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Original Article
Özgün Araştırma

Agents isolated from horses with respiratory system infection signs

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Abstract: In this study, it was aimed to isolate the agents and to determine the antibiotic susceptibility of the agents by taking nasal swabs from horses with respiratory system problems. Nasal swab samples were taken from 30 horses of different age groups with respiratory system symptoms in South Marmara Region. The samples were cultured on Blood agar, MacConkey agar, Mycoplasma agar and, Sabouraud Dextrose agar. Colony morphology, gram staining and biochemical tests were performed for identification. Disk diffusion method was used to determine the antimicrobial susceptibility of the agents. Ten different microorganism species were isolated from nasal swabs taken from horses of different ages with respiratory system problems. As a result of the biochemical tests, 9 *Streptococcus zooepidemicus* (27.27%), 4 *Staphylococcus aureus* (12.12%), 4 Coagulase-negative staphylococci (CoNS) (12.12%), 4 *Klebsiella pneumoniae* (12.12%), 3 *Pasteurella* spp. (9.09%), 2 *Streptococcus pyogenes* (6.06%), 2 *Bacillus* spp. (6.06%), 2 *Corynebacterium* spp. (6.06%), 2 *Candida* spp. (6.06%) and 1 *Rhodococcus equi* (3.03%) were identified. The most *Streptococcus zooepidemicus* (27.27%) and the least *Rhodococcus equi* (3.03%) were identified from nasal swab samples. The most sensitive antibiotic of the isolates was Amoxicillin / clavulanic acid (96.87%), and the most resistant was Penicillin-G (18.75%).

Keywords: Antibiogram test, bacteria, fungi, horse, nasal swab, respiratory diseases

Solunum sistemi infeksiyon semptomlu atlardan izole edilen etkenler

Özet: Bu çalışmada solunum sistemi sorunu olan atlardan nasal svaplar alınarak etkenlerin izolasyonu ve antibiyotik duyarlılıklarının belirlenmesi amaçlanmıştır. Güney Marmara Bölgesi'nde solunum sistemi semptomları olan farklı yaş gruplarındaki 30 attan nasal svap örnekleri alındı. Örnekler Blood agar, MacConkey agar, Mycoplasma agar ve Sabouraud Dextrose agarda kültüre edildi. İdentifikasyon için koloni morfolojisi incelemesi, gram boyama ve biyokimyasal testler yapıldı. Etkenlerin antimikrobiyal duyarlılıklarını belirlemek için disk difüzyon yöntemi kullanıldı. Solunum sistemi sorunları olan farklı yaşlardaki atlardan alınan nasal svaplardan 10 farklı mikroorganizma türü izole edildi. Biyokimyasal testler sonucunda 9 *Streptococcus zooepidemicus* (%27,27), 4 *Staphylococcus aureus* (%12,12), 4 Koagülaz negatif stafilokok (KNS) (%12,12), 4 *Klebsiella pneumoniae* (%12,12), 3 *Pasteurella* spp. (%9.09), 2 *Streptococcus pyogenes* (%6.06), 2 *Bacillus* spp. (%6.06), 2 *Corynebacterium* spp. (%6.06), 2 *Candida* spp. (%6.06) ve 1 *Rhodococcus equi* (%3.03) tespit edildi. Nasal svap örneklerinden en fazla *Streptococcus zooepidemicus* (%27.27) ve en az *Rhodococcus equi* (%3.03) izole edildi. İzolatların en duyarlı olduğu antibiyotik Amoksisilin/klavulanik asit (%96.87), en dirençli olduğu ise Penisilin-G (%18.75) idi.

Anahtar Kelimeler: Antibiogram test, bacteria, fungi, horse, nasal swab, respiratory diseases

Introduction

Respiratory problems are among the most important disorders that limit the athletic performance of horses. For this reason, early detection of respiratory problems, adequate and appropriate treatment is very important, especially in race horses, to maximize the chances of recovery, to reduce economic losses and performance losses (Van Erck-Westergren et al. 2013). Infectious agents cause clinical and subclinical respiratory problems in horses. Environmental factors play a very important role in the development of respiratory diseases. Horses housed indoors are exposed to high levels of organic dust, including bacteria and fungi. Materials such as straw and straw naturally contain an important native microflora that can grow further when stored or used as feed or bedding (Carvallo et al. 2017). In addition, respiratory system infections are an important cause of death in foals (Vitale et al. 2019). Generally, viral and bacterial upper respiratory tract infections occur in foals aged 1, inflammatory respiratory tract disease and pleuropneumonia occur in race horses aged 2 or more. Respiratory tract neoplasms generally occur in horses 5 years and older (Ainsworth et al. 2010).

