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. ruminalis, and M. varigena*). The trehalose-negative organisms are now classified as M haemolytica. (Jaworski *et a*l., 1998).

M. haemolytica, formerly Pasteurella,(*P. haemolytica*) is the primary aetiological agent of pneumonic 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 pneumonic 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; Bowland and Shewen, 2000). *M. haemolytica* has been recognized as the principal cause of death from pneumonic 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, managemental and/or infectious factors (Thompson *et at.*, 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 Ghani 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 *Maheinemia 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

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).

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 37^0C 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*

Characteristics	*M.haemolytica*	*M.glucosida*	*M.granulomatis*	*M.ruminalis*	*M.varigena*
β- hemolysis(bovine blood)	+	+	_	_	+
Ornithine decarboxylase	_	d	_	_	d
L(+)Arabinose	_	_	_	_	+
D(+)Xylose	+	+	d	_	+
Meso-Inositol	d	+	d	_	d
D(-)Sorbitol	+	+	+	_	_
L(+)Rhammnose	_	_	_	_	d[b]
β--Glucosidase(NPG)	_	+	+	_	_b
Glycosides[c]	_	d	d	_	_b
α-Fucosidase	+	+	_	_	_b
β--Xylosidase(ONPX)	_	d	d	_	_

+, 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. [b]strains of Bisgaard Taxon are positive. [c]Glycosides: cellobiose, esculin, amygdalin, arbutin, gentiobiose, and salicin.

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 H_2S, 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%).

Table2: Incidence of *M. haemolytica* in respiratory tract of apparently healthy & diseased animals.

Species	Apparently healthy animals			Diseased animals			Total		
	No.of samples	No.of isolates	%	No. ofsamples	No.of isolates	%	No. of animals	No.of isolates	%
Cattle	175	4	2.30	50	4	8	225	8	3.60
Buffaloes	143	4	2.80	10	2	20	153	6	3.90
Sheep	187	22	11.80	54	12	22.20	241	34	14.10
Goats	158	16	10.10	60	16	26.70	218	32	14.70

Buffaloes

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.

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

The distribution and recovery rate of M. haemolytica according to the site of respiratory tract from which they were recovered are illustrated in Table (3) and Figure (2).

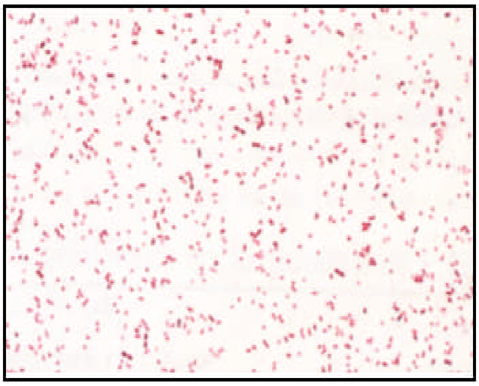

Fig.(1): *Mannheimia haemolytica* on blood agar, revealed grey, medium sized colonies with zones of β-haemolysis.

Fig.(2): Gram stained *M. haemolytica*, revealed gram – ve coccobacilli (x1000)

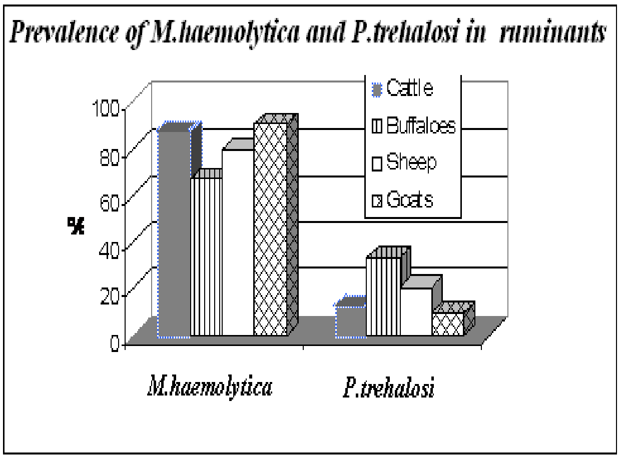

Fig. (3): Prevalence and distribution of *M. haemolytica* in apparently healthy & diseased animals.

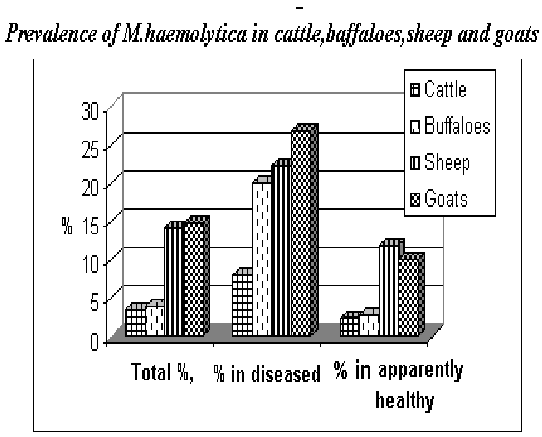

Fig. (4): Prevalence and distribution of *M. haemolytica* in respiratory tracts of apparently healthy & diseased animals.

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:

Type of sample	Cattle				Buffaloes				Sheep				Goats			
	M. haemolytica		*P. trehalosi*		*M. haemolytica*		*P. trehalosi*		*M. haemolytica*		*P. trehalosi*		*M. haemolytica*		*P. trehalosi*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Nasal Swabs	2	25	1	12.50	4	66.70	1	16.66	21	61.80	5	14.70	26	81.30	3	9.40
Lungs	3	37.50	0	0	0	0	1	16.66	6	17.60	2	5.90	3	9.40	0	0
Tonsils	2	25	0	0	0	0	0	0	-	-	-	-	-	-	-	-
Totals	7	87.50	1	12.50	4	66.70	2	33.32	27	79.40	7	20.60	29	90.70	3	9.40

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, septicaemia 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 Pasturella 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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