Journal homepage:
http://www.bsu.edu.eg/bsujournals/JVMR.aspx

Online ISSN: 2357-0520 Print ISSN: 2357-0512

Original Research Article

# Bacteriological studies on calves affected with respiratory manifestations

**El-Seedy, F.R.[a], Abed, A.H.[a,*]; Hassan, H.M.[b]; Nabih, A.M.[b]; Khalifa, E.[c] and Salem, S.E.[b]**

[a] Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.
[b] Animal Reproduction Research Institute, Giza, Egypt.
[b] Microbiology Department, Faculty of Veterinary Medicine, Matrouh University, Egypt.

## ABSTRACT

Bovine respiratory disease (BRD) is the most common and costly disease affecting beef cattle calves in the world. The objectives of this study were to put a high light on epidemiology of *P. multocida* and *M. haemolytica* as important causes of bovine respiratory disease (BRD) in Egypt Governorates; Giza, El-Fayoum, Beni-Suef, Assiut and Sohag. A total number of 406 deep nasal swabs and blood samples were collected from 406 bovine calves suffered from respiratory manifestations reared in different Governorates in Egypt. Bacteriological examination was achieved and isolation of *P. multocida* and *M. haemolytica* were attained. The overall prevalence of both *P. multocida* and *M. haemolytica* was of 26.6%; 18.2% for *P. multocida* and 8.4%for *M. haemolytica*. EL-Fayoum Governorate showed the highest prevalences while Beni-Suef Governorate showed the lowest prevalences. *P. multocida* was singly isolated from 4.9% of cases. While it was mixed with *S. aureus*, *E. coli*, *Streptococcus* spp., both *S. aureus* and *E. coli*, both *S. aureus* and *Streptococcus* spp. and both *E. coli* and *Streptococcus* spp. with percentages of 4%, 1.2%, 2.2%, 1.7%, 3.2% and 1.0%, respectively. Meanwhile, *M. haemolytica* was isolated as a single isolate from 1.7% of cases while it was mixed with *S. aureus*, *Streptococcus* spp., both *S. aureus* and *Streptococcus* spp. and both *E. coli* and *Streptococcus* spp. with percentages of 2.7%, 1.2%, 2.5% and 0.2%, respectively. In conclusion respiratory manifestations are very important disorders in bovine calves. *P. multocida* and *M. haemolytica* are the most common bacteria causing infection.

## ARTICLE INFO

Article history:

Received 3/2019

Accepted 4/2019

Online 4/2019

***Keywords:***

*P. multocida*, *M. haemolytica*, Bovine respiratory disease, prevalence

[*]**Corresponding author:** *Department* of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.
**Email:** aboabedelmasry@yahoo.com, ahmed.moawad@vet.bsu.edu.eg

## 1. Introduction

Bovine respiratory disease (BRD) is one of the most common diseases affecting beef cattle calves all over the world causing great economic losses due to reduction of average daily gain, feed efficiency, overall performance of beef calves and finally calves mortality (Härtel *et al.,* 2004 and Taylor *et al.,* 2010). The incidence of BRD has been reported with variability from 5% to 66% in feedlot cattle and it is the most costly beef cattle disease as the costs are associated with BRD prevention, treatment, morbidity, and mortality rate (Snowder *et al*., 2006).

BRD in calves is often referred to as a 'multifactorial disease' meaning that besides infectious agents, a multitude of environmental and managemental factors and their interactions are responsible for the outbreak of disease (Kabeta *et al*., 2015). Many of the infectious agents commonly involved in calf pneumonia are common inhabitants of the nasal passages of healthy animals. Many factors can weaken the host's immune system and/or damage the lining of the respiratory tract to such an extent that these pathogens are able to progress deeper into the respiratory tract and cause disease (Lopez, 2001). Any one or a combination of the environmental and management factors can make calves more susceptible to disease. Bacterial infections causing pneumonia in calves can possibly be fatal.

*Pasteurella (P.) multocida* and *Mannheimia (M.) haemolytica* are the two bacteria most commonly associated with pneumonia in cattle calves. Numerous other bacteria can also cause pneumonia including Mycoplasma, Pseudomonas, Corynebacteria, Staphylococcus, Hemophilus, *E. coli*, Streptococcus, Bordetella, Neisseria, Erysipelothrix and Fusobacterium (Aly *et al*., 1990 and Asaye *et al.,* 2015).

Pneumonic pasteurellosis refers to any of the disease conditions caused by bacteria of the genera Pasteurella or Mannheimia especially *P. multocida* and *M. haemolytica* are most commonly associated with pneumonia in cattle calves (Adamu and Ameh, 2007 and Asaye *et al.,* 2015). The disease manifests itself most often in calves within four weeks of weaning, when calves are sorted and often sold to different farms. This gives it a common nickname, "Shipping Fever" (Snowder *et al*., 2006).