Respiratory infection causes significant economic losses, especially for young performance horses (Dyson et al. 2008). Infectious diseases affecting the upper and lower respiratory tract of horses are common (Gilkerson et al. 2015). These

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are usually associated with pathogenic bacterial invasions (Reuss and Giguère 2015), viral infections (Singh et al. 2018), and rarely fungal agents (Stewart and Cuming 2015). In respiratory system infections in horses; *Rhodococcus equi (R. equi), Streptococcus equi subsp. equi (S. equi subsp. equi), Streptococcus equi* subsp. *zooepidemicus (S. equi subsp. zooepidemicus), Staphylococcus aureus (S. aureus), Streptococcus pneumoniae (S. pneumoniae), Pasteurella* spp., *Actinobacillus* spp., *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Bordetella bronchiseptica (B. bronchiseptica)* and *Mycoplasma* spp. (Chapman et al. 2000; Christley et al. 2001; Wood et al 2005; Couetil et al 2016; Kasap et al 2018; Pusterla et al 2020; Nehal et al. 2021) isolated. *S. equi* subsp. *zooepidemicus* is a normal commensal of healthy horses. However, it is an important opportunistic pathogen in respiratory tract infections in foals and horses due to exercise, temperature, transport or viral infections (Velineni et al. 2014).

Viral pathogens such as Equine herpes virus (EHV), Equine Influenza (EI), Equine Viral Arteritis (EVA) and Equine Rhinovirus A (ERAV) and B (ERBV) (Ainsworth and Cheetham 2010, Laing et al 2020) and fungal pathogens such as *Blastomyces* spp., *Histoplasma* spp., *Cryptococcus* spp., *Coccidioides* spp. and *Candida* spp. are also important for the respiratory system (Cordeiro et al. 2013; Stewart and Cuming 2015). These agents may cause infection alone or may cause mixed infection (Kulkarni et al. 2019).

Although clinical findings show differences, exercise intolerance, cough, runny nose, fever, respiratory problems, increased respiratory effort, general depression, anorexia, lethargy and weight loss can be observed (Couetil et al. 2016).

Pneumonia cases in adult horses mostly develop as a result of bacteria aspirated from the environment, nose or oropharynx reaching the lower respiratory tract and suppression of pulmonary defense mechanisms. Numerous opportunistic pathogens have been isolated in cases of pneumonia in horses, and mixed aerobic and anaerobic bacteria are present in many cases (Reuss and Giguère 2015). In race horses, pneumonia most commonly occurs in association with aerobic bacteria that form one or more microflora of the upper respiratory tract mucosa (Chapman et al. 2000). Opportunistic respiratory pathogens frequently isolated in cases of pneumonia are *Streptococcus equi* subsp. *zooepidemicus*, *Pasteurella* spp. and *Bordetella bronchiseptica*, but *Pseudomonas aeruginosa* and *Escherichia coli* are also commonly detected in respiratory tract samples (Fonseca et al 2020).

Although some fungal species are accepted as primary agents of respiratory tract infections in many mammals and the nasal cavity is important for the colonization of fungal species, research on mycotic agents in respiratory infections of horses is limited. *Candida* spp., as an opportunistic pathogen, causes many infections in adult horses and foals (Riley et al. 1992; Reilly and Palmer 1994; Stewart and Cuming 2015, Dauvillier atl al 2018).

Cytological examination of respiratory tract samples and bacterial culture are useful and reliable methods for diagnosing these infections, determining their etiology and determining the appropriate antibiotic treatment (Sweeney et al. 2005; Reuss and Giguere 2015). It is important to correctly identify and diagnose bacterial respiratory tract infections. Because correct and effective treatment can be performed by using antimicrobials suitable for the agents isolated and identified according to the laboratory results (Wilson 2001; Morley et al. 2005; Bowen 2013).

In cases where respiratory system infections are suspected in horses, agent isolation and antibiogram tests are generally not used. Empirical therapy is usually initiated when a bacterial infection is suspected (Christley et al. 2000). Empirical treatment may cause deterioration of prognosis, increase in clinical findings and antibiotic resistance. It is very important to perform antibiogram tests in respiratory system infections of horses in order to obtain more successful results from the treatment. Proper selection of antibiotics; It shortens the treatment time and provides a good prognosis. It also reduces the cost of treatment and prevents antibiotic resistance that will occur as a result of unnecessary antibiotic use.

