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ISOLATION AND IDENTIFICATION OF *Rhodococcus equi* BACTERIAL STRAIN IN A PUREBREAD ARABIAN HORSES FARM IN ROMANIA

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Abstract

Rhodococcus equi is a Gram-positive, encapsulated, pleomorphic, intracellular bacillus, which mainly affects foals. Despite often being fatal it is still a neglected pathogen. The main objective of this study is to establish the etiological diagnosis in a clinical case of pneumonia in foals using basic bacteriology techniques. The strain referred to has been collected from a 32-day-old foal with the following symptoms: fever, dyspnea, abdominal breathing, cough and a deeply altered general condition. The isolation of the bacteria has been performed on Lowenstein- Jensen selective medium and the identification has been done based on morpho-cultural and biochemical features. Consequently, an antibiogram was performed and it showed that the isolated R. equi strain is sensitive to rifampicin and azithromycin thus the therapeutic protocol was corrected.

Key words*: bacteria, foal, Rhodococcus equi.*

INTRODUCTION:

Rhodococcus equi is the most important cause of pneumonia in foals aged between 1-5 months (Johns, 2013). It is not the most frequent etiology for this age group, still has the most significant economic consequences on the account of the death rate, prolonged treatment, screening programs for early detection and relatively expensive prophylactic strategy (Rush, 2014). The disease is rarely encountered in horses older than 8 months. Epidemiological data indicate that the pulmonary infection has its origins, supposable, in the first week of life (Merck et al, 2014). *R. equi* belongs to the *Nocardiaceae* family that also comprises *Nocardia, Mycobacterium, Corynebacterium* and *Gordonia* with some similarities among the group. Due to this fact, initially is rather easy to misdiagnose, so a proper correlation between the clinical symptoms and the results of the bacteriologic examination are indispensable (Ayoade, 2020). *R. equi* (formely *Corynebacterium equi*) is a pleomorphic, Gram-positive bacterium that was first isolated in 1923 from the lungs of foals in Sweden. Though not a very frequently found pathogen, *R. equi* has proven to be one of the most important that affects foals (Manzat, 2001).

Highly effective methods for preventing *R. equi* pneumonia in foals are lacking (Cohen, 2014).

Initially the bacteria was named *Corynebacterium equi* by H. Magnusson and later, in 1977, was transferred to *Rhodococcus* genus by Goodfellow and Alderson (Vasquez-Boland, 2019).

Apart from clinical symptoms specific for pneumonia such as fever, labored breathing, cough, a rattling sound in the windpipe and depression, one of the most severe manifestation of *R. equi* infection are abscesses, mainly located pulmonary, but also can be found elsewhere in the body (Young, 2020).

Prolonged treatment is required for the affected foals because of the increased persistence of the bacteria in the lung abscesses. The elective treatment consists in a combination of the following antibiotics: erythromycin and rifampicin (Wright, 1990). The main objective

of this study was to establish the etiological diagnosis in a clinical case of pneumonia in foals using basic bacteriology techniques and the correlation of treatment with the results obtained from the bacteriological examination performed.

MATERIALS AND METHODS

The bacterial strain was isolated from a 32 day old foal that presented the following symptoms: fever, dyspnea, abdominal breathing, cough, deeply altered general condition.

Among the differential diagnosis for this clinical case were mentioned also parasite infestations such as Ascarids: Ascarid worms have a high prevalence in foals causing irritation of the digestive tract, decreased feed absorbtion and colic, but also damage lung tissue due to migrating larvae (Mitrea, 2011).

Parascaris equorum was detected mainly in foals (5/6; 83.88%) (Buzatu, 2014).

After the collection of samples for the bacteriological examination has been finalized, the foal started to receive treatment with antibiotics, gentamycin combined with cephalosporin. This treatment was followed for one week until the all the performed exams were finalized. Unfortunately, the treatment with gentamycin and cephalosporin had no significant results, on the contrary, the health status of the foal had worsened. Moreover, a ultrasonography examination was performed and it revealed that the pulmonary abscesses have increased.

