Occurrence of Mannheimia haemolytica and Pasteurella trehalosi Among Ruminants in Egypt

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Abstract: Prevalence of haemolytic Mannheimia species in cattle, buffaloes, sheep and goats in both apparently healthy and diseased animals was investigated. Nasal swabs were collected from some farms in different Governorates. Samples of lung tissues, tonsils, retropharyngeal lymph nodes, and nasal swabs were also collected from freshly slaughtered cattle, buffaloes, sheep and goats at abattoirs of Egypt. (A total of 837 samples). Typical β-haemolytic Pasteurellaceae were isolated from nasal swabs and tissue samples and identified biochemically. Bacterial isolates identified as P. trehalosi and M. haemolytica. The Prevalence rate of M. haemolytica which isolated from the respiratory tracts of cattle and buffaloes were 3.60% and 3.90%, respectively. M. haemolytica was isolated from sheep and goats in prevalence rate of 14.10% and 11.80%, respectively. We demonstrate that a relatively high number of apparently healthy animals seem to carry the potentially pathogenic M. haemolytica. In case of buffaloes, the recovery rate of P. trehalosi was higher than that in cattle (P. trehalosi are rare in cattle). M. haemolytica isolates were predominate over P. trehalosi in both sheep and goats. [New York Science Journal 2010;3(5):135-141]. (ISSN 1554 – 0200).

KEY WORDS: Mannheimia haemolytica, Pasteurella trehalosi, Epidemiology

1. Introduction

Two biotypes have been recognized for the taxon Pasteurella haemolytica: biotype A, an isolates that ferment L-arabinose, and biotype T isolates that ferment trehalose. The trehalose-positive isolates were found to represent a distinct species (P. trehalosi). The trehalose-negative organisms were found to represent a distinct genus (Mannheimia) with five species (M. glucosida, M. granulomatis, M. haemolytica, M. ruminatis, and M. varigena). The trehalose-negative organisms are now classified as M haemolytica. (Jaworski et al., 1998).

M. haemolytica, formerly Pasteurella(P. haemolytica) is the primary aetiologic agent of pneumatic pasteurellosis (one of the most important respiratory diseases in cattle and sheep) (Ewers et al., 2004). M. haemolytica is a commensal of cattle, sheep, and other ruminants, but it also causes bovine and ovine pneumatic pasteurellosis, which is responsible for considerable economic losses to the cattle, sheep and other livestock industries in many parts of the world (Frank, 1989; Gilmour et al., 1989; Bowerland and Shewen, 2000). M. haemolytica has been recognized as the principal cause of death from pneumatic pasteurellosis affecting cattle, sheep and goats and septicemic pasteurellosis in sheep and goats (Janet et al., 2008). This bacterium is an opportunist pathogen which has been recovered from the mucous membranes of the nasopharyngeal and oral regions of clinically healthy cattle, sheep and goats. Most species of Mannheimia are known as opportunistic pathogens (Ewers et al., 2004) and are frequently isolated from asymptomatic carriers (Biberstein et al., 1978; Gilmour et al., 1989; Gilmour and Gilmour, 1989; Trevor et al., 2008).

In sheep, disease caused by Mannheimia species has mainly included pneumonia and septicaemia (Gilmour and Gilmour, 1989) although isolates have been reported from the myocardium and the brain of healthy animals (Ewers et al., 2004). Outbreaks of Mannheimia are thought to occur when local and systemic defense mechanisms are impaired and virulent strains of the organism undergo massive proliferation prior to invading the nasopharyngeal mucosa or being inhaled in large numbers into the lungs. Various forms of stress factors have been incriminated as predisposing causes. These include environmental, management and/or infectious factors (Thompson et al., 1977; Frank, 1989).

Respiratory disorders in animal production units in Egypt were reported to cause a considerable loss due to lower productivity and death (Abdel Ghanai et al., 1990; El- Battrawy, 1991; Ismael et al., 1993), but the role of P. haemolytica and its relative importance is still equivocal.

The aim of this work is to advance knowledge of the epidemiology of Mannheimia haemolytica (P. haemolytica) and P. trehalosi among farm animals in...
Egypt through the studying prevalence of the organism in cattle, buffaloes, sheep and goats in both apparently healthy and diseased animals.

2. Material and Methods

2.1. Samples

Nasal swabs were collected from some farms in different Governorates. Samples of lung tissues, tonsils, retropharyngeal lymph nodes, and nasal swabs were also collected from freshly slaughtered cattle, buffaloes, sheep and goats at abattoirs of Egypt (A total of 837 samples).