The aim of this study is to isolate bacterial and fungal agents from nasal swabs taken from horses showing signs of respiratory system infection and to determine the antibiogram sensitivity of the isolated bacterial agents.

Material and Methods

Samples

Thirty nasal swab samples were taken from 30 race horses aged 3 months to 4 years with clinical findings such as nasal discharge, anorexia and cough. Nasal swabs were advanced deeply (> 30 cm) into the ventral nasal concha. Swabs were placed into a semi-solid Amies transport medium. Swabs were delivered to the laboratory by providing a cold chain.

Bacteriological examination

Nasal swaps were incubated in nutrient broth (Oxoid, CM0001B) at 37°C for 8-12 hours under aerobic conditions (Broux et al. 2016; Espindola et al. 2022). Blood Agar No.2 with 5% defibrinated sheep blood (Oxoid, CM0271), MacConkey Agar (Oxoid, CM0007), Mycoplasma Agar (Oxoid, CM0401) were used for bacterial isolations. Samples were incubated at 37°C for 1-5 days under microaerophilic and aerobic conditions. Growing colonies were examined according to colony morphology and hemolysis characteristics. In addition, Gram staining was performed and evaluated according to their staining properties and microscopic morphology. Pure cultures were prepared and identified by routine biochemical tests. Biochemical tests for oxidation/fermentation, coagulase, catalase, cytochrome oxidase, urease and indole production were applied for identification. Bile solubility and optochin susceptibility tests were performed to identify *S. pneumoniae*. Trehalose and Sorbitol utilization tests were performed to distinguish among *S. equi* and *S. zooepidemicus*. Colonies suspected to be *R. equi* were subcultured and identified by colony morphology, Gram stain, biochemical tests and CAMP test. Bacterial identification was performed using standard API 20E (REF 20160), API20 NE (REF 20050), API Staph (REF 20500), API Strep (REF 20600), API Coryne kits (REF 20600) (BioMerieux).

Fungal examination

All samples were cultured on Sabouraud dextrose agar with Chloramphenicol (Oxoid, CM41). It was incubated at 25°C for 2-4 weeks. In addition, Brain heart infusion agar (Oxoid, CM1136B) containing 0.05 mg/ml of Chloramphenicol (Oxoid, SR0078) and Cycloheximide (1%) (Oxoid, SR0222C) was used. Cultures were incubated at 37°C for 2-4 weeks and examined every 3 days for the presence of fungal organisms.

Fungal colonies were evaluated according to their macroscopic properties. The shape, texture and color of fungal colonies in SDA and BHIA were examined In addition, the microscopic properties of were stained with lactophenol cotton blue. Fungal colonies were identified according to their macroscopic and microscopic features.

Antibiogram test

Kirby-Bauer Disk Diffusion were used to determine antibacterial susceptibility. It was performed on Mueller-Hinton agar using the standard disk diffusion method, equivalent to the 0.5 McFarland turbidity standard. Testing and evaluation were done according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) (Anonymous 2002; CLSI 2017).

Commercially available antibiotic discs (Oxoid) were used in the study. These discs; Gentamicin (10mµg), Cephazolin (30 mµg), Erythromycin (10 mµg), Oxytetracycline (30 mµg), Streptomycin (10 mµg), Amoxycilin / Clavulanic acid (30 mµg), Enrofloxacin (5 mµg), Penicillin-G (10 U), Ampicillin / Sulbactam (30 mµg) and Trimethoprim / Sulfamethoxazole (25 mµg).

All collected clinical samples were inoculated into the brain hearth infusion broth and incubated at 37°C until the turbidity reached the 0.5 McFarlands standard. The inoculum was spread over the entire surface of the medium of Mueller-Hilton agar with a drigalski spatula and left for 10-15 minutes. Antibiotic discs were placed under aseptic conditions using sterile forceps. Plates were then incubated at 37°C for 14-18 hours. After incubation, inhibition zone diameters were measured in millimeters and standard zone diameters were compared with the standards specified by the "Clinical and Laboratory Standards Institute (CLSI)" and evaluated as "susceptible" or "resistant" according to results.

