawson TR, Horohov DW, Meijer WG, Muscatello G, 2010. Current understanding of the equine immune response to Rhodococcus equi. An immunological review of R. equi pneumonia. Vet Immunol Immunopathol, 135(1-2), 1-11.

Erganiş O, Sayin Z, Hadimli HH, Sakmanoglu A, et al., 2014. The effectiveness of anti-R. equi hyperimmune plasma against R. equi challenge in thoroughbred Arabian foals of mares vaccinated with R. equi vaccine. Turkish J Vet Anim, 2014.

Erganiş O, Hadimli HH, Sayin Z, Sakmanoğlu A, et al., 2015. Efficacy of experimental inactivated and live Rhodococcus equivaccines for thoroughbred Arabian mares in mice. Turkish J Vet Anim, 39(3), 295-301.

Giguère S, Polkes AC, 2005. Immunologic disorders in neonatal foals. Vet Clin North Am Equine Pract, 21(2), 241-272.

Jacks S, Giguère S, Crawford PC, Castleman WL, 2007. Experimental infection of neonatal foals with Rhodococcus equi triggers adult-like gamma interferon induction. Clin Vaccine Immunol, 14(6), 669-677.

Khan MY, Ali S, Baqi S, 2013. Rhodococcus equi pneumonia in a live related renal transplant recipient. J Pak Med Assoc, 63(5), 635-638.

Kirkan Ş, Parin U, Dolgun HTY, Oral EO, 2021. Detection of Rhodococcus equi by PCR from foals and determination of antimicrobial susceptibility. Turkish J Vet Anim, 45(3), 396-403.

Lazovskaya A, Levanova G, Kashnikov SY, Vorob'ev Z, 2010. Detection of virulence plasmids in Rhodococcus equi strains isolated from pigs and cattle. Russ Agric Sci, 36(4), 309-311.

Liu H, Wang Y, Yan J, Wang C, et al., 2014. Appearance of multidrug-resistant virulent Rhodococcus equi clinical isolates obtained in China. J Clin Microbiol, 52(2), 703-703.

Lobar Khasanova, Kambaralieva M, Alimova B, Pulatova O, et al., (2022). Isolation and Characterization of a Bacterium – Producer of Amidase. Главный редактор. 91: 12.

Lopez AM, Hines MT, Palmer GH, Alperin DC, et al., 2002. Identification of pulmonary T-lymphocyte and serum antibody isotype responses associated with protection against Rhodococcus equi. Clin Vaccine Immunol, 9(6), 1270-1276.

Lönker NS, Fechner K, Abd El Wahed A, 2020. Horses as a Crucial Part of One Health. Vet Sci, 7(1), 28.

Muscatello G, Anderson G, Gilkerson J, Browning G, 2006. Associations between the ecology of virulent Rhodococcus equi and the epidemiology of R. equi pneumonia on Australian thoroughbred farms. Appl Environ Microbiol, 72(9), 6152-6160.

Muscatello G, Leadon D, Klay M, Ocampo-Sosa A, et al., 2007. Rhodococcus equi infection in foals: the science of 'rattles'. Equine Vet J, 39(5), 470-478.

Muscatello G, 2012. Rhodococcus equi pneumonia in the foal–Part 1: Pathogenesis and epidemiology. Vet J, 192(1), 20-26.

Patterson-Kane JC, Donahue JM, Harrison LR, 2002. Placentitis, fetal pneumonia, and abortion due to Rhodococcus equi infection in a Thoroughbred. J Vet Diagn Invest, 14(2), 157-159.

Prescott JF, 1991. Rhodococcus equi: an animal and human pathogen. Clin Microbiol Rev, 4(1), 20-34.

Puthucheary S, Sangkar V, Hafeez A, Karunakaran R, 2006. Rhodococcus equi-an emerging human pathogen in immunocompromized hosts: a report of four cases from Malaysia. Southeast Asian J Trop. Med Public Health, 37(1), 157.