The objective of this study was to put a high light on epidemiology of *P. multocida* and *M. haemolytica* as important causes of bovine respiratory disease in Egypt.

## 2. Materials and methods

### 2.1. Animals

A total number of 406 pneumonic bovine calves reared in different Governorates (Giza, EL-Fayoum, Beni-Suef, Assiut and Sohag) were examined during the period from January 2017 till December 2017 (Table 1).

### 2.2. Samples

A total number of 406 deep nasal swabs collected under aseptic conditions for bacteriological examination from 406 calves affected with respiratory manifestation. Also, blood samples were collected from each calf for bacteriological investigation using Leishman's stain for detection of Pasteurella bipolarity.

**Table 1. Number of animal and samples collected from respiratory affected bovine calves from different Governorates of Egypt.**

| Governorates | Number of affected animals | Number of collected samples | |
|---|---|---|---|
| | | Nasal swab | Blood samples |
| **Giza** | **69 (17.0%)** | **69** | **69** |
| **El-Fayoum** | **106 (26.2%)** | **106** | **106** |
| **Beni-Suef** | **83 (20.4%)** | **83** | **83** |
| **Assiut** | **87 (21.4%)** | **87** | **87** |
| **Sohag** | **61 (15.0%)** | **61** | **61** |
| **Total** | **406** | **406** | **406** |

### 2.3. Bacteriological examination

Isolations of *P. multocida*, *M. haemolytica* and other bacteria were done according to Collee *et al.* (1996) and Quinn *et al.* (2002). The collected nasal swabs were inoculated under aseptic conditions into brain heart infusion broth (BHIB) and incubated aerobically at 37C for 6-8 hrs. A loopful from broth was cultured onto blood agar, MacConkey's agar and DAS media then incubated aerobically at 37C for 24 hrs. Cultivation of other bacteria were achieved

using nutrient agar, MacConkey's agar, blood agar; eosin methylene blue media (EMB), mannitol salt agar; Baird Parker agar and modified Edward's media then incubated aerobically at 37C for 24-48 hrs. All the recovered isolates were identified morphologically using Gram's stain.

**2.4. Blood smears**

Two blood films were freshly prepared from each examined calf and stained with Leishman's stain for detection of Pasteurella bipolarity.

**2.5. Biochemical identification of the bacterial isolates**

All the recovered isolates were identified biochemically according to schemes described by Kreig and Holt (1984), Collee *et al.* (1996) and Quinn *et al* (2002). The suspected isolates of *P. multocida* and *M. haemolytica* were tested for haemolysis on blood agar and growth on MacConkey's agar as well as biochemical test; oxidase, catalase, indole, triple sugar iron agar medium, citrate utilization and sugar fermentation (glucose, lactose, sucrose and mannitol) tests.

## 3. Results

**3.1. Results of isolation and characterization of *P. multocida* and *M. haemolytica***

*P. multocida* and *M. haemolytica* bacteria appear under microscope with Gram's stained smears as Gram-negative coccobacilli or rods and non-spore forming. Meanwhile by Leishman's stain of blood smears bacteria appears light blue with dark blue bipolarity in both sides of coccobacilli or rods**.**

**3.2. Biochemical and other characters of *P. multocida* and *M. haemolytica***

Results of biochemical characters of both *P. multocida* and *M. haemolytica* were recorded in table (2).

**Table 2. Biochemical and other characters of *P. multocida* and *M. haemolytica*.**

| Biochemical test | *P. multocida* | *M. haemolytica* |
|---|---|---|
| **Haemolysis on blood agar** | **- (No haemolysis)** | **Beta-haemolysis** |
| **Growth on MacConkey** | - | + |
| **Indole production** | + | - |
| **Oxidase** | + | + |
| **Catalase** | + | + |
| **Citrate** | - | - |
| **Glucose fermentation** | + | + |
| **Sucrose fermentation** | + | + |
| **Lactose fermentation** | - | + |
| **Mannitol fermentation** | + | + |
| **$H_2S$ production** | - | - |

**3.3. Prevalence of *P. multocida* and *M. haemolytica* in different Governorates**

The overall prevalence of both *P. multocida* and *M. haemolytica* was 108/406 with a percentage of 26.6% arranged as 74/406 for *P. multocida* with a percentage of 18.2% and 34/406 for *M. haemolytica* with a percentage of 8.4%. The detailed data for Governorates were shown in table (3).