Results

Bacterial isolation and identification

As a result of the examination performed from nasal swabs taken from 30 horses between 3 months and 4 years of age, showing signs of nasal discharge and cough, 9 different bacterial species and 1 yeast species were isolated (Table 1). Two different bacteria were isolated as mixed culture from two nasal swabs, and one species of bacteria was isolated from 28 as pure culture.

Table 1. Bacterial and fungal species isolated from nasal swabs

Microorganism	Number of isolates (%)
S. equi subsp. zooepidemicus	9 (26.47)
Coagulase-negative staphylococci (CoNS)	5 (14.70)
S. aureus	4 (11.76)
Klebsiella pneumoniae	4 (11.76)
Pasteurella spp.	3 (8.82)
Bacillus spp.	2 (5.88)
S. pyogenes	2 (5.88)
Corynebacterium spp.	2 (5.88)
R. equi	1 (2.94)
Candida spp.	2 (5.88)
Total	**34 (100)**

The distribution of isolated factors according to age groups is presented in Table 2.

R. equi was isolated from a 4 month old foal. Fifteen different agents were isolated from the foals in the 3-12 months age group, and 18 different agents were isolated from the horses and foals in the 2-4 age group. More species of bacteria have been isolated from horses and foals over 2 years of age. *Candida* spp. was isolated from animals aged 2-4 years.

The distribution of isolated agents by age groups is presented in Table 2.

Table 2. Distribution of isolated agents by age groups.

Age Groups	Species	Number of isolates	Total
3-6 months	*S. equi subsp. zooepidemicus*	2	7
	S. aureus	1	
	Coagulase-negative *Staphylococci*	2	
	Pasteurella spp.	1	
	R. equi	1	
6-12 months	*S. equi subsp. zooepidemicus*	2	9
	S. aureus	1	
	Coagulase-negative Staphylococci	3	
	Klebsiella pneumoniae	1	
	Bacillus spp.	1	
	Corynebacterium spp.	1	
2-4 years	S. equi subsp. zooepidemicus	5	18
	S. aureus	2	
	Klebsiella pneumoniae	3	
	Pasteurella spp.	2	
	Bacillus spp.	1	
	S. pyogenes	2	
	Corynebacterium spp.	1	
	Candida spp.	2	

Antibiogram test

Amoxycilin / Clavulanic acid and Ampicillin / Sulbactam were determined as the antibiotics to which the agents were most sensitive, Penicillin-G was the antibiotic to which the isolated agents were most resistant. The results are shown in Table 3.

Streptococcus spp. was found to be highly sensitive to Amoxycilin / Clavulanic acid (30 mµg), Ampicillin / Sulbactam (30 mµg) and Trimethoprim / Sulfamethoxazole (25 mµg). *Staphylococcus* spp. was more sensitive to Amoxycilin / Clavulanic acid (30 mµg), Ampicillin / Sulbactam (30 mµg) and Enrofloxacin (5 mµg).Other isolated agents were generally susceptible to Amoxycilin / Clavulanic acid and Ampicillin / Sulbactam. The results are shown in Table 4.

Table 3. Antibiotic sensitivity/resistance of agents

Antibiotic	Sensitivity (%)	Resistance (%)
Amoxycilin/ Clavulanic acid	96.87	3.12
Ampicillin/ Sulbactam	93.75	6.25
Trimethoprim/ Sulfamethoxazole	87.50	12.50
Enrofloksasin	84.37	15.62
Oxytetracycline	84.37	15.62
Cephazolin	65.62	34.37
Gentamisin	53.12	46.87
Erythromycin	37.50	62.50
Streptomycin	34.37	65.62
Penisilin-G	18.75	81.25

Table 4. Antibiotic susceptibility of isolated bacterial strains

Antibiotic	Sensitivity-S / Resistance R (%)	*S. equi* subsp. *zooepidemicus*	*S. aureus*	Coagulase negative Staphylococci	*Klebsiella pneumoniae*	*Pasteurella* spp.	*Bacillus* spp.	*S. pyogenes*	*Corynebacterium* spp.	*R. equi*
Gentamisin	S/R	66.66/33.33	25/75	80/20	50/50	66.66/33.33	50/50	0/100	50/50	0/100
Cephazolin	S/R	44.44/55.55	75/25	100/0	75/25	33.33/66.66	100/0	0/100	100/0	100/0
Erythromycin	S/R	11.11/88.88	50/50	80/20	25/75	33.33/66.66	50/50	0/100	100/0	0/100
Oxytetracycline	S/R	77.77/22.22	75/25	100/0	75/25	100/0	50/50	100/0	100/0	100/0
Streptomycin	S/R	22.22 /77.77	25/75	60/40	25/75	33.33/66.66	50/50	50/50	50/50	0/100
Amoxycilin/ Clavulanic acid	S/R	88.88/11.11	100/0	100/0	100/0	100/0	100/0	100/0	100/0	100/0
Enrofloksasin	S/R	66.66/33.33	100/0	100/0	75/25	100/0	100/0	50/50	100/0	100/0
Penisilin-G	S/R	0/100	25/75	20/80	25/75	33.33/66.66	50/50	0/100	50/50	0/100
Ampicillin/ Sulbactam	S/R	88.88/11.11	100/0	100/0	100/0	100/0	100/0	50/50	100/0	100/0
Trimethoprim/ Sulfamethoxazole	S/R	88.88/11.11	75/25	100/0	75/25	100/0	100/0	100/0	50/50	100/0

