doi: 10.17221/117/2017-VETMED

Gram-negative aerobic and microaerophilic microorganisms isolated from pathological processes and lesions of horses

J. Bzdil[1]*, O. Holy[2], J. Toporcak[3]

[1]State Veterinary Institute Olomouc, Olomouc, Czech Republic
[2]Palacky University Olomouc, Olomouc, Czech Republic
[3]University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovak Republic

*Corresponding author: vetmed@seznam.cz

ABSTRACT: The pathogenicity of bacterial strains isolated from pathological processes and lesions of horses, strategies for their treatment and the choice of appropriate antimicrobials are frequently a challenging problem for private veterinarians who seek help in our laboratory. Therefore, the aim of this study was to map genera and species of Gram-negative aerobic and microaerophilic microorganisms isolated from pathological processes in horses and to identify the most effective antimicrobial agents for therapy based on antibiotic susceptibility. Between 2009 and 2014 a total of 449 clinical samples (n = 449) were examined; 229 (51%) of them were obtained from the respiratory tract, 121 (27%) from the skin, 40 (8.9%) from the digestive tract, 40 (8.9%) from the eyes, eight (1.8%) from the urinary system, six (1.3%) from the musculoskeletal system, four (0.9%) from the lymphatic system and one (0.2%) from milk. The examination was performed using conventional microbiological culture methods. The identification of isolates was confirmed using MALDI-TOF molecular phenotyping (Bruker Daltonics GmbH, Bremen, Germany). From the 276 Gram-negative isolates (prevalence of 61.5%), the most frequently detected strains were *Enterobacter* spp., *Escherichia* spp., *Acinetobacter* spp., *Pseudomonas* spp. and *Actinobacillus* spp. with prevalence rates of 7.6%, 6.7%, 6.7%, 6.0% and 5.8%. In addition, another 20 genera of microorganisms were detected. Susceptibility to antimicrobial agents was determined using the disc diffusion method. The most effective agents were gentamicin (94.1%), enrofloxacin (91.7%), colistin (87.0%), florfenicol (86.2%), neomycin (85.5%), streptomycin (82.4%) and tetracycline (78.5%). A good knowledge of the spectrum of bacterial species participating in pathological processes and lesions in horses and their antimicrobial susceptibility may be of great importance not only in treatment but also in deciding which prophylactic antibiotics to administer after surgical interventions.

Keywords: prevalence; pathogenicity; clinical; immunity; barrier; susceptibility; effective; therapy

Many species of microorganisms isolated from pathological lesions in various animals are not commonly referred to as pathogens in the veterinary literature. The aim of this study was to follow up on our previous work (Bzdil et al. 2017) in which Gram-positive species of microorganisms isolated from pathological processes and lesions of horses and their antimicrobial susceptibilities have been described. In this work, the genera and species of Gram-negative aerobic and microaerophilic microorganisms are mapped in detail. In previous work (Bzdil et al. 2017), we found that Gram-positive isolates obtained from horses, such as *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp. with prevalence rates of 33.9%, 19.8% and 7.6%, respectively, predominated not only in terms of the frequency of occurrence but also with respect to species diversity. Among

Supported by the Palacky University Olomouc, Czech Republic (Project No. LF_2016_025).

doi: 10.17221/117/2017-VETMED

Gram-negative microorganisms, *Actinobacillus equulii* is considered to be the most common cause of horse diseases (Wintzer 1999). Infections with this microorganism are considered septic, affecting the respiratory, gastrointestinal, lymphatic, urogenital and musculoskeletal systems and are fatal in most foals. In addition to *A. equulii*, Wintzer also described the occurrence of *Actinobacillus suis* in horses. The *Pasteurella* (*P. multocida*, *P. caballi*) and *Bordetella* (*B. bronchiseptica*) genera, which can cause bronchopneumonia in horses, are less important in the European region. The members of the genus *Haemophilus* are rather the domain of human medicine. Specifically, *H. influenzae* is described as the cause of otitis, sinusitis, conjunctivitis, pneumonia and invasive infections, especially in children (Murphy et al. 2009). The genus *Moraxella*, with the exception of *M. bovis*, is described in various animal species as a secondary pathogen (Quinn et al. 2011). For example, in racehorses a case of keratoconjunctivitis caused by *Moraxella bovoculi* was described (Liu et al. 2014). *Flavobacterium* spp. is usually isolated from water; while mainly considered as a cause of fish disease, in some cases it can also infect humans (Bernardet et al. 1996). Microorganisms of the genus *Stenotrophomonas* have also been described as infectious agents in the literature. For example, Berger and Frohlich (2011) reported a case of cystitis and endocarditis caused by *S. maltophilia*.