Afterwards, samples were collected from the foal's nasal cavities and transported to the laboratory to further proceed with the bacteriological examination. A primary seeding on blood agar and BHI broth was performed.

Isolation of the pathogen was intiated on specific culture media (Blood Gelosis, blood agar, Muller-Hinton agar, MacConkey agar, BHI agar, Chapman agar, Lowenstein-Jensen agar, BHI broth).

Isolation and identification: the following materials were used culture media.

The obtained cultures has been investigated for the macroscopical morphology and submitted to passages from the selected colonies on both, BHI broth and agar culture media. The primary identification of the bacterial genera has been performed by bacterioscopic examination of the isolate, the catalase test and selective media seeding on MacConkey agar, Chapman agar and Lowenstein-Jensen agar. Based on this the suspicion of *R. equi* infection was issued.

The antibiogram and the lecture of the results has been performed according to Liofilchem and EUCAST standards.

The Identification of the isolated strain has been carried out on API Coryne (Biomerieux) tests, in duplicates.

RESULTS AND DISCUSSIONS

After the collection of samples for the bacteriological examination has been finalized, the foal started to receive treatment with antibiotics, gentamycin combined with cephalosporin. This treatment was followed for one week until the all the performed exams were finalized. Unfortunately, the treatment with gentamycin and cephalosporin had no significant results, on the contrary, the health status of the foal had worsened. Moreover, a ultrasonography examination was performed and it revealed that the pulmonary abscesses have increased.

Besides pneumonia symptoms, in the literature can also be found extra-pulmonary disorders that are present in the infection with *R. equi.*

Diarrhea, can be caused by pyogranulomatous typhlocolitis or caused as a result of antimicrobial treatment. Abdominal lesions are identified in approximately 50% of the foals with *R. equi* infection.

Pyogranulomatous lymphadenitis of the mesenteric or colonic lymph nodes and large intra-abdominal abscesses can be also present.

Septic inflammation of growth cartilage and osteomyelitis are less frequent localizations of the infection.

There have also been reported cases of panophthalmia, sinusitis, pericarditis, nephritis, uveitis and hepatic and renal abscesses caused by *R. equi* infection (Giguére, 2011).

The cultures made from the samples collected from the foal presented polymorphic flora, in which predominate mucoid, whitish, transparent colonies, with weak alpha-hemolysis.

In Table 1 are presented different morphological, cultural and biochemical characteristics of the isolated cultures on different types of culture media.

Table 1. Morphological, cultural and biochemical characteristics of *R. equi* on different types of culture media

Cultural Aspect Gelosis+blood	Cultural Aspect MacConkey	Cultural Aspect Chapmann	Cultural Aspect Lowenstein-Jensen	Morphologic Aspect	Catalasis
Mucoid, whitish, transparent, confluent colonies with weak alpha-hemolysis. (Figure 1)	Medium colonies, transparent, round, glossy, lactose-positive.	-	White, mucous culture, poorly developed 24 and 48 hours from seeding (Figure 2)	Coccoid forms, coccobacilary, rare chains, isolated, diplo or groups, Gram-positive. (Figure 3)	Positive

Based on the characteristics presented in the table above, the suspicion of *R. equi* infection was issued.

The antibiogram that was performed had the following results (Figure 5):

- Susceptible to: gentamicin, doxycycline, streptomycin, norfloxacin, rifampicin;
- Intermediate sensitive to: spectinomicin;
- Resistant to: cefixima, vancomycin, oxacillin,amikacin,trimethoprim+sulfomethoxazole, colistin sulfate, cephalotin, clindamycin.

For further confirmation, API Coryne tests (Biomerioux), in duplicates, were executed. The results based on the biochemical characteristics are presented in Table 2.

