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THREE NEW SEROTYPES OF *RHODOCOCCUS EQUI* IN PRESCOTT'S SEROTYPING SYSTEM – SHORT COMMUNICATION

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Three new serotypes were found among *Rhodococcus equi* strains, which could not be assigned into any of the seven serotypes of Prescott's system. Forty-three *R. equi* strains out of 44 previously nontypable ones isolated in Hungary could be allocated into one of the three new serotypes using the agar gel immunodiffusion (AGID) test. The three new suggested serotypes are serotype 8 (proposed reference strain: HNCMB-138003), serotype 9 (proposed reference strain: HNCMB-138004) and serotype 10 (proposed reference strain: HNCMB-138005). Hyperimmune sera produced in rabbits against the new serotypes and reference strains gave precipitation only with their homologous antigens, and no cross-reactions were observed. All of the previously nontypable isolates from clinical samples of horses (lung abscesses, intestinal lymph nodes, mediastinal lymph nodes) proved to be serotype 8, while strains of serotypes 8, 9 and 10 could be isolated from nasal and rectal swabs of horses and from the soil. Serotype 9 dominated among the previously nontypable strains of swine origin. One of the previously nontypable human strains was serotype 10. This serotype was also isolated from pigs, horses and the soil. The description of the three new serotypes can help us reveal new correlations between the host species, geographical origin and serotype of *R. equi* isolates.

Key words: *Rhodococcus equi*, serotyping, AGID

Rhodococcus equi is the causative agent of severe purulent pneumonia of foals (Vázquez-Boland et al., 2013). This bacterium had been first described as *Corynebacterium equi* nearly one hundred years ago (Magnusson, 1923). As a result of comparative studies the species was later transferred as *Rhodococcus equi* into the genus *Rhodococcus* (Goodfellow and Alderson, 1977). Recently, reclassification under the name of *Prescotella equi* has been suggested (Jones et al., 2013*a*, 2013*b*); however, the validity of this questioned (Garrity, 2014).

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From the end of the 1930s, several attempts were made to study the serological features of *R. equi* strains (Magnusson, 1938; Bruner et al., 1939; Karlson et al., 1940; Bruner and Edwards, 1941; Woodroofe, 1950; Carter and Hylton, 1974), and two serotyping systems (Prescott's and Nakazawa's) were introduced in the laboratory practice.

Prescott's serotyping system (Prescott, 1981) classifies *R. equi* strains into seven serotypes on the basis of capsular polysaccharide antigens using the agar gel immunodiffusion (AGID) test. The exact structure of the polysaccharide capsule of several serotypes was also described (Leitch and Richards, 1990; Severn and Richards, 1990; Finnerty, 1992; Masoud and Richards, 1994). On the other hand, Nakazawa et al. (1983) later assigned *R. equi* strains into 27 serotypes using the slide agglutination test.

The above two serotyping systems were compared by several authors. Prescott's seven type strains were serotyped in Nakazawa's system and Prescott's type strains 1, 2, 3, 4, 5, 6 and 7 were classified as serotype 4, 16, 2, 12, 21, 1 and 9, respectively, in Nakazawa's system. The two systems use different serological methods (agglutination and precipitation tests) for the classification and detect different antigens, so they do not correspond with each other (Katsumi et al., 1991; Zimmermann, 1996; Lämmler et al., 1997; Soedarmanto et al., 1998).

Because of its simplicity and specificity, Prescott's serotyping system is much more frequently used for the serotyping of *R. equi* strains. During the last 25 years, a large number of *R. equi* strains were serotyped using Prescott's serotyping system by several authors. In one of our previous studies, 90 *R. equi* strains (23.75%) out of 379 strains isolated from different sources (swine, soil, horse, human) could not be typed in Prescott's serotyping system (Makrai et al., 2005, 2008). By re-examining the previously untypable *R. equi* strains using freshly produced hyperimmune sera, 46 of them could be assigned into one of Prescott's serotypes (data not presented).

The aim of the present study was to re-examine strains that repeatedly could not be serotyped, and to describe new capsular serotypes of *R. equi* found among previously untypable strains isolated in Hungary.

Materials and methods

The 44 untypable strains were isolated from 21 settlements located throughout Hungary. The samples originated from the submandibular lymph nodes of swine (19), from the lungs (3), mediastinal (1) and mesenteric (2) lymph nodes, nasal (4) and rectal swabs (10) of horses, from humans (1) and from the soil (4).

Thirteen *R. equi* strains were selected from our collection of 44 nontypable isolates representing a wide range of sources (different host species, geographical origin, clinical and environmental samples).