Discussion and conclusion

Respiratory diseases have been reported to be among the most common diseases in horse breeding in countries around the world (Sharma et al 2017; Kasap et al 2018; Vitale et al 2019). In particular, unsuitable shelter conditions, crowded environments and unfavorable climatic conditions increase the exposure of horses to different respiratory pathogens (Wood et al 2005).

S. zooepidemicus, *S. aureus*, *S. pneumoniae*, *Pasteurella* spp., *Actinobacillus* spp., *E. coli*, K. *pneumonia*, *B. bronchiseptica* and *Enterobacter* spp. are the most isolated bacterial respiratory pathogens from horses. *Str. zooepidemicus* is the most frequently isolated bacteria (Chapman et al 2000; Bianchi et al 2018; Kasap at al 2018; Preziuso et al 2019; Bianchi et al 2020). In a study conducted by Broux et al (2016), nasal swaps taken from 103 horses showing signs of respiratory system infection were found to be 30% *S. equi* subsp. *zooepidemicus* has been isolated (Broux et al. 2016). In a study of racehorses with pleuropneumonia, bronchopneumonia, embolic pneumonia and pleuritis, *S. equi* subsp. *zooepidemicus* 72%, *Pasteurella* spp. 5%, *Klebsiella* spp. and *Staphylococcus* spp. 4% were isolated. (Carvallo et al. 2017). In nasopharyngeal swabs taken from horses with acute respiratory problems, 45.7% coagulase negative *Staphylococcus* spp., 18.5% *S. equi* subsp. *zooepidemicus* and 3.3% coagulase positive *Staphylococcus* spp. were isolated (Carman et al. 1997). *S. equi* subsp. *zooepidemicus* is an opportunistic pathogen of the upper respiratory tract. (Christley et al 2001; Pusterla et al 2020). In another study, *R. equi* was isolated in 10 of nasal swap samples taken from 1010 horses (Gressler et al 2018). In a study conducted by Fonseca et al (2020), the highest rate of isolated *S. zooepidemicus* was 22.9%. Other agents isolated from the upper respiratory tract of horses were found as *S. equi* (14.1%), *E coli* (17.5%), coagulase-negative *staphylococci* (17.3%), respectively. Bianchi et al. (2018) examined 50 lungs with bronchopneumonia and identified *S. zooepidemicus* in 21 of them. *S. zooepidemicus* (27.4%) and *E. coli* (15.6%) were isolated primarily from racehorses with lower respiratory tract disorders (Kasap et al. 2018). In our study, *S. zooepidemicus* was the agent with the highest rate with 27.27%, and then, coagulase negative *Staphylococcus* spp., *Klebsiella* spp. and *S. aureus* were isolated in 12.12%.

The agents and isolation rates in our study were similar to those in other studies. *R. equi* is one of the most important causes of pneumonia in foals between 1 and 6 months of age. *R. equi* infection is usually characterized by an acute respiratory problem in foals followed by death within a few hours or days. Therefore, accurate and early diagnosis of *R. equi* infection is important. In a study conducted in northern Brazil, *R. equi* was detected in all cases of pyogranulomatous pneumonia in young foals. (Bianci et al. 2020).

In a study, it was reported that *Candida* spp. was isolated from 35 nasal swabs from 97 horses (Cordeiro et al. 2013). *Penicillium* spp. (53%), *Aspergillus* spp. (34%), *Rhizomucor* spp. (5%), and *Candida* spp. (5%) were isolated from horses with respiratory infection (Dauvillier et al. 2019). In this study, only 2 *Candida* spp. (6.06%) were isolated. It has been reported that fungal spores in stored hay, which are used as feed and bedding in respiratory system infections, may cause mycotic respiratory infections in horses by alveolar inhalation. Fungal agents in the respiratory system can have infective, toxic, allergic or a combination of all three effects (Dauvillier et al. 2019).