Using a newly developed method for the detection of equine respiratory pathogens called the LAMP assay, other authors have described the same agent to be linked to equine respiratory diseases (Kinoshita et al. 2015). Some authors indicate that Enterobacteriaceae, especially *Escherichia coli*, *Klebsiella* spp. or *Salmonella* sp., are less significant in horses than in calves and piglets (Wintzer 1999). However, even in horses, these microorganisms can cause severe diseases of the digestive, respiratory and urogenital tracts and can also lead to the development of sepses. *Acinetobacter* spp. are characterised as opportunistic pathogens of humans and animals; however, they are also isolated from other clinical conditions including post-operative wound infections (Quinn et al. 2011). In horses, this genus, along with coagulase-negative staphylococci, is reported to be dominant and is detected in up to 45% of cases (Boguta et al. 2002). Microorganisms of the genus *Alcaligenes* are considered to be saprophytes and are occasionally isolated from the digestive tracts of vertebrates (Quinn et al. 2011). These organisms have been linked with chronic laminitis in horses. However, their role in causing the disease remains unclear (Onishi et al. 2012). *Aeromonas* spp., *Vibrio* spp. and *Plesiomonas* spp. are bacteria with numerous common attributes. They are found in the aquatic environment (*Vibrio* spp.), and are mainly pathogens of fish and reptiles, while on rare occasions they also infect mammals. In addition to aquatic animals, *A. hydrophila* is also the cause of abortions in cattle, septicaemia and food poisoning; *Vibrio* spp. and *Plesiomonas shigelloides* are the cause of diarrhoeal diseases of birds and humans (Quinn et al. 2011). Other authors point out that *Aeromonas* spp. as well as their co-pathogens such as *Salmonella* spp. can be isolated from the gastrointestinal tract of horses with no symptoms of diarrhoea (Waldridge et al. 2011). *Burkholderia* members may cause dangerous zoonotic diseases in horses, such as glanders (*Burkholderia mallei*) and pseudomalleus (*Burkholderia pseudomallei*) (Wintzer 1999). Glanders was eliminated in Europe and is now found in Southeast Asia and the Arabian Peninsula (Wintzer 1999; Dominguez et al. 2016). Other species of *Burkholderia* spp. can also cause endotoxaemia due to their lipopolysaccharide structures (Hsueh et al. 2016). Similarly, *Pseudomonas* spp., especially *P. aeruginosa*, is isolated from dermatitis, rhinitis, haemorrhagic pneumonia and sepsis in farmed mink, as well as from bovine mastitis and may be the cause of nosocomial infections in humans (Quinn et al. 2011). In addition, it is a major pathogen in cystic fibrosis patients (Quinn et al. 2011). Other pseudomonads (e.g., *P. mendocina*) could be the cause of disease or sepsis as well (Ruggiero et al. 2016).

It is important to carefully determine the appropriate antibiotic therapy in cases of antibiotic intervention. In our previous work, we found florfenicol and amoxycillin with clavulanic acid to be the most effective antibiotics against Gram-positive infections of horses (Bzdil et al. 2017). Aminoglycosides such as gentamicin, kanamycin, neomycin, streptomycin, sulphonamides potentiated by trimethoprim and tetracyclines (Wintzer 1999) are frequently used as antibiotic therapies against Gram-negative microorganisms. Other authors believe that combinations of penicillins (penicillin, ampicillin, ticarcillin) and cephalosporins (ceftiofur) with aminoglycosides such as gentamicin and amikacin (Furr and Mogg 2003)

doi: 10.17221/117/2017-VETMED

are particularly suitable for the treatment of newborn foals.

Due to the growing variety of bacteriological findings from pathological processes and lesions in horses and the resistance of the isolated microorganisms, we are increasingly asked by private veterinarians to provide them with information on the pathogenicity of these bacterial strains, treatment options and the choice of appropriate antimicrobials. Therefore, the aim of this work was to describe the prevalence of individual isolated taxa and their susceptibility to antimicrobials that could be used in treatment or in prophylactic regimens, for example, after surgical interventions.