Production of hyperimmune sera in rabbits against the selected 13 untypable strains, preparation of antigen for AGID and the AGID test were carried out as described earlier (Makrai et al., 2008). Each newly produced serum was examined with each of the 13 antigens. Sera that gave identical precipitation bands with these 13 antigens were placed in one group and one serum was used to further examinations. These sera were examined with the accepted seven (1 to 7) *R. equi* type strains (ATCC 33701–33707), too.

Results

Three distinct new groups were detected among the selected 13 strains that were used for hyperimmune serum production in rabbits. One serum was selected from each of the three groups for further study. Each of the three selected sera gave precipitation with its homologous antigen and did not react with any of the previously known *R. equi* serotypes from 1 to 7. Based on these results, three new serotype candidates of *R. equi* (serotypes 8, 9 and 10) are proposed. The three suggested type strains have been deposited at the Hungarian National Collection of Medical Bacteria as *R. equi* strains HNCMB-138003 (serotype 8), HNCMB-138004 (serotype 9) and HNCMB-138005 (serotype 10). All but one untypable strain (43 strains) gave precipitation bands with one of the three sera; they could be assigned into one of the suggested new serotypes.

Serotype 9 dominated among the previously nontypable 44 strains (47.73%), but serotypes 8 and 10 also represented a significant proportion (serotype 8: 36.36%, serotype 10: 13.64%), while one strain (2.27%) could not be typed.

Most of the strains isolated from horses belonged to serotype 8, while strains from the submandibular lymph nodes of pigs were mainly of serotype 9. The previously nontypable human isolate belonged to serotype 10. The distribution of previously nontypable *R. equi* strains according to origin and serotype is shown in Table 1.

Discussion

Two systems are used for serotyping of *R. equi* strains: Prescott's (1981) and Nakazawa's (Nakazawa et al., 1983) systems. These two systems are not compatible, since they detect different antigens in different serological tests (agar gel precipitation and slide agglutination test, respectively). Certain type strains of both systems can be assigned into one of the serotypes of the other one, but there is no correlation between them (Zimmermann, 1996; Lämmler et al., 1997).

When using either of the two systems, some strains prove to be untypable (Mutimer et al., 1982; Zimmerman, 1996; Lämmler et al., 1997). Ninety-eight *R. equi* strains of different origin were serotyped in both serotyping systems by

Lämmler et al. (1997). When using Prescott's system, his strains could be assigned into five serotypes and 12 strains were not typable, while in Nakazawa's system they could be assigned into 11 serotypes and only three strains were not typable. Although much fewer nontypable strains were detected in Nakazawa's system than in Prescott's system, the latter system has become much more widely used since it is easy to carry out, the reactions are clear and there are no cross-reactions. With the addition of the three new serotypes the efficacy of Prescott's system has increased considerably.

Table 1

Assignment of previously nontypable *Rhodococcus equi* strains into three new serotypes according to their origin

Origin		New serotypes			Non-typable	Total
		8	9	10		
Horse	lung abscesses	3	–	–	–	3 (6.82%)
	intestinal lymph nodes	2	–	–	–	2 (4.55%)
	mediastinal lymph nodes	1	–	–	–	1 (2.26%)
	nasal swabs	3	–	1	–	4 (9.1%)
	rectal swabs	5	3	2	–	10 (22.73%)
Soil	from horse environment	1	1	1	1	4 (9.1%)
Swine	submandibular lymph nodes	1	17	1	–	19 (43.18%)
Human		–	–	1	–	1 (2.26%)
Total		16 (36.36%)	21 (47.73%)	6 (13.64%)	1 (2.27%)	44 (100%)

Among the previously not typable 44 *R. equi* strains three new serotypes were found. They are proposed to represent serotypes 8, 9 and 10 of *R. equi*, respectively. They proved to be independent, the proposed type strains of these new suggested serotypes reacted only with their homologous sera and these sera did not show cross-reactions with the seven accepted type strains of *R. equi*. The suggested reference strains for these serotypes are *R. equi* strain HNCMB-138003 (serotype 8), HNCMB-138004 (serotype 9) and HNCMB-138003 (serotype 10). Forty-three out of 44 formerly untypable *R. equi* could be serotyped using the three new type sera of these three suggested new serotypes.

The newly serotyped strains isolated from clinical samples of different species showed a characteristic serotyping pattern, while strains isolated from nasal and rectal swabs and soil samples showed a significant diversity, like in the case of other serotypes. Virulence markers (vapA, vapB plasmid profile) of the strains of the three new serotypes are to be examined in the future, as has been performed with the serotypes described previously (Makrai et al., 2005).

Among the previously nontypable strains isolated from horses serotype 8, while among those isolated from swine serotype 9 was the dominant serotype, and the previously nontypable human isolate belonged to serotype 10.

The classification of previously nontypable *R. equi* strains into the three new serotypes can reveal new relationships between the host species, geographical origin and serotype of *R. equi* isolates.

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