In a study examining the antibacterial susceptibilities of agents isolated in respiratory system infections in horses, it was reported that *Staphylococcus* spp. was sensitive to Ceftifour, Enrofloxacin, Gentamicin and Tetracyclines at a rate of 81.5% to 97.5%, and to Penicillin at a lower rate (57.1%). *Streptococcus* spp. isolated in the study was found to be susceptible to Ceftifour, Enrofloxacin, Gentamicin and Penicillin at a rate of 80% -97% and Tetracycline at a rate of 59% (Toombs-Ruane et al. 2015). In a study, *R. equi* was found to be sensitive to streptomycin, erythromycin, neomycin, norfloxacin, amoxicillin and sulfadiazine. It was determined that the isolated *Staphylococcus* species were sensitive to ampicillin, amoxicillin, neomycin, doxycycline, gentamicin, oxytetracycline erythromycin and streactomycin (Sharma et al., 2017). In another study, all isolates of β-hemolytic, group C *Streptococci* were found susceptible to Ceftiofur and Erythromycin, but especially lower respiratory tract sample isolates were found resistant to Tetracycline (*S. equi* subsp. zooepidemicus more than 90% and *S. equi* subsp. *equi* 66.7%) (Fonseca et al. 2020). It was determined that all *S. zooepidemicus* (100%) were susceptible to cefotaxime, meropenem and doxycycline.

In our study, similar results were obtained with other studies. Our isolates were highly susceptible to Amoxycilin / Clavulanic acid, Ampicillin / Sulbactam, Trimethoprim / Sulfamethoxazole, Enrofloxacin and Oxytetracycline. Tetracyclines are recommended as alternative agents for the treatment of upper

respiratory tract infections in horses (British Equine Veterinary 2016). In this study, we found oxytetracycline effective.

The use of antibiotics for the treatment of respiratory infections in horses remains controversial. Studies on effective and correct use of antibiotics in respiratory system infections of horses are not sufficient. There is a need for prospective studies that monitor horses with and without antibiotic therapy. Antibiotic resistance is quite common in bacterial agents of infectious respiratory infections in horses. In addition, it can have serious effects such as higher morbidity and mortality due to treatment failure and increase treatment costs. In recent years, empirical and inexpensive antibiotic application studies in respiratory system infections in horses have led to the development of resistance against the antibiotics used, prolonging the treatment and increasing the cost. In order to prevent these negative results, it will be more appropriate to isolate your agent and perform an antibiogram test.

Respiratory system infections cause serious performance losses especially in race horses. The causative agents of infection should be identified correctly. In addition, accurate and effective antibiotics should be determined for the agent isolated by antibiotic susceptibility tests. Incorrect and ineffective antibiotic administration adversely affects the prognosis of infection in horses and causes performance losses. In addition, it causes the progression of the infection and prolongation of the treatment period by causing antibiotic resistance. By taking a nasal swab, rapid agent identification can be done without causing pain to the animal and without the need for anesthesia.

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Ethical statement: This study is not subject to HADYEK permission according to Article 8 (k) of the "Regulation on the Working Procedures and Principles of Animal Experiments Ethical Committees".

References

Ainsworth DM, Cheetham J. (2010) Disorders of the respiratory disease. Reed SM, Bayly WM, Sellon DC. eds. *Equine Internal Medicine*, 3th edition, Saunders Elsevier, St. Louis, p. 290 – 371.

Anonymous. (2002) Performance Standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard 2nd ed. Wayne, PA, USA: National Committee for Clinical Laboratory Standards M31-A2.

Bianchi MV, Mello LS, Lorenzo CD, Lopes BC, Snel GGM, Driemeier D, Pavarini SP. (2018) Lung lesions of slaughtered horses in southern Brazil. *Pesq. Vet. Bras.* 38(11):2056-2064.

Bianchi MV, Mello LS, Ribeiro PR, Wentz MF, Stolf AS, Lopes BC, de Andrade CP, .Snel GGM Sonne L, Driemeier DS, Pavarini SP. (2020) Causes and Pathology of Equine Pneumonia and Pleuritis in Southern Brazil. *J Comp Pathol*. 179: 65-73.

