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SHORT COMMUNICATION

Isolation and Identification of Microorganisms from the Upper Respiratory Tract of Equines in Himachal Pradesh

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ABSTRACT

The present investigation was conducted to identify different bacterial agents associated with the respiratory infections of equines in the submountaineous region of Himachal Pradesh. Nasal swabs were collected from a total of 119 animals, including horses (n=59) and mules (n=60), exhibiting respiratory disease manifestations (n=20) as well as apparently healthy animals (n=99); and subjected to routine cultural, staining and biochemical examinations for the identification of the isolated bacteria. The bacterial genera thus isolated and identified included *Staphylococcus* spp. 12 (38.71%), *Corynebacterium* spp. 6 (19.35%), *Bacillus* spp. 3 (9.68%), *Streptococcus* spp. 2 (6.45%), *Micrococcus* spp. 2 (6.45%), *Pseudomonas* sp. 1 (3.23%) and others 5 (16.13%) from the 15 samples from diseased equines; and *Staphylococcus* spp. 83 (44.62%), *Corynebacterium* spp. 30 (16.13%), *Bacillus* spp. 33 (17.74%), *Micrococcus* spp. 12 (6.45%), *Streptococcus* spp. 6 (3.23%) and others 22 (11.83%) from the 81 samples from apparently healthy animals, whereas, 23 samples were bereft of any bacterial growth. Further speciation of *Staphylococcus* spp., *Corynebacterium* spp. and *Bacillus* spp. was also carried out by means of biochemical tests.

Keywords: Respiratory, Bacteria, Identification, Biochemical, Equines

The equines including horses, ponies, mules and donkeys, are susceptible to a variety of infectious diseases, including the respiratory infections, which may be manifested as mild, moderate or severe forms depending upon the aetiological agents involved. These respiratory infections result from a complex interaction of parasitic, bacterial and viral factors, as well as environmental conditions. The equine population of Himachal Pradesh is frequently affected by Respiratory Distress Syndrome (RDS) manifested in the form of polypnoea, inappetance, pyrexia and low tolerance to work (Chahota *et al.,* 2001). So it became imperative to study the association of various bacteria in aetio-pathology of the respiratory infections in equine species so that prompt diagnosis, timely treatment and control measures could be undertaken.

In the present study, a total of 119 nasal swab samples were collected from horses (n=59) and mules (n=60), exhibiting respiratory manifestations (n=20) as well as apparently healthy animals (n=99). The samples were collected from various parts of Himachal Pradesh including district Kangra (Dadi, Paprola, Paror, Utrala, Dharamshala, Baijnath, Saddu, Sungal, Shahpur, Kangra and Veterinary Clinical Complex, Dr. GC Negi College of Veterinary and Animal Sciences, CSK HPKV, Palampur), district Mandi (Chauntra), district Chamba (Khajjiar, Chamba, Sultanpur, Obri, Pakkatala and Mangala). Before sampling, the exterior part of the nasal mucosa was cleaned and disinfected with 70% alcohol. Then, a 20 to 25cm long sterile swab was directed through the ventral nasal meatus into the nasal tract and mucosal exudates were collected. All the samples were collected in sterile vials in Phosphate Buffered Saline (PBS, pH 7.2). Collected samples were transported to the laboratory in insulated cold boxes containing ice packs and stored at -20°C until further processing.

The isolation of the bacterial agents was attempted by directly streaking the swabs on blood agar plates containing 5 to 10 percent defibrinated sheep blood, in duplicate to have discrete colonies of the bacteria. Samples were also streaked on various differential media like MacConkey lactose agar and Eosin methylene blue agar. The streaked plates were incubated at 37°C aerobically for 24 to 48 hrs (Quinn *et al.*, 1994).

The cultures resulting from the streaking on sheep blood agar invariably contained mixed populations of various bacterial genera/species or their variants, therefore, different colonies were picked up and re-streaked on freshly prepared blood agar plates and further incubated at 37°C for 24 to 48 hrs. The plates were examined for detailed colonial morphology and staining characteristics. The smears were prepared from the purified colonies and Gram's staining was done. Tests like catalase and oxidase were performed taking single isolated colonies. A single colony of each isolate was inoculated in nutrient broth and incubated at 37°C for 48 hours to obtain broth cultures for biochemical testing (Quinn *et al.*, 1994).