MATERIAL AND METHODS

All samples were collected from pathological lesions and processes of ill animals from stud farms in the Czech Republic and the following materials were included:

Samples from the digestive tract. Faeces, rectal swabs and swabs taken from the stomach lining were examined routinely using conventional methods of cultivation on meat peptone blood agar (MPBA), endo agar (EA) and xylose lysine deoxycholate citrate agar (XLD) (Trios s.r.o., Prague, Czech Republic) and plates were incubated aerobically at 37 ± 1 °C for 24 hours. Moreover, parallel cultivation focusing on organisms of the genus *Salmonella* was also carried out by non-selective enrichment of 1 g of material in 9 ml buffered peptone water (BPW) at 37 ± 1 °C for 18 h, selective enrichment of 0.1 ml of the incubated BPW on semisolid Rappaport-Vassiliadis agar (MSRV) at 41.5 ± 1 °C for 24 hours and subsequently double inoculated onto XLD agar and Rambach agar (RA) (Trios s.r.o., Prague, Czech Republic). The incubation of plates was again conducted at 37 ± 1 °C for 24 hours.

Samples from skin and the urinary system. The cultivation of hair, swabs, scrapings of skin, urine and swabs from the urinary tract was performed on MPBA, EA, Edward's agar (EDW) and Sabouraud agar with chloramphenicol (SAC) (All Trios s.r.o., Prague, Czech Republic) and the plates were again incubated aerobically at 37 ± 1 °C for 24 hours. SAC was incubated for 120 hours at 21 ± 1 °C. In indicated cases, these materials also underwent microaerophilic cultivation.

Samples from the oral cavity, eyes, respiratory, musculoskeletal and lymphatic systems. Swabs and the lavage of respiratory tract, pharynx, conjunctiva, oral mucosa, the puncture of chest, lymph nodes and joints were cultured as materials from the skin; in addition, microaerophilic incubation of inoculated plates with MPBA and Haemophilus test medium (HTM) (Trios s.r.o., Prague, Czech Republic) was also carried out. Microaerophilic cultivation took place in a 2.5-l volume plastic box for microaerophilic cultivation (GEN box) (BioMerieux is, Marcy l'Etoile, France) with CO_2 generated by the GENbox microaer (BioMerieux is, Marcy l'Etoile, France) for 48 hours at 37 ± 1 °C.

Mammary gland and milk samples. Colostrum was cultivated on MPBA (Trios s.r.o., Prague, Czech Republic), and incubation was carried out at 37 ± 1 °C for 42–48 hours. At the same time the samples were cultivated on SAC (Trios s.r.o., Prague, Czech Republic). These plates were incubated for 120 hours at 21 ± 1 °C. In parallel, milk samples were incubated after inoculation on MPBA in culture tubes at 37 ± 1 °C followed by 18–24 hours of incubation after inoculation onto EDW (Trios s.r.o., Prague, Czech Republic). EDW plates were incubated at 37 ± 1 °C for a further 18–24 hours.

Bacteriological confirmation and susceptibility determination. The intensity of the growth of microorganisms and the homogeneity of bacterial growth on each incubated plate were evaluated. All types of colonies on plates were isolated, suspicious Gram-negative organisms were isolated and subsequently confirmed with a molecular phenotyping method using mass detector MALDI-TOF and the original Bruker library of spectrums (Bruker Daltonics GmbH, Bremen, Germany). Clinical strains were tested for antibiotic susceptibility using the disc diffusion method. Mueller-Hinton agar (Trios s.r.o., Prague, Czech Republic) and antibiotic discs were used for testing (Oxoid Ltd., Basingstoke, UK). The tested antibiotics were penicillin G, streptomycin, neomycin, gentamicin, florfenicol, tetracycline, erythromycin, clindamycin, amoxycillin/clavulanic acid, enrofloxacin, bacitracin, cephalothin and co-trimoxazole. Tests were assessed after 18–24 hours of incubation at 37 ± 1 °C. Values were interpreted in accordance with CLSI standards (CLSI 2016) and Standard Operating Procedure SOP BAK 10/03 (Bzdil 2003). All used discs and media were tested with the ref-

doi: 10.17221/117/2017-VETMED

erence strains *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923).