Russell DA, Byrne GA, O'Connell EP, Boland CA, et al., 2004. The LysR-type transcriptional regulator VirR is required for expression of the virulence gene vapA of Rhodococcus equi ATCC 33701. J Bacteriol Res, 186(17), 5576-5584.

Sanada Y, Noda H, Nagahara H, 1992. Serological survey of Rhodococcus equi infection in horses in Hokkaido. J Vet Med Sci, 54(4), 649-652.

Sheoran AS, Timoney JF, Holmes MA, Karzenski SS, et al., 2000. Immunoglobulin isotypes in sera and nasal mucosal secretions and their neonatal transfer and distribution in horses. Am J Vet Res, 61(9), 1099-1105.

Takai S, Kawazu S, Tsubaki S, 1985. Enzyme-linked immunosorbent assay for diagnosis of Corynebacterium (Rhodococcus) equi infection in foals. Am J Vet Res, 46(10), 2166-2170.

Takai S, 1997. Epidemiology of Rhodococcus equi infections: a review. Vet Microbiol, 56(3-4), 167-176.

Takai S, Higuchi T, Matsukura S, Tamada Y, et al., 2000a. Some epidemiological aspects of Rhodococcus equi infection in foals in Japan: a review of 108 cases in 1992-1998. J Anim Sci, 11(1), 7-14.

Takai S, Hines SA, Sekizaki T, Nicholson VM, et al., 2000b. DNA sequence and comparison of virulence plasmids from Rhodococcus equi ATCC 33701 and 103. Infect Immun, 68(12), 6840-6847.

Takai S, Ogawa K, Fukunaga N, Sasaki Y, et al., 2001. Isolation

of virulent Rhodococcus equi from native Japanese horses. Comp Immunol Microbiol Infect Dis, 24(2), 123-133.

Takai S, Son W-G, Lee D-S, Madarame H, et al., 2003. Rhodococcus equi virulence plasmids recovered from horses and their environment in Jeju, Korea: 90-kb type II and a new variant, 90-kb type V. J Vet Med Sci, 65(12), 1313-1317.

Takai S, Sengee S, Madarame H, Hatori F, et al., 2005. The absence of Rhodococcus equi in Mongolian horses. J Vet Med Sci, 67(6), 611-613.

Takai S, Sudo M, Sakai M, Suzuki K, et al., 2021. Isolation of Rhodococcus equi from the gastrointestinal contents of earthworms (family Megascolecidae). Lett Appl Microbiol, 74, 27-31.

Tel O, Arserim N, 2011. Seroepidemiological survey of Rhodococcus equi infection in asymptomatic horses and donkeys from southeast Turkey. J S Afr Vet Assoc, 82(4), 224-226.

West E, 2017. The Impact of Horse Culture. The Gilder Lehrman Institute of American History. 4, 1-4

Willingham-Lane JM, Coulson GB, Hondalus MK, 2018. Identification of a VapA virulence factor functional homolog in Rhodococcus equi isolates housing the pVAPB plasmid. PloS one, 13(10), e0204475.

Witkowski L, Rzewuska M, Takai S, Kizerwetter-Świda M, et al., 2016. Molecular epidemiology of Rhodococcus equi in slaughtered swine, cattle and horses in Poland. BMC Microbiol, 16(1), 1-6.

Author Contributions

Motivation/Concept: KY, OE; Design: OE; Control/ Supervision: AU, OE; Data Collection and/or Processing: KY, OB, IY, IGD, AU; Analysis and/or Interpretation: AI, AS; Literature Review: AU, ZS; Writing the Article: OE; Critical Review: AU, ZS, OE

Ethical Approval

Blood collections were performed with owners' consent and all procedures used in this study were carried out according to the ethical guidelines for the use of animal samples permitted by (The Bioethics Commission of LIP KazSRVI approval no 15, Date: 20.05.2022).