Identification of various genera of bacterium was done by cultural and staining characteristics and biochemical examinations by standard protocols as described by Cruickshank *et al.* (1975), Carter (1995) and Forbes *et al.* (2007).

In the present study, the association of aetiological agents *viz.* bacteria both in apparently healthy as well as diseased animals were detected. A total of 9 horses and 11 mules were found to manifest respiratory disease. A total of 99 nasal swabs were obtained from apparently healthy animals showing no clinical signs of the respiratory disease, including 50 horses and 49 mules. The horses and mules had to meet any of the following criteria, either alone or in combination, to be considered as diseased animal exhibiting clinical manifestations: respiratory distress, coughing and nasal discharge. Similar clinical conditions were studied by Fretz *et al*. (1979) in equines in Western Canadian racetracks, who categorized the respiratory disease into mild, moderate and severe form based on clinical signs ranging from slight depression, anorexia, serous nasal discharge and a dry cough to severe depression, copious mucopurulent discharge, fever and bronchopneumonia, regardless of the agents involved.

In the present investigation, a total of 96 (80.67%) samples out of 119 samples of nasal swabs yielded a variety of bacteria, whereas, 23 samples were bereft of any bacterial growth. From the 15 samples from the respiratory tract of equines exhibiting respiratory disease manifestations, a variety of bacteria were isolated including *Staphylococcus* spp. 12 (38.71%), *Corynebacterium* spp. 6 (19.35%), *Bacillus* spp. 3 (9.68%), *Streptococcus* spp. 2 (6.45%), *Micrococcus* spp. 2 (6.45%), *Pseudomonas* sp. 1 (3.23%) and others 5 (16.13%). The bacterial isolates from 81 samples from the respiratory tract of apparently healthy animals included *Staphylococcus* spp. 83 (44.62%), *Corynebacterium* spp. 30 (16.13%), *Bacillus* spp. 33 (17.74%), *Micrococcus* spp. 12 (6.45%), *Streptococcus* spp. 6 (3.23%) and others 22 (11.83%).

The bacteria isolated in this study are similar to a previous study on the equines of Himachal Pradesh conducted by Deshwal *et al*. (2002). They studied Respiratory Disease Syndrome (RDS) and a total of 48 isolates were obtained from the nasal swabs collected from 28 horses, mules and ponies on the basis of cultural and biochemical tests, including *Staphylococcus* spp. (17), *Streptococcus* spp. (10), *Bacillus* spp. (9), *Corynebacterium* spp. (6), *Nocardia* spp. (2) and *Pseudomonas* spp. (1).

Similar study on the isolation and identification of aerobic bacterial flora from the upper respiratory tract was carried out by Gutema *et al*. (2009), who collected samples from 80 apparently healthy donkeys and 20 donkeys with respiratory tract disease and recovered the bacterial isolates *Streptococcus* spp. (28.1%), *Corynebacterium* spp. (15.4%), *Staphylococcus* aureus (13.2%), coagulase negative *Staphylococcus* spp. (9.5%), *Bacillus* spp. (9.0%), *Klebsiella pneumoniae* (5.8%), E. coli (4.2%), *Micrococcus* spp. (4.2%), *Rhodococcus* spp. (2.7%), Proteus vulgaris (2.1%), *Actinomyces pyogenes* (2.1%), *Pasteurella caballi* (1.6%), Actinomyces spp. (1.1%), *Pseudomonas* spp. (0.5%) and *Pasteurella haemolyticum* (0.5%). Apart from this, various microbial studies have been carried out for the isolation and identification of bacteria from the respiratory tract of equines (Carman *et al*., 1997; Racklyeft and Love, 2000; Boguta *et al*., 2002; Vijayasarathi *et al*., 2002; Ihler *et al*., 2003; Munoz *et al*., 2003; Wood *et al*., 2004; Bohra *et al*., 2008; Jannatabadi *et al*., 2008).