Table 2. Biochemical characteristics of *R. equi* strain

NIT	PYZ	PyrA	PAL	ßGUR	ßGAL	αGLU	ßNAG	ESC	URE	GEL	0	GLU	RIB	XYL	MAN	MAL	LAC	SAC	GLYG	CAT
+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+
+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+

Legend: NIT - Potassium nitrate; PYZ - Pyrazinamidase; PyrA - Pyrolidonylarylamidase; PAL - Alkaline phosphatase; Bgur - β-glucuronidase; βGAL - β-galactosidase; αGLU - α-glucosidase; βNAG - N-Acetyl-β-glucosaminidase; ESC - Esculin; URE - Urease; GEL - Gelatin; 0 - negative control; GLU - Glucose; RIB - Ribose; XYL - Xylose; MAN - Mannitol; MAL - Maltose; LAC - Lactose; SAC - Sacchrarose; GLYG - Glycogen; CAT - Catalase.

Figure 1. Blood Gelosis - pure culture of *R. equi*, after 24 hours incubation. Colonies are irregular, semitransparent and mucoid with weak alpha-hemolysis. After several days in culture, colonies produce a characteristic salmon-pink pigment

Figure 2. Cultural Aspect of *R. equi* on Lowenstein-Jensen: white, mucous culture, poorly developed 24 and 48 hours from seeding

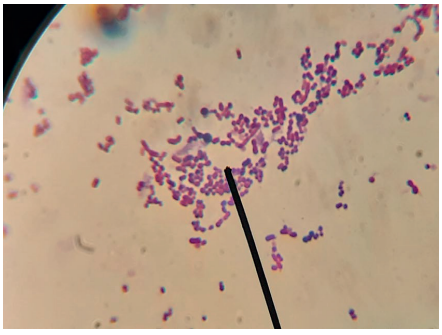

Figure 3. Morphologic aspect of *R. equi* isolate, Gram stain, 1000x, Gram-variable pleomorphic, coccoid, and bacillary bacteria

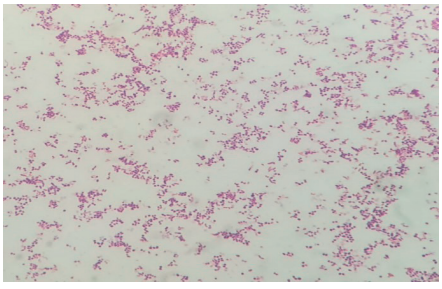

Figure 4. Microscopic aspect of *R. equi* on Lowenstein-Jensen Media (Gram stain, 700x)

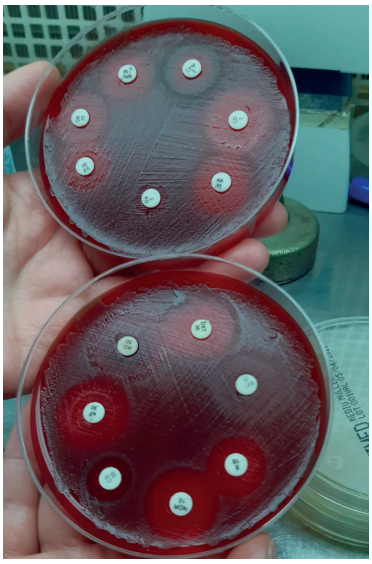

Figure 5. Antibiogram results

The successful early diagnosis and treatment of *R. equi* pneumonia in foals and management of the foals environment to reduce risks of contracting the disease are, arguably, among the most challenging experiences currently facing the equine clinician.
The incidence of the disease is increasing, as a reflection of intensification of managerial practice. High successful farms can therefore become victims of their own success. (Muscatello et al. 2007).

CONCLUSIONS

From the nasal exudate samples collected from the foal, one bacterial strain with possible involvement in the pathological process was isolated. It is presenting morphological and cultural properties characteristic for the *Rhodococcus* genus. This result was confirmed by performing API Coryne tests.
R. equi was diagnosed by correlating the results of the bacteriologic examination with the clinical symptoms presented by the foal and the anamnesis.
Currently, the treatment of choice for *R. equi* pneumonia is a member of the class of antibiotics known as macrolides combined with another antibiotic known as rifampin. Examples of macrolide antibiotics are azithromycin clarithromycin and erythromycin. This combination has been used to treat foals for about 40 years (Cohen, 2021).