**Table 3. Prevalence of *P. multocida* and *M. haemolytica* isolates in different Governorates.**

| Governorates | No. of samples | *P. multocida* | | *M. haemolytica* | | Total | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| **Giza** | 69 | 13 | 18.8 | 6 | 8.7 | 19 | 27.5 |
| **El-Fayoum** | 106 | 22 | 20.8 | 10 | 9.4 | 32 | 30.2 |
| **Beni-Suef** | 83 | 11 | 13.3 | 5 | 6.0 | 16 | 19.3 |
| **Assiut** | 87 | 16 | 18.4 | 8 | 9.2 | 24 | 27.6 |
| **Sohag** | 61 | 12 | 19.7 | 5 | 8.2 | 17 | 27.9 |
| **Total** | 406 | 74 | 18.2 | 34 | 8.4 | 108 | 26.6 |

%: Percentages were calculated according to the corresponding No. of samples.

### 3.4. Prevalence of single and mixed *P. multocida* infections in different Governorates

Totally in all studied Governorates, *P. multocida* was singly isolated from 20 cases (4.9%). While in case of mixed infection; it was isolated with *S. aureus* from 16 infected calves (4%). Also, it was mixed with *E. coli*, *Streptococcus* spp., both *S. aureus* and *E. coli*, both *S. aureus* and *Streptococcus* spp. and both *E. coli* and *Streptococcus* spp. as follow: 5 cases (1.2%), 9 cases (2.2%), 7 cases (1.7%), 13 cases (3.2%) and 4 cases (1.0%), respectively (Table 4).

**Table 4. Prevalence of single and mixed *P. multocida* infections in different Governorates.**

| Governorates / Isolates | Giza (*No.= 69*) | | El-Fayoum (*No.= 106*) | | Beni-Suef (*No.= 83*) | | Assiut (*No.= 87*) | | Sohag (*No.= 61*) | | Total (*No.= 406*) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NO. | % | NO. | % | NO. | % | NO. | % | NO. | % | NO. | % |
| ***P. multocida* (Single isolate)** | **4** | **5.8** | **6** | **5.7** | **3** | **3.6** | **5** | **5.7** | **2** | **3.3** | **20** | **4.9** |
| ***P. multocida*+ *S. aureus*** | **3** | **4.4** | **5** | **4.7** | **3** | **3.6** | **3** | **3.5** | **2** | **3.3** | **16** | **4.0** |
| ***P. multocida*+ *E. coli*** | **1** | **1.4** | **2** | **1.9** | **0** | **0** | **1** | **1.1** | **1** | **1.6** | **5** | **1.2** |
| ***P. multocida*+ Streptococci.** | **1** | **1.4** | **3** | **2.8** | **1** | **1.2** | **2** | **2.3** | **2** | **3.3** | **9** | **2.2** |
| ***P. multocida*+ *S. aureus*+ *E. coli*** | **2** | **2.9** | **2** | **1.9** | **1** | **1.2** | **1** | **1.1** | **1** | **1.6** | **7** | **1.7** |
| ***P. multocida*+ *S. aureus*+ Streptococci** | **2** | **2.9** | **3** | **2.8** | **2** | **2.4** | **3** | **3.5** | **3** | **4.9** | **13** | **3.2** |
| ***P. multocida*+ *E. coli* + Streptococci** | **0** | **0** | **1** | **0.9** | **1** | **1.2** | **1** | **1.1** | **1** | **1.6** | **4** | **1.0** |
| *Total* | 13 | 18.8 | 22 | 20.8 | 11 | 13.3 | 16 | 18.4 | 12 | 19.7 | 74 | 18.2 |

%: Percentages were calculated according to the corresponding No. of samples in each Governorate.

### 3.5. Prevalence of single and mixed *M. haemolytica* infections in different Governorates

The results were presented in table (5) where *M. haemolytica* was isolated as a single isolate from 7 cases (1.7%). There was no mixed *M. haemolytica* isolates neither with *E. coli* nor with both *E. coli* and *S. aureus* in all Governorates. Totally, it was mixed with *S. aureus*, *Streptococcus* spp., both *S. aureus* and *Streptococcus* spp. and both *E. coli* and *Streptococcus* spp. as follow: 11 cases (2.7%), 5 cases (1.2%), 10 cases (2.5%) and one case (0.2%), respectively.