2.2. Sampling techniques

**Nasal Swabs:**

Cotton-tipped, 15 cm long, sterile swabs were used. With the attempt to avoid picking contamination from the external nares, the swab was carefully inserted into each nostril, and then placed back into its jacket. The swabs were kept in an ice-box and taken to the laboratory.

**Tissue samples:**

Samples from both healthy and pneumonic lungs were obtained at slaughterhouse of freshly-slaughtered animals. The samples were collected in separate plastic bags, labeled and kept cooled in the ice-chest until being transported to the laboratory (Collins et al., 1989).

**Isolation of the organism**

The swabs were removed from the transport media and inoculated onto bovine blood agar containing antibiotics (Becton-Dickinson). Plates were incubated at 37°C in 5–10% carbon dioxide atmosphere and inspected after 24 and 48 hrs of incubation. Based on morphology, suspected colonies were chosen for further identification by using gram staining and biochemical reactions. There was no limitation on the selection and retention of types and number of colonies per plate. Bacterial isolates identified as *P. trehalosi* and *M. haemolytica* were evaluated for the presence of hemolysis on blood agar and were classified using previously described biogrouping (Bisgaard and Mutters, 1986; Blanco et al., 1995) and biovariant systems (Jaworski et al., 1998).

**Identification of M. haemolytica**

Cells are Gram-negative, non-motile, small rods. Colonies are regular, smooth and grayish on blood agar and are 1-2 mm in diameter after 24 hrs on incubation.

**Biochemical activity**

All strains of *Mannheimia* ferment mannitol, glucose; maltose, sorbitol and sucrose are fermented without gas production. Indol, urease, methyl blue (MB) and Voges-Proskauer (VP) reactions are negative. Catalase (always) and oxidase are positive. Typically they do not ferment trehalose, but ferment L-arabinose. *Mannheimia* can be separated from genus *Pasteurella* by being not producing acid from D-mannose, from genus *Actinobacillus* (almost) by being urease negative, from genus *Haemophilus* by being mannitol positive and from genus *Lonepinella* by being VP negative (Angen et al., 2002).

### Table1: Phenotypic characters separating existing species of *Mannheimia*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th><em>M. haemolytica</em></th>
<th><em>M. glucosida</em></th>
<th><em>M. granulomatis</em></th>
<th><em>M. ruminalis</em></th>
<th><em>M. varigena</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>β-hemolysis (bovine blood)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>_</td>
<td>d</td>
<td>_</td>
<td>_</td>
<td>d</td>
</tr>
<tr>
<td>L(+)Arabinose</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>L(+)-Xylose</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Meso-Inositol</td>
<td>d</td>
<td>+</td>
<td>d</td>
<td>_</td>
<td>d</td>
</tr>
<tr>
<td>Dr(+)-Sorbitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>L(+)Rhammose</td>
<td>_</td>
<td>_</td>
<td>+</td>
<td>-</td>
<td>d&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>β-Glucosidase(NPG)</td>
<td>_</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycosides&lt;sup&gt;c&lt;/sup&gt;</td>
<td>_</td>
<td>d</td>
<td>d</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>α-Fucosidase</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>β-Xylosidase(ONPX)</td>
<td>_</td>
<td>d</td>
<td>d</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

+, 90% or more of the strains positive within 1-2 days; (+), 90% or more of the strains positive within 3-14 days; -, 10% or less of the strains are positive within 14 days; d, 11-89% of the strains are positive. *Strains of Bisgaard Taxon are positive.*<sup>c</sup>Glycosides: cellobiose, esculin, amygdalin, arbutin, gentiobiose, and salicin.

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P. trehalosi were Gram-negative, non-motile (at 22 and 37 °C) rods. All isolates were positive in the nitrate reduction test and were negative in Simmons’ citrate, methyl red and Voges–Proskauer tests. No isolates produced H₂S, urease or gelatinase. Indole was not produced. The isolates were all negative in the ornithine decarboxylase tests. The isolates showed variable reactions in the catalase and oxidase tests. Variable results were obtained for haemolysis (on bovine blood agar). Acid was produced from (–)-D-ribose, (–)-D-mannitol, (–)-D-sorbitol, (–)-D-fructose, (+)-D-glucose, (+)-D-mannose, maltose, sucrose, (+)-D-trehalose and dextrin. Acid was not produced from (+)-L-arabinose, (–)-D-arabinose, (+)-D-galactose, (–)-L-rhamnose, (–)-L-sorbose, lactose. The isolates varied in their ability to produce acid from aesculin, amygdalin, arbutin, gentiobiose and salicin. All isolates were negative in the β-galactosidase (ONPG) test. All the isolates were negative in tests for α-fucosidase, and β-xylosidase. Variable results were obtained in the α-glucosidase, β-glucosidase and β-glucuronidase tests.