RESULTS

A total of 449 clinical samples from horses were examined in the period 2009–2014; 229 (51.0%) of them were obtained from the respiratory tract, 121 (27.0%) from the skin, 40 (8.9%) from the digestive tract, 40 (8.9%) from the eyes, 8 (1.8%) from the urinary tract, six (1.3%) from the musculoskeletal system, four (0.9%) from the lymphatic system and one (0.2%) from milk. The complete list of tested samples is shown in Table 1. A total of 276 strains of Gram-negative aerobic or microaerophilic isolates (61.5% prevalence) were isolated from these samples (n = 449). These microorganisms were classified into 25 bacterial genera, with 12 genera (149 isolates) belonging to Enterobacteriaceae, i.e., 54.0% of all Gram-negative isolates. The remaining 46.0% of Gram negative isolates were from genera such as *Acinetobacter*, *Actinobacillus*, *Aeromonas*, *Alcaligenes*, *Bordetella*, *Burkholderia*, *Flavobacterium*, *Haemophilus*, *Moraxella*, *Pasteurella*, *Pseudomonas*, *Stenotrophomonas* and *Vibrio*. The most frequently isolated microbial strains were *Enterobacter* spp., *Escherichia* spp., *Acinetobacter* spp., *Pseudomonas* spp. and *Actinobacillus* spp. with prevalence rates of 7.6%, 6.7%, 6.7%, 6.0% and 5.8%. The most diverse genera were *Aeromonas* (six species), *Acinetobacter* (five species) and *Pseudomonas* (four species). The prevalence of particular isolated species is shown in Figure 1. Table 2 shows the percentage of the total number of tested bacterial strains that were susceptible to the selected antibiotics. The most effective agents were gentamicin (94.1%), enrofloxacin (91.7%), colistin (87.0%), florfenicol (86.2%), neomycin (85.5%), streptomycin (82.4%) and tetracycline (78.5%). A lower efficacy was observed with amoxycillin/clavulanic acid and co-trimoxazole: 69.4% and 64% of the tested strains, respectively, were susceptible. In contrast, very poor efficacy was observed with bacitracin, clindamycin, erythromycin and cephalothin: *in vitro* activity was only 5.0%, 6.0%, 13.5% and 38.9%, respectively. The most susceptible microorganisms were *Flavobacterium*, *Pasteurella* and *Bordetella* strains with susceptibility to 9, 8 and 7 antimicrobial agents, respectively. The greatest variability in susceptibility testing was observed for *E. coli*, which did not show 100% sensitivity to any of the tested antimicrobials.

DISCUSSION

The most frequently investigated samples were from the respiratory tracts of horses, which are apparently most often attacked by various microbial agents. Material from the skin constituted the second-placed group, which is in agreement with the data of Wintzer (1999). Samples from the digestive tract and from eyes were the third and fourth most frequent types of samples. On the other hand, urinary, musculoskeletal, lymphatic and milk samples occurred only sporadically. More than half of the isolated strains belonged to 12 genera of Enterobacteriaceae (prevalence 33.2%), which is probably due to their predominance in the stable microecosystem. The prevalence of this family is comparable to the proportion of Gram-positive microorganisms constituted by *Staphylococcus* spp. (33.9%) (Bzdil et al. 2017), and, therefore, its medical significance should not be underestimated in horses. Wintzer (1999) considers these

Table 1. Numbers and types of clinical samples from horses examined in the period 2009–2014

Sample type	Samples examined (%)	Sample type	Samples examined (%)	Sample type	Samples examined (%)
Nasal swab	187 (41.65)	urine	7 (1.56)	colostrum	1 (0.22)
Skin swab	98 (21.83)	articular punctate	6 (1.34)	pectoral punctate	1 (0.22)
Conjunctival swab	40 (8.91)	upper respiratory tract wash	6 (1.34)	urinary bladder swab	1 (0.22)
Throat swab (tonsils)	25 (5.57)	tracheal swab	5 (1.11)	hair	1 (0.22)
Anal swab	23 (5.12)	lymph nodes swab	4 (0.89)	oral swab	1 (0.22)
Skin scraping	22 (4.9)	sputum	3 (0.67)	stomach swab	1 (0.22)
Faeces	15 (3.34)	laryngeal swab	2 (0.45)	total	449 (100)