A total of 21 isolates of *Staphylococcus* spp. were further processed for identification upto the species level on the

Table 1: Speciation of *Staphylococcus* spp. based on biochemicaltests

Priyanka	No. of Isolates	Beta hemolysis	Pigment	Catalase	Oxidase	Maltose	Mannitol	Glucose	Novobiocin	DNase
S. aureus	7	+	+	+	_	+	+	+	S	+
S. intermedius	8	+	_	+	_	±	±	+	S	±
S. saprophyticus	4	_	±	±	_	±	±	_	R	_
S. epidermidis	2	±	±	+	_	+	_	+	S	_

Table 2: Speciation of *Bacillus* spp. based on biochemical tests

Species	No. of Isolates	Glucose	Xylose	Mannitol	Lactose	Maltose	Salicin	NB with 6% NaCl	Nitrate reduction	Indole	TSI	VP
B. subtilis	2	+	_	+	+	±	NA	+	±	_	V/A	±
B. cereus	8	+	_	_	±	+	+	±	±	_	V/A	±
B. megaterium	3	+	±	+	+	±	±	±	_	_	V/V	_

Table 3: Speciation of *Corynebacterium* spp. based on biochemical tests

Species	No. of Isolates	Beta hemolysis	Nitrate	Glucose	Maltose	Sucrose	Mannitol	Xylose
C. pseudotuberculosis	1	+	±	+	+	_	_	_
C. haemolyticum	1	+	_	+	+	±	_	_
C. matruchotii	1	NA	+	±	±	±	NA	NA
C. afermentans	2	NA	_	_	_	_	NA	NA
C. peudodiphtheriticum	2	NA	+	_	_	_	NA	NA
C. ulcerans	2	+	_	+	+	_	_	_
C. xerosis	2	_	+	+	±	+	_	_
C. kutscheri	1	±	+	+	+	+	_	_
C. flavescens	1	NA	_	+	+	±	_	_

basis of biochemical tests. The species identified include *S. aureus* (7), *S. intermedius* (8), *S. saprophyticus* (4) and *S. epidermidis* (2). In one of the study, Chahota *et al*. (2001) studied 24 cases of respiratory distress syndrome among equines in H.P. and isolated various bacteria including two species of *Staphylococcus* spp. viz. *S. aureus* and *S. saprophyticus*.

Further speciation of 13 isolates of *Corynebacterium* spp. was done revealing *C. pseudotuberculosis* (1), *C. haemolyticum* (1), *C. matruchotii* (1), *C. afermentans* (2), *C. pseudodiphtheriticum* (2), *C. ulcerans* (2), *C. xerosis* (2), *C. kutscheri* (1) and *C. flavescens* (1). A total of 13 isolates of *Bacillus* spp. were identified upto the species level including *B. subtilis* (2), *B.cereus* (8) and *B. megaterium* (3). The results of the biochemical tests conducted for the identification of *Staphylococcus* spp., *Bacillus* spp. and *Corynebacterium* spp. are shown in Table 1, 2 and 3 respectively.

The microbial isolation studies yielded a variety of bacterial isolates from the upper respiratory tract of equines exhibiting respiratory disease manifestations, as well as apparently healthy animals, including *Staphylococcus* spp., *Corynebacterium* spp., *Bacillus* spp., *Streptococcus* spp., *Micrococcus* spp., *Pseudomonas* sp. and some other sp. Further speciation of *Staphylococcus* spp., *Corynebacterium* spp. and *Bacillus* spp. carried out by means of biochemical tests also revealed a spectrum of species within each of these genera. Thus, various bacteria

are involved in the multiple etiology of the respiratory infections. Also, it is a known fact that most of the bacterial agents, which are normally present as commensals in the respiratory tract of equines, act as pathogens under certain adverse conditions like environmental stress, viral infections, parasitic infestations as well as infections by specific bacterial pathogens; and this reflects the multi-aetiological aspect of the respiratory disease. Therefore, the diagnostic, treatment and preventive measures which are being undertaken for the control of respiratory disease in equines should necessarily consider the involvement of these bacteria, so that prompt diagnosis and effective treatment can be given and thus the respiratory infections can be controlled.

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