3. Results

Prevalence of M. haemolytica in respiratory tract of farm animals

Cattle

Examination of 225 samples collected from the respiratory tracts of cattle revealed the recovery of 8 isolates of M. haemolytica with a prevalence rate of 3.6% as shown in Table (2). Four isolates out of the 8 isolates were obtained from 175 samples of apparently healthy animals (2.3%) and the other 4 came from 50 samples of diseased animals (8%).

Sheep

Bacteriological examination of 241 samples collected from the upper and lower respiratory tracts of apparently healthy and diseased sheep of different ages, revealed the isolation of 34 M. haemolytica isolates (14.10%). Out of 187 samples collected from the respiratory tracts of apparently healthy sheep, 22 M. haemolytica strains were recovered (11.80%), while the examination of 54 samples from diseased sheep revealed an isolation rate of 22.20% as shown in Table (2).

Goats

In the present study, the frequency of M. haemolytica isolation from apparently healthy and diseased goats is 10.10% and 26.70%, respectively as shown in Table (2) and figure (1). In comparison with the situation in cattle and buffaloes, it is evident from the results that M. haemolytica may play an important role in the respiratory disease in goats and sheep more than that in cattle and buffaloes.

Prevalence of M. haemolytica biotypes in the respiratory tract of animals

Table 2: Incidence of M. haemolytica in respiratory tract of apparently healthy & diseased animals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Apparently healthy animals</th>
<th>Diseased animals</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>No. of isolates</td>
<td>%</td>
</tr>
<tr>
<td>Cattle</td>
<td>175</td>
<td>4</td>
<td>2.30</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>143</td>
<td>4</td>
<td>2.80</td>
</tr>
<tr>
<td>Sheep</td>
<td>187</td>
<td>22</td>
<td>11.80</td>
</tr>
<tr>
<td>Goats</td>
<td>158</td>
<td>16</td>
<td>10.10</td>
</tr>
</tbody>
</table>

Out of 153 samples collected from respiratory tracts of buffaloes, 6 M. haemolytica strains were recovered (3.90%). Four of these isolates were from apparently healthy animals with a prevalence rate of 2.80%, while the other two strains were from diseased calves (20%) as shown in Table (2).

Comparing the results obtained for apparently healthy cattle and buffaloes, it appears that both animals showed nearly the same prevalence rate of infection. However, diseased buffaloes showed higher prevalence rate of M. haemolytica recovery than diseased cattle.
The biochemical typing of the isolates recovered from cattle revealed that 7 isolates (87.50%) were *M. haemolytica* strains and one isolate was *P. trehalosi* (12.50%), while those recovered from buffaloes were 4 isolates of *M. haemolytica* (66.70%) and 2 isolates *P. trehalosi* (33.30%), Table (3). No *P. trehalosi* strains were isolated from the lungs and tonsils of cattle, while the only strain isolated from the lungs of a buffalo-calf was *P. trehalosi*. The distribution and recovery rates of *M. haemolytica* in the respiratory tracts of sheep and goats as well as the recovery rate in the nasal passages vs. the lungs of these animals are illustrated in Table (3).
Table 3: The distribution and recovery rate of M. haemolytica and P. trehalosi from the respiratory tract:

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Cattle</th>
<th>Buffaloes</th>
<th>Sheep</th>
<th>Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. haemolytica</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>P. trehalosi</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Nasal Swabs</td>
<td>2</td>
<td>25</td>
<td>1</td>
<td>12.50</td>
</tr>
<tr>
<td>Lungs</td>
<td>3</td>
<td>37.50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tonsils</td>
<td>2</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>7</td>
<td>87.50</td>
<td>1</td>
<td>12.50</td>
</tr>
</tbody>
</table>

4. Discussions
Prevalence of M. haemolytica and P. trehalosi in respiratory tract of ruminants

Cattle and Buffaloes

Frequencies of Mannheimia strain isolates are very variable and can sometimes be high and differ depending on the source of isolation: Great Britain reports 24% in pneumonic calves lungs (Quirie et al., 1986) and Denmark, 25% in diseased cattle (Angen et al., 2002).