doi: 10.17221/117/2017-VETMED

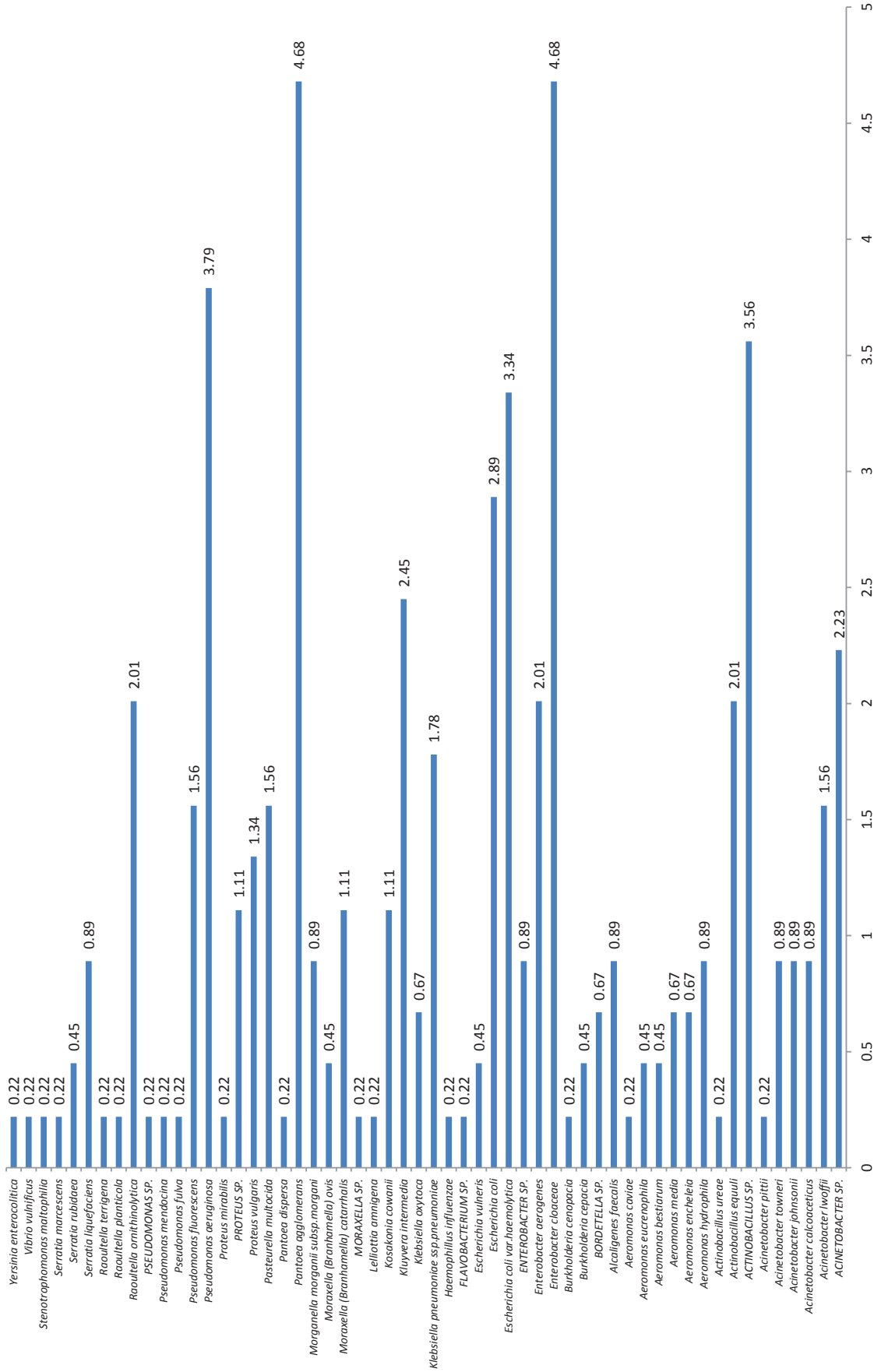

Figure 1. Prevalence of Gram-negative microorganisms isolated from pathological processes and lesions among horses in the period 2009–2014

doi: 10.17221/117/2017-VETMED

Table 2. Percentages of Gram-negative strains found to be susceptible to the tested antimicrobials

