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BACTERIAL PATHOGENS PROFILE ASSOCIATED WITH RESPIRATORY MANIFESTATIONS IN EQUINE

By

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ABSTRACT

The current study aimed to characterize the bacterial causes of respiratory diseases in clinical, subclinical and carrier horses of different ages .159 nasal swabs were collected from the internal nares of horses suffered from respiratory manifestation which classified into three groups; foreign breed (29), native breed (73), and Arabian breed (57). The main isolate was of *S. equi subspp. Equi* recovered from foreign breed in an incidence of 15.7%, and from native breed in an incidence of 30.7%, while the incidence of other detected bacteria of the foreign breed was *E. coli*, and *k. pneumoniae* in an incidence of 66.6% and 16.6%, *respectively.* The native breed and Arabian horse breeds recorded only *E. coli* in an incidence of 100% for each. It could be concluded that the respiratory manifestations of bacterial caused by *S. equi* is commonly identified mixed with *E. coli* and *k. pneumoniae.*

Key words:

(Horses**,** *Streptococcus,* Respiratory diseases, Incidence).

INTRODUCTION

Infectious upper respiratory disease (IURD) of horses has been a frequent problem. Risk factors for IURD include the season with a high transfer rate (summer and fall), the stabling period (≤ 3 months), and age (2 to 3 years old), suggesting that, the movement and new environment may have depressed the immune system of the horses and decreased their ability to respond properly to pathogens. The bacterial strains isolated from IURD horses included *Pseudomonas* spp*., E. coli*, *Staphylococcus* spp., *Streptococcus equi subspp. Equi* and *zooepidemicus* **(Ryu *et al.,* 2011)**. *Streptococcus equi* is the etiologic agent of a highly infectious upper respiratory disease of horses known as strangles. Asymptomatic carriers in the population may result in the spread of disease via introduction of *S. equi* to native horse

populations. Bacterial culture and polymerase chain reaction (PCR) of nasopharyngeal (NP) washes and guttural pouch (GP) lavages have been used for both diagnostic testing and detection of *S. equi* in clinical cases and carrier animals but no definitive or gold standard test method has been shown to be optimal **(Boyle *et al*., 2012; Holland *et al.,* 2006; Sweeney *et al.,* 2005; Timoney and Artiushin, 1997**).

Other pathogens such as *E.coli* became responsible for secondary bacterial pneumonia following the administration of antimicrobials **(Racklyeft and Love, 2000)**. The mortality rate from pleuropneumonia and secondary bacterial pneumonia caused by these agents is high **(Sweeney *et al.,* 1985; Racklyeft and Love, 2000).** So the current study aimed to characterize most common bacterial pathogens associated with respiratory diseases in clinical, subclinical and carrier foreign, native, arabian horses of different ages**.**

MATERIAL AND METHODS

Sample:

Physical examination was employed for horses, which showed strangles signs clinically as respiratory distress, nasal discharge, followed by collection of swabs from nasal discharge. One hundred fifty-nine swabs as shown in (Table 1) were collected from horses suffered clinically from respiratory symptoms.

Table (1): Numbers and types of samples collected from horses suffered from respiratory manifestation.

Type of horses		n= horses examined	Total
Foreign breed	Gelding(Castrated horses)	12	29
	Mare	17	
Native breed	Stallion	16	73
	Mare	24	
	Foals (male)	14	
	Foals (female)	19	
Arabian breed	Stallion	13	57
	Mare	15	
	Foals (male, colt)	18	
	Foals (female, filly)	11	
Total			159

Isolation and identification of bacterial pathogens profile (Quinn *et al.*, 2011).

Nasal swabs were inoculated into brain-heart infusion broth overnight at 37ºC / 24hr then cultured on blood agar and MacConkey's agar and incubated at 37ºC for 24 -48h.The colonies were examined for their morphological characters, appearance and hemolytic activity. Smears from suspected colonies were prepared and stained with Gram's stain to be examined microscopically before being transferred into semisolid agar for further biochemical and serological identification.

RESULTS

As shown in (Table 2), the result of isolation of Gram positive bacteria were 55 isolates (19 isolates from foreign breed, 26 isolates from native breed and 10 isolates from Arabian breed), while Gram negative bacteria were 26 isolates (6 isolates from foreign breed, 15 isolates from native breed and 5 isolates from Arabian breed). The results of biochemical identification gave the biochemical characteristic according to (Table 3 and 4)**.**

Table (3) shows that there were seven suspected isolates of *S. equi subsp*. *Equi* recovered from foreign with incidence of 15.7%. There are also eight suspected isolates of *S. equi subsp. Equi* recovered from native breed. Such result represents an incidence of 30.7%. From Table (4), the incidence of detected bacteria of the foreign breed recovered 4 *E. coli*, and one *Klebsiella pneumoniae* in an incidence of 66.6% for *E. coli* and in an incidence of 16.6% for *Klebsiella pneumoniae* .The Native breed and Arabian breed recorded only *E.coli* in an incidence of 100% for each. The serotyping of *E. coli* (Table 5) isolated from native breed included four serotypes (O1, O157, O166, and O146).