After interpreting the antibiogram resulted that the isolate is for sensitive to rifampicin, norfloxacin, azithromycin and the foal has been treated accordingly by using a combination of rifampicin and azithromycin. This treatment was maintained for 5 weeks.
Positive results appeared soon after the treatment was initiated.
The foal presented improved general condition, temperature between physiological limits. By the end of the treatment, the pulmonary abscesses had gradually reduced, cough was no longer present and the appetite had returned to normal.

REFERENCES:

Aiello E. Susan, Michael A. Moses, Melinda D. Merck, (2014) *The Merck Veterinary Manual*, Ed. X, Callisto Publishing House, 1336-1338.

Bulgaru Anca, Tudor Poliana (2016) *The prevalence of Helminth Parasites in Horses Raised in Modern Conditions*, Scientific Works. Series C. Veterinary Medicine, Vol. LXI, (http://veterinarymedicinejournal.usamv.ro/pdf/2015/issue_2/Art54.pdf) 271-276.

Buzatu Marius Cătălin, Ioniță Mariana, Mitrea L.I. (2014) *Coprological Prevalence of Intestinal Parasites and Strngyle Epg Profiles of orking Horses from North-Eastern and South-Eastern Romania,* Scientific Works, Series C Veterinary Medicine, Vol. 19 Issue 3, 62-67 (http://veterinarymedicinejournal.usamv.ro/pdf/2013/vol19_3/art11.pdf).

Cohen D. Noah, 2021, *Rhodococcus equi Pneumonia in foals: an update on Epidemiology, Diagnosis, Treatment and Prevention*, AAEP (https://aaep.org/horsehealth/rhodococcus-equi-pneumonia-foals-update-epidemiology-diagnosis-treatment-and-prevention).

Cohen D. Noah, 2014, *Rhodococcus equi foal pneumonia*, Elsevier, V. 30, I. 3, 609-622.

Folusakin Ayoade, Mohammed U. Alam, *Rhodococcus equi*, (2020), Statpearls, from: RhodococcusEqui - StatPearls - NCBI Bookshelf (nih.gov).

Giguère S. *Treatment of Infections Caused by Rhodococcusequi*. Vet Clin North Am Equine Pract. 2017 Apr 33 (1) 67-85.

Johns I. (2013), *Management of Rhodococcus equi pneumonia in foals,* Veterinary Research and Report, 27 Nov. 2013, V. 2013:4, 49-59.

Jose A. Vazquez-Boland, Wim G. Meijer (2019*) The pathogenic actinobacterium Rhodococcus equi: what's in a name*? Molecular microbiology, V 112, I. 1, 1-15.

Liofilchem - Antibiotic Disc Interpretative Criteria and Quality Control - Rev.13 / 20.11.2017 (http://www.liofilchem.net/pdf/disc/disc_interpretative_table.pdf).

Manzat R. M., (2001) *Infectious Diseases of the Animals - Bacteriosis*, Timisoara, Brumar Publishing House, 390-393.

Mitrea L.I. (2011) *Parazitologie și Boli Parazitare*, Ceres Publishing House, Bucharest, 413-426.

Muscatello G., D. P. Leadon, M. Clay, A. Ocampo-Sosa, D. A. Lewis, U. Fogarty, T. Buckley, J. R. Gilkerson, W. G. Meijer and J. A. Vasquez-Boland. (2007*), Rhodococcusequi infection in foals: the science of rattles*, Eqvine Veterinary Journal*,* 39 (5) 470-478.

Rush Bonnie R. (2014), *Rhodococcus equi pneumonia in foals*, MSD Manual, Jan. 2014.

Wright B., *Rhodococcusequi pneumonia of foals,* 1990, Factsheet.

Young A., (2020) *Rhodococcus equi*, UC Davis Veterinary Medicine- Equine health, from: Rhodococcusequi | School of Veterinary Medicine (ucdavis.edu).