Active substance microorganism	Number examined/number susceptible (susceptible %)												
	colistin	streptomycin	neomycin	gentamicin	florfenicol	tetracycline	erythromycin	clindamycin	amoxycillin/ clavulanic acid	enrofloxacin	bacitracin	cephalothin	co-trimoxazole
Acinetobacter spp.	2/2 (100)	9/7 (77.8)	10/9 (90)	22/22 (100)	7/5 (71.4)	21/20 (95.2)	20/7 (35)	22/5 (22.7)	24/20 (83.3)	21/20 (95.2)	11/0 (0)	17/1 (5.9)	2/2 (100)
Actinobacillus spp.	1/1 (100)	10/5 (50)	8/6 (75)	20/19 (95)	6/6 (100)	20/18 (90)	19/4 (21.1)	20/2 (10)	18/15 (83.3)	20/20 (100)	8/0 (0)	20/16 (80)	2/2 (100)
Aeromonas spp.	1/1 (100)	2/2 (100)	4/4 (100)	11/11 (100)	5/4 (80)	11/7 (63.6)	11/0 (0)	11/0 (0)	11/2 (18.2)	11/10 (90.9)	3/0 (0)	11/0 (0)	1/1 (100)
Alcaligenes spp.	NT	NT	1/1 (100)	2/2 (100)	NT	2/1 (50)	2/0 (0)	2/0 (0)	2/1 (50)	2/2 (100)	1/0 (0)	2/0 (0)	NT
Bordetella spp.	NT	NT	NT	1/1 (100)	NT	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	NT	1/1 (100)	NT
Burkholderia spp.	NT	NT	1/1 (100)	NT	NT	1/0 (0)	1/0 (0)	1/0 (0)	1/1 (100)	1/1 (100)	1/0 (0)	1/0 (0)	NT
Enterobacter spp.	4/3 (75)	5/5 (100)	11/8 (72.7)	15/15 (100)	3/2 (66.7)	15/13 (86.7)	15/0 (0)	15/0 (0)	15/5 (30)	15/15 (100)	7/0 (0)	15/4 (26.7)	NT
Escherichia coli	30/27 (90)	12/11 (91.7)	13/11 (84.6)	53/50 (94.3)	25/23 (92)	53/38 (71.7)	27/0 (0)	43/0 (0)	53/42 (79.2)	53/49 (92.5)	10/0 (0)	28/7 (25)	27/20 (74.1)
Flavobacterium spp.	NT	1/1 (100)	1/1 (100)	1/1 (100)	NT	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/0 (0)	NT
Haemophillus spp.	1/1 (100)	NT	NT	NT	1/1 (100)	1/1 (100)	NT	1/0 (0)	1/1 (100)	1/1 (100)	NT	NT	1/1 (100)
Klebsiella/ *Raoultella* spp.	5/5 (100)	12/12 (100)	1/1 (100)	17/17 (100)	6/6 (100)	16/14 (87.5)	15/0 (0)	16/0 (0)	16/10 (62.5)	19/18 (94.7)	1/0 (0)	15/9 (60)	5/4 (80)
Kluyvera spp.	NT	1/1 (100)	NT	1/1 (100)	NT	1/1 (100)	1/0 (0)	1/0 (0)	1/0 (0)	1/1 (100)	NT	1/0 (0)	NT
Kosakonia spp.	NT	2/2 (100)	NT	2/2 (100)	1/1 (100)	2/2 (100)	2/0 (0)	2/0 (0)	2/1 (50)	2/2 (100)	NT	2/1 (50)	NT
Lelliottia spp.	NT	NT	NT	1/1 (100)	1/0 (0)	1/1 (100)	1/0 (0)	1/0 (0)	1/0 (0)	1/1 (100)	NT	1/0 (0)	NT
Moraxella/ *Branhamella* spp.	NT	NT	2/2 (100)	6/0 (0)	2/2 (100)	6/6 (100)	6/4 (66.7)	6/2 (33.3)	6/5 (83.3)	6/6 (100)	2/1 (50)	6/5 (83.3)	1/1 (100)
Morganella spp.	1/0 (0)	NT	2/2 (100)	2/2 (100)	1/1 (100)	2/1 (50)	2/0 (0)	2/0 (0)	2/0 (0)	2/0 (0)	2/0 (0)	2/0 (0)	NT
Pantoea spp.	1/1 (100)	8/7 (87.5)	6/5 (83.3)	16/16 (100)	3/3 (100)	9/9 (100)	16/0 (0)	16/0 (0)	16/15 (93.8)	16/16 (100)	5/1 (20)	16/12 (75)	NT
Pasteurella spp.	NT	4/4 (100)	1/1 (100)	6/6 (100)	2/2 (100)	6/6 (100)	6/5 (83.3)	6/0 (0(	6/6 (100)	6/6 (100)	1/0 (0)	6/6 (100)	NT
Proteus spp.	1/0 (0)	NT	3/3 (100)	4/4 (100)	NT	4/0 (0)	4/0 (0)	4/0 (0)	4/4 (100)	4/2 (50)	3/0 (0)	4/1 (25)	1/0 (0)
Pseudomonas spp.	6/6 (100)	3/1 (33.3)	2/2 (100)	15/14 (93.3)	2/0 (0)	6/4 (66.7)	6/0 (0)	6/0 (0)	6/1 (16.7)	15/9 (60)	2/0 (0)	6/0 (0)	9/0 (0)
Serratia spp.	NT	3/2 (66.7)	1/1 (100)	5/5 (100)	NT	5/2 (40)	5/0 (0)	5/0 (0)	4/1 (25)	5/5 (100)	1/0 (0)	5/0 (0)	NT
Stenotrophomonas spp.	1/0 (0)	NT	NT	1/1 (100)	NT	NT	NT	NT	NT	1/1 (100)	NT	NT	1/1 (100)
Vibrio spp.	NT	1/1 (100)	1/1 (100)	1/1 (100)	NT	1/0 (0)	1/0 (0)	1/0 (0)	1/1 (100)	1/1 (100)	1/0 (0)	1/0 (0)	NT
Yersinia spp.	NT	1/0 (0)	1/0 (0)	1/0 (0)	NT	1/0 (0)	1/0 (0)	1/0 (0)	1/1 (100)	1/1 (100)	NT	1/0 (0)	NT
Total	54/47 (87)	74/61 (82.4)	69/59 (85.5)	203/191 (94.1)	65/56 (86.2)	186/146 (78.5)	163/22 (13.5)	184/11 (6)	193/134 (69.4)	206/189 (91.7)	60/3 (5)	162/63 (38.9)	50/32 (64)