Table (2): The results of bacterial isolation recovered from nasal swab samples of foreign, native and Arabian horse breeds.

Horses breed	*Streptococcus*		*Rhdococcus spp*	*E.coli*
	S.eqi spp. Equi	*S.eqi spp.Zooepdicus*		
Foreign (29)	**11**	**8**	**2**	**8**
Native (73)	**31**	**12**	**9**	**21**
Arabic (57)	**28**	**8**	**6**	**15**

Table(3): The results of biochemical identification on the suspected 55 *Streptococcus* bacteria recovered from nasal swab samples from foreign, native and Arabic horse breeds.

Test	Isolates from Foreign breed (19)		Isolates from Native breed (26)		Isolates from Arabic breed(10)	
	n= of positive isolates	%	n= of positive isolates	%	n= of positive isolates	%
Catalase	0	0	0	0	0	0
Haemolysis	3	15.7	8	30.7	0	0
Oxidase	0	0	0	0	0	0
Lancefield classification +ve group c	3	15.7	8	30.7	0	0

Table (4): The results of biochemical identification on the suspected 26-Gram negative bacteria recovered from nasal swab samples from foreign, native and Arabic horse breeds.

Test	Isolates from Foreign breed (6)		Isolates from Native breed (14)		Isolates from Arabic breed (5)	
	n= of positive isolates	%	n= of positive isolates	%	n= of positive isolates	%
Indole production	4	66.6	14	100	5	100
Methyl red test	5	83.3	14	100	5	100
Voges-proskauer	1	16.6	0	0	0	0
Citrate utilization test	2	33.3	0	0	0	0
H_2S production in TSI agar	0	0	0	0	0	0
Urease activity	2	33.3	0	0	0	0

Table (5): Results of serotyping of four *E. coli* recovered from native breed.

Isolate number	Polyvalent	Monovalent
1	3	O 157
2	1	O1
3	2	O166
4	2	O146

DISCUSSION

Current literature in equine medicine encompasses a broad range of topics in both clinical and research microbiology, including nosocomial infections, zoonotic transmission of disease and multi-drug resistance. Efforts to describe the microbiological environment encountered in equine practice range from broad retrospective surveys of multiple pathogens to detailed molecular characterization of individual isolates. Salient characteristics including disease presentations, reported trends in antimicrobial resistance and zoonotic concerns are summarized below for the principal bacterial organisms encountered in equine clinical practice **(Moodley *et al.,* 2010)**.

Opportunistic infection of *S. zooepidemicus* in horses typically results in pneumonia, rhinitis, placentitis, endometritis, cervicitis, abortion, keratitis, mastitis, cellulitis and non-strangles lymph node abscessation. *S. zooepidemicus* has long been recognized as an important pathogen in equine reproduction and presently is the most frequently isolated pathogen associated with abortion in mares **(Erol *et al*., 2012**). The pathogenesis of *S. zooepidemicus* placentitis is not completely understood. The external genitalia of the mare and stallion are suggested as sources of the organism, which opportunistically penetrate the cervix via natural or artificial insemination, iatrogenically, or due to poor conformation of the internal and external genitalia of the mare causing infection. Little is known about the pathogenesis of *S. zooepidemicus* in the lower respiratory tract of horses. The organism has been associated with lower airway disease of young horses with compromised or overwhelmed immune system due to stress (intense training, transportation) or secondary infection **(Sweeney, 1991)**. In the present study small translucent colonies, some of which were mucoid by Gram's stain and showed Gram positive cocci that occurred singly, in pairs, in short chain when examined under light microscope with a total 55 isolates (19 isolates from foreign breed, 26 isolates

from native breed and 10 isolates from Arabian breed). In addition, when colonies from MacConkey's agar plates were stained by Gram's stain it showed Gram-negative bacilli when examined under light microscope with a total 26 isolates (6 isolates from foreign breed, 15 isolates from native breed and 5 isolates from Arabian breed). As previously reported the determination of the causative agent of an upper respiratory disease can be difficult but is important for several reasons, where the treatment and prognosis in an individual horse is only one part. Often several horses are at risk of being infected in an outbreak and therefore we must know the characteristics of the pathogenesis and epidemiology of the disease-causing agent. To minimize the number of horses affected in a strangles outbreak, measures to avoid exposure of non-infected horses to possible transmission risks must be taken immediately. When strangles is suspected, a bacteriological verification of *S. equi* infection must be taken to support the action in controlling spreading of the disease by isolating the diseased horses, placing a stable or facility under quarantine, and applying restrictions on the movement of horses and contact with sick horses **(Lindahl, 2013)**.

In light of this, an in-depth understanding of the institutional, local and regional microbial population is a prerequisite for effective and responsible antimicrobial use by veterinary hospital clinicians and field practitioners. Consideration of the potential impact of biosecurity and antibiotic protocols on the health of patients, staff, clients, and the public is of paramount importance **(Brosnahan, 2008)**.

CONCLUSION

Our study concluded that *Streptococcus spp.; E.coli and K. pneumoniae* represent the main pathogens in upper respiratory tract in equines

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