These results are in general agreement with those recorded by Wray and Thompson, (1971); Biberstein, (1978); Allan et al., (1985); Bali et al., (1993); Odendaal and Henton,(1995) who all reported the preponderance of M. haemolytica in cattle respiratory tracts. These results may reinforce the idea that P. trehalosi are rare in cattle and play no part in epidemic disease. The present results are only partially comparable with other national and international studies, since the samples used in other studies are different in origin and characteristics. The prevalence of M. haemolytica was lower than that found in calf nasal exudates by Frank and Smith, (1983) (17%) and Wray and Thompson, (1971) (87.70%).

In case of buffaloes, the recovery rate of P. trehalosi was higher than that in cattle (33.30% vs. 12.50%) but lower than that reported by El-Shahedy, (1985); Youssef,(1989) ; El-Battrawy, (1991). A part from species susceptibility, no explanation could be given, and further investigations will be needed to clarify the situation in buffaloes.

It has been stated that isolates frequency is low in nasal cotton swabs of healthy, non-stressed animals and high in calf with respiratory tract disease (Frank and Smith, 1983). This agrees with the prevalence values found in this study. Mannheimia undergoes explosive growth to become the dominant nasopharyngeal isolate in cattle subjected to stressful management practice, viral respiratory infections, or change in environmental conditions (Purdy et al., 1986). Once extensive colonization of the upper respiratory tract has been established, M. haemolytica 1 invades the lung through repeated aspiration of infected droplets or sloughed tissue.

Sheep and Goats

Previous investigations on the prevalence of M. haemolytica have shown a considerable variation. Ranges between 8.9% and 96.2% of healthy sheep that carry these organisms in the nasal cavity have been reported (AL-Tarazi and Dagnall 1997; Biperstein et al., 1966, 1970). The variation is likely to be caused by several factors including different isolation techniques, misidentification, and seasonal variation. Swabbing of the tonsils and nasal cavity of slaughtered sheep showed that M. haemolytica could be isolated from 95% of the tonsils and 64% of the nasopharyngeal swabs (Gilmour et al., 1974). Furthermore, it has been found that the prevalence of M. haemolytica in temperate climates varies seasonally with a higher prevalence in spring and early summer (Gilmour and Gilmour, 1989).

Frequencies of Mannheimia strain isolates are very variable and can sometimes be high and differ depending on the source of isolation: United States, 15.8% in sheep nasal exudates (Frank, 1982), United Kingdom, recorded a prevalence of 52% for Mannheimia and 42% for P. trehalosi in sheep and goats respiratory system and Turkey, 8.3% in ovine lungs (Kirkan and Kaya, 2005). The prevalence of M. haemolytica was lower than that found in ovine nasal exudates recorded by Blanco et al., 1995 (25%); Pijoan et al., (1999) (35%).

It is evident that M. haemolytica isolates predominate over P. trehalosi in both species. Bali et al., 1993 reported that, although M. haemolytica was the most frequent serotype isolated from sheep in Northern Ireland, P. trehalosi outnumbered M. haemolytica. They constituted 45.4% vs. 38.8% for M. haemolytica, while untypable strains represented 15.8%. Odendaal and Henton, 1995 reported that, although most serotypes were present in sheep and goats in South Africa, M. haemolytica serotypes predominated over the P. trehalosi strains (49.8% vs. 16.4%). The majority of serotypes were associated with pneumonia, followed by gangrenous mastitis.

"blue udder" and septicemia. β-haemolytic Mannheimia species were isolated from 24% to 64% of the sheep in four flocks of sheep in Norway, a total of 26 haemolytic M. ruminalis-like strains were isolated among which, a considerable genetic diversity was found (Poulsen et al., 2006). M. haemolytica causes sporadic cases and small outbreaks of acute pneumonia and pleuritis in goat kids (Jubb et al., 1993). However, little is known about the epidemiology of pasteurellosis in goats.

According to Gilmour and Gilmour, 1989 M. haemolytica is normally associated with pneumonia in cattle and sheep, septicemia in lambs and mastitis in ewes. These observations have subsequently been supported by Angen et al., 2002; Garcia et al., 2009. However, the present investigation clearly demonstrated that these organisms also can be obtained from the upper respiratory tract of apparently healthy sheep.

5. Conclusion
The present study demonstrates that M. haemolytica and Pasteurella trehalosi are found in the upper respiratory tract of healthy animals as well as in diseased animals. It is evident that M. haemolytica isolates predominate over P. trehalosi in sheep and goats. These results may reinforce the idea that P. trehalosi are rare in cattle and play no part in epidemic disease. In case of buffaloes, the recovery rate of P. trehalosi was higher than that in cattle, no explanation could be given, and further investigations will be needed to clarify the situation in buffaloes.

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6. References

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