NT = not tested

doi: 10.17221/117/2017-VETMED

microorganisms less important in horses than in calves and piglets. Conversely, the prevalence of *Actinobacillus* spp. was in our case only 5.8%. These findings confirm that, for instance, *Pasteurella* spp. or *Bordetella* spp. are in our climatic conditions actually less significant in terms of prevalence (Wintzer 1999). The situation is similar in *Haemophilus* spp. (Murphy et al. 2009), *Alcaligenes* spp., *Moraxella* spp. (Quinn et al. 2011; Onishi et al. 2012; Liu et al. 2014) and *Stenotrophomonas* spp. (Berger and Frohlich 2011; Kinoshita et al. 2015). The finding of *Flavobacterium* spp., which is described as a fish pathogen (Bernardet et al. 1996), is very interesting, as are those of *Aeromonas* spp. and *Vibrio* spp. (Quinn et al. 2011; Waldridge et al. 2011). Six species of *Aeromonas* were examined in this study. The results indicate a high frequency of genus *Acinetobacter* with a prevalence of 6.7%. Five species of *Acinetobacter* spp. were isolated. A similar situation was surprisingly observed for *Pseudomonas* spp., which was in this study isolated in 6.0% cases; four different species were identified (Quinn et al. 2011). The occurrence of *Burkholderia* genera was negligible. Species causing glanders and melioidosis were not detected (Wintzer 1999; Dominguez et al. 2016). It is encouraging that we have detected neither agents causing dangerous equine infections (*Taylorella equigenitalis*) nor important zoonotic agents such as *B. mallei*, *B. pseudomallei*, *Brucella* spp., *Francisella* spp., *Salmonella* spp. and *Campylobacter* spp. etc. among our examined Gram-negative microorganisms. Overall, these results indicate that the most effective antimicrobials for the treatment of pathological processes caused by Gram-negative microorganisms are gentamicin, enrofloxacin, colistin, florfenicol, neomycin, streptomycin and tetracycline, which is consistent with literature data (Wintzer 1999; Furr and Mogg 2003; Bzdil et al. 2017). Compared to our previous work (Bzdil et al. 2017), we have here reported significant resistance to tetracyclines and to co-trimoxazole. It is interesting that those resistances were similar among Gram-positive and Gram-negative microorganisms. These Gram-positive and Gram-negative microorganisms were resistant to tetracycline in 19.1% and 21.5% of cases and to co-trimoxazole in 37.0% and 38.0% of cases, respectively. Preparations combining beta-lactams with aminoglycosides, quinolones (enrofloxacin) and phenicols (florfenicol) should target most Gram-positive and Gram-negative microorganisms. Therefore, they are suitable for use in horses both for the treatment of bacterial diseases and for so-called antibiotic prophylaxis after surgical procedures or injuries.