Bowen M. (2013) Antimicrobial stewardship: Time for change. *Equine Vet J.* 45, 127–129.

British Equine Veterinary. Protect Me - Practice policy. (2016) Available: https://www. beva. org. uk/ Portals/ 0/ Documents/ Resources/ 1beva- antimicrobial- policy- templatedistributed. pdf.

Broux B, Gryspeerdt A, Amory H, Frippiat T, Pardon B, Gasthuys F, Legrand L, Deprez P. (2016) Prevalence of respiratory pathogens in nasal swabs from horses with acute respiratory disease in Belgium. *Vlaams Diergeneeskd Tijdschr.* 85, 221–224.

Carman S, Rosendal S, Huber L, Gyles C, McKee S, Willoughby RA, Dubovi E, Thorsen J, Lein D. (1997) Infectious agents in acute respiratory disease in horses in Ontario. *J Vet Diagn Invest.* 9, 17–23.

Carvallo FR, Uzal FA, Diab SS, Hill AE, Arthur RM. (2017) Retrospective study of fatal pneumonia in racehorses. *J Vet Diagn Invest.* 29, 450–456.

Chapman PS, Green C, Main JP, Taylor PM, Cunningham FM, Cook AJ, Marr CM. (2000) Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. *Vet Rec.* 146, 91–95.

Christley RM, Rose RJ, Hodgson DR, Reid SW, Evans S, Bailey C, Hodgson JL. (2000) Attitudes of Australian veterinarians about the cause and treatment of lower-respiratory-tract disease in racehorses. *Prev Vet Med.* 46, 149–159.

Christley RM, Hodgson DR, Rose RJ, Wood JL, Reids SW, Whitear KG, Hodgson JL. (2001) A case-control study of respiratory disease in Thoroughbred racehorses in Sydney, Australia. *Equine Vet J.* 33, 256–264.

Cordeiro Rde A, Bittencourt PV, Brilhante RS, Teixeira CE, Marques FJ, Bittencourt PV, Carvalho VL, Bandeira T, Brilhante RS, Moreira JL, Pereira-Neto W, Sidrim JJ, Rocha, MF. (2013) Species of *Candida* as a component of the nasal microbiota of healthy horses. *Med Mycol.* 51, 731-736.

Couëtil LL, Cardwell JM, Gerber V, Lavoie JP, Léguillette R, Richard EA. (2016)Inflammatory Airway Disease of Horses-Revised Consensus Statement. *J Vet Intern Med*. 30, 503–515.

Clinical and Laboratory Standards Institute (CLSI) (2017). Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. M100-S26, 27th ed, p. 32, 52, 54.

Dauvillier J, Ter Woort F, van Erck-Westergren E. (2019) Fungi in respiratory samples of horses with inflammatory airway disease. *J Vet Intern Med.* 33, 968–975.

Dyson PK, Jackson BF, Pfeiffer DU, Price JS. (2008) Days lost from training by two- and three-year-old thoroughbred horses: A survey of seven UK training yards. *Equine Vet J.* 40, 650–657.

Esp ´ındola JP, Machado G, Diehl GN, Dos Santos LC, De Vargas AC, Gressler GT. (2022) Culturable microbial population from the upper respiratory tract of 1,010 clinically healthy

horses in Southern Brazil, J Equine Vet Sci. doi:https://doi.org/10.1016/j.jevs.2022.103946Fonseca JD, Mavrides DE, Morgan AL, Na JG, Graham PA, McHugh TD. (2020) Antibiotic resistance in bacteria associated with equine respiratory disease in the United Kingdom. *Vet Rec.* 187, 189.

Gilkerson JR, Bailey KE, Diaz-Méndez A, Hartley CA. (2015) Update on viral diseases of the equine respiratory tract. *Vet Clin North Am Equine Pract.* 33, 91-104.

Gressler LT, Machado G, da Silveira BP, Cohen ND, Corbellini LG, Leotti VB, Diehl GN, Dos Santos LC, de Vargas AC. (2018) Prevalence of *Rhodococcus equi* from the nasal cavity of 1010 apparently healthy horses. *Equine Vet J.* 50, 667–671.

Kasap S, Kennerman E, Göçmen H, Cihan H, Ulgen M. (2018) Results of cytological and microbiological examination using tracheal aspiration in race horses with lower respiratory tract disease. *Acta Vet Brno*. 87: 339–345.