In conclusion, we have described the isolation of a relatively wide spectrum of Gram-negative aerobic and microaerophilic microorganisms from clinical material from horses suffering from various pathological processes and lesions. Enterobacteriaceae clearly prevailed among the isolates over the monitored period. In the literature, most of our isolated strains are associated with pathological processes in various animal species. Therefore, the involvement of these microorganisms in equine diseases cannot be excluded. Similar as in Gram-positive microorganisms, a decisive factor in the pathogenesis might be the functional impairment of natural somatic barriers and the weakening of the animal immune system. Even in the case of Gram-negative microorganisms, a good knowledge of their spectrum, their ability to evoke or participate in pathological lesions and processes in horses and their sensitivity to antimicrobial agents is of great practical value. Our data on antibiotic susceptibility can greatly facilitate the choice of a suitable antibiotic therapy.

Acknowledgements

We are thankful to the director of the State Veterinary Institute Olomouc, associate professor MVDr. Jan Bardon, Ph.D., M.B.A. for providing material and financial support during the preparation and publication of this work.

REFERENCES

Berger S, Frohlich W (2011): Stenotrophomonas maltophilia as a causative agent of bacterial infections in the horse: two clinical cases. Pferdeheilkunde 27, 381–385.

Bernardet JF, Segers P, Vancanneyt M, Berthe F, Kersters K, Vandamme P (1996): Cutting a Gordian knot: emended classification and description of the genus Flavobacterium, emended description of the family Flavobacteriaceae, and proposal of Flavobacterium hydatis norn. nov. (basonym, Cytophaga aquatilis Strohl and Tait 1978). International Journal of Systematic Bacteriology 46, 128–148.

doi: 10.17221/117/2017-VETMED

Boguta L, Gradzki Z, Borges E, Maurin F, Kodjo A, Winiarczyk S (2002): Bacterial flora in foals with upper respiratory tract infections in Poland. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health 49, 294–297.

Bzdil J (2003): Determination of Susceptibility of Microorganisms to Antimicrobials by Disc Diffusion Method. Standard Operating Procedure SOP BAK 10/03, SVI Olomouc. p 4.

Bzdil J, Holy O, Chmelar D (2017): Gram-positive aerobic and microaerophilic microorganisms isolated from pathological processes and lesions of horses. Veterinarni Medicina 62, 1–9.

CLSI – Clinical and Laboratory Standards Institute (2016): Performance Standards for Antimicrobial Susceptibility Testing– M100-S26. Twenty-six informational supplement. Wayne, Pensylvania. 296 pp.

Dominguez M, Munstermann S, de Guindos I, Timoney P (2016): Equine disease events resulting from international horse movements: Systematic review and lessons learned. Equine Veterinary Journal 48, 641–653.

Furr M, Mogg TD (2003): Antimicrobial treatment of neonatal foals. Compendium 25, 302–309.

Hsueh PT, Liu CL, Wang HH, Ni WF, Chen YL, Liu JK (2016): A comparison of the immunological potency of Burkholderia lipopolysaccharides in endotoxemic BALB/c mice. Microbiology and Immunology 60, 725–739.

Kinoshita Y, Niwa H, Katayama Y (2015): Use of loop-mediated isothermal amplification to detect six groups of pathogens causing secondary lower respiratory bacterial infections in horses. Microbiology and Immunology 59, 365–370.

Liu H, Yan J, Wang Y, Yan Q, Zhao L, Yan R, He H (2014): Isolation of Moraxella bovoculi from racehorses with keratoconjunctivitis. Journal of Veterinary Diagnostic Investigation 26, 585–587.

Murphy TF, Faden H, Bakaletz LO, Kyd JM, Forsgren A, Campos J, Virji M, Pelton SI (2009): Nontypeable Haemophilus influenzae as a pathogen in children. Pediatric Infectious Diseases Journal 28, 43–48.

Onishi JC, Park JW, Haggblom MM, Fennell MJ, Fugaro MN (2012): Chronic laminitis is associated with potential bacterial pathogens in the laminae. Veterinary Microbiology 158, 329–336.

Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S, Hartigan PJ (2011): Veterinary Microbiology and Microbial Disease. 2nd edn. Blackwell Publishing, Oxford. 912 pp.

Ruggiero NA, Chang M, Brown S (2016): Pseudomonas mendocina Bacteremia, A Case Study and Review of Literature. Clinical Infectious Diseases 24, 314–317.

Waldridge BM, Stewart AJ, Taylor DC, Saville WJ (2011): The Incidence of Aeromonas species in the feces of non-diarrheic Horses. Journal of Equine Veterinary Science 31, 700–702.

Wintzer HJ (1999): Horse diseases, study and practice guide. Slovak/Czech edition. Hajko and Hajkova, Bratislava. 538 pp.

Received: September 7, 2017
Accepted after corrections: December 29, 2017