Kulkarni MB, Deshmukh SS, Agiwale SM, Awandkar SP. (2019) Antibiogram of pathogens from nasal affections in equine. *INTAS POLIVET.* 20, 385-389.

Laing G, Christley R, Stringer A, Ashine T, Cian F, Aklilu N, Newton R, Radford A, Pinchbeck G. (2021) Pathology, infectious agents and horse- and management-level risk factors associated with signs of respiratory disease in Ethiopian working horses. *Equine Vet J.* 53, 670–681.

Morley PS, Apley MD, Besser TE, Burney DP, Fedorka-Cray PJ, Papich MG, Traub-Dargatz JL, Weese JS, American College of Veterinary Internal Medicine (2005) Antimicrobial drug use in veterinary medicine. *J Vet Intern Med*. 19, 617–629.

Nehal MF, Kamelia MO, Azza NF, Shaimaa RAAE, Soumaya SAES, Shahein MA, Ibraheem EM (2021). Phenotypic Study on the Bacterial Isolates from Equine with Respiratory Disorders regarding Antimicrobial Drug Resistance. *World Vet J.* 11(1), 98–109.

Preziuso S, Moriconi M, Cuteri V. (2019) Genetic diversity of Streptococcus equi subsp. zooepidemicus isolated from horses. *Comp Immunol Microbiol Infect Dis*. *65*, 7–13.

Pusterla N, Rice M, Henry T, Barnum S, James K. (2020). Investigation of the Shedding of Selected Respiratory Pathogens in Healthy Horses Presented for Routine Dental Care. *J Vet Dent. 37*(2), 88–93.

Reilly LK, Palmer JE. (1994) Systemic candidiasis in four foals. *J Am Vet Med Assoc.* 205, 464–466.

Reuss SM, Giguère S. (2015) Update on bacterial pneumonia and pleuropneumonia in the adult horse. *Vet Clin North Am Equine Pract*. 31, 105–120.

Riley CB, Yovich JV, Robertson JP, O'Hara FL. (1992) Fungal arthritis due to infection by *Candida famata* in a horse. *Aust Vet J.* 69, 65–66.

Sharma AK, Randhawa CS, Narang A, Sood NK, Rai TS. (2017). Cytological and Bacteriological Evaluation of Tracheal Aspirates for the Diagnosis of Lung Affections in Horses. *J Anim Res.* 7(3); 759-464.

Singh RK, Dhama K, Karthik K, Khandia R, Munjal A, Khurana SK, Chakraborty S, Malik, YS, Virmani N, Singh R, Tripathi BN, Munir M, van der Kolk JH. (2018) A comprehensive review on equine influenza virus: etiology, epidemiology, pathobiology, advances in developing diagnostics, vaccines, and control strategies. *Front Microbiol.* 9, 1941.

Stewart AJ, Cuming RS. (2015) Update on the fungal respiratory disease in horses. *Vet Clin North Am Equine Pract*. 33, 43-62.

Sweeney CR, Timoney JF, Newton JR, Hines MT. (2005) *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles. *J Vet Intern Med*. 19, 123–134.

Toombs-Ruane LJ, Riley CB, Kendall AT, Bolwell CF, Benschop J, Rosanowski SM. (2015) Antimicrobial Susceptibilities of Aerobic Isolates from Respiratory Samples of Young New Zealand Horses. *J Vet Intern Med.* 29, 1700–1706.

Van Erck-Westergren E, Franklin SH, Bayly WM. (2013) Respiratory diseases and their effects on respiratory function and exercise capacity. *Equine Vet J.* 45, 376-387.

Velineni, S., Desoutter, D., Perchec, A. M., & Timoney, J. F. (2014). Characterization of a mucoid clone of Streptococcus zooepidemicus from an epizootic of equine respiratory disease in New Caledonia. *Veterinary journal (London, England: 1997)*, *200*(1), 82–87.

Vitale V, Sgorbini M, Cuteri V, Preziuso S, Attili AR, Bonelli F. (2019) Cytological findings in bronchoalveolar lavage fluid of foals with pneumonia caused by *Rhodococcus equi* and other bacteria. *J Equine Vet Sci.* 79, 9–12.

Wilson WD. (2001) Rational selection of antimicrobials for use in horses. *Ann Conven Am Assoc Eq Prac*. 47, 75–93.

Wood JL, Newton JR, Chanter N, Mumford JA. (2005) Association between respiratory disease and bacterial and viral infections in British racehorses. *J Clin Microbiol.* 43, 120–126.