**Table 5. Prevalence of single and mixed *M. haemolytica* infections in different Governorates.**

| Governorates / Isolates | Giza (*No.= 69*) | | El-Fayoum (*No.= 106*) | | Beni-Suef (*No.= 83*) | | Assiut (*No.= 87*) | | Sohag (*No.= 61*) | | Total (*No.= 406*) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NO. | % | NO. | % | NO. | % | NO. | % | NO. | % | NO. | % |
| ***M. haemolytica* (Single isolate)** | **1** | **1.4** | **3** | **2.8** | **1** | **1.2** | **2** | **2.3** | **0** | **0** | **7** | **1.7** |
| ***M. haemolytica* + *S. aureus*** | **2** | **2.9** | **3** | **2.8** | **1** | **1.2** | **3** | **3.5** | **2** | **3.3** | **11** | **2.7** |
| ***M. haemolytica* + Streptococci.** | **1** | **1.4** | **1** | **0.9** | **1** | **1.2** | **1** | **1.1** | **1** | **1.6** | **5** | **1.2** |
| ***M. haemolytica*+ *S. aureus*+ Streptococci** | **2** | **2.9** | **2** | **1.9** | **2** | **2.4** | **2** | **2.3** | **2** | **3.3** | **10** | **2.5** |
| ***M. haemolytica* + *E. coli* + Streptococci** | **0** | **0** | **1** | **0.9** | **0** | **0** | **0** | **0** | **0** | **0** | **1** | **0.2** |
| ***Total*** | **6** | **8.7** | **10** | **9.4** | **5** | **6.0** | **8** | **9.2** | **5** | **8.2** | **34** | **8.4** |

%: Percentages were calculated according to the corresponding No. of samples in each Governorate.

## 4. Discussion

Bovine Respiratory Disease (BRD) is a multi-factorial disease, usually resulting from the interaction of bacterial and viral agents, combined with stress factors resulting in bronchopneumonia (Ellis, 2009 and Kabeta *et al*., 2015). One of the challenges of bovine respiratory medicine is early detection of clinical cases of BRD.

In current investigation, the prevalences of *P. multocida* and *M. haemolytica* in different Governorates were investigated and presented in table (3). The results revealed that the overall prevalence of both *P. multocida* and *M. haemolytica* was 26.6% arranged as 18.2% and 8.4% for *P. multocida* and *M. haemolytica*, respectively. These results were nearly similar to those reported by Zaki *et al*. (2002) who isolated *P. multocida* in a high prevalence rate (19.9%) in comparison to *P. haemolytica* (8.8%) from 226 lung sample taken from pneumonic calves aging 1 day to 2 month old in Kaliobia and Sharkia Governorates. Higher prevalences were obtained by El-Jakee *et al*. (2016) who isolated 88 *P. multocida* isolates from 256

nasopharyngeal swabs and lung tissues samples (34.4%) from different Governorates in Egypt. It was found that dead calves showed the highest percentage of *P. multocida* isolation followed by the emergency slaughtered calves, diseased calves then apparently healthy ones. Also, Ahmed *et al*. (2015) estimated the prevalence of *Pasteurella* spp. in upper respiratory tract of cattle. The prevalence of *Pasteurella* spp. isolation was 38.88%. *P. multocida* prevalence was 30% while *M. haemolytica* prevalence was 20%.

In the same context Abera *et al*. (2014) found that the overall percentage of *P. multocida* (39.3%) and *M. haemolytica* (46.4%) , this results were higher than that mentioned by this study but this may possibly be due to the fact that The etiology of pneumonia is complex and multifactorial, thus the low rate of isolation of *P. multocida* and *M. haemolytica* from the examined animals in the current investigation may be due to other incriminated causes this results is supported by the results obtained by Garoia *et al*. (1982). On the other hand, lower prevalences were recorded by Lasisi *et al*. (2016) who investigated the prevalence of pneumonic pasteurellosis-caused by *P. multocida* and *M. haemolytica* in cattle and isolated *P. multocida* from six unhealthy lung tissue samples (7.22%) while *M. haemolytica* was isolated from one (1.22%) unhealthy lung tissue sample only.

Regarding to the Governorates, EL-Fayoum Governorate showed the highest overall prevalence as 30.2% meanwhile, Beni-Suef Governorate showed the lowest as 19.3%. The highest prevalence of *P. multocida* infection was in EL-Fayoum Governorate (20.8%) while lowest prevalence was in Beni-Suef Governorate (13.3%). Also, the highest prevalence of *M. haemolytica* infection was in El-Fayoum as 9.4% and the lowest prevalence was in Beni-Suef as 6.0%. These findings may be attributed to the different hygienic measures and stress factors in different Governorates.

Prevalence of single and mixed *P. multocida* and *M. haemolytica* infections in different Governorates was illustrated in tables (4& 5). Totally, *P. multocida* and *M. haemolytica* were singly isolated from 4.9% and 1.7% of cases, respectively. Regarding mixed infections, mixing of *P. multocida* and *M. haemolytica* isolates with *S. aureus* was the most Prevalent as 4% and 2.7%, respectively, followed by the mixed infections with both *S. aureus* and *Streptococcus* species as 3.2% and 2.5%, respectively. Then, the mixed infections with *Streptococcus* species as 2.2% and 1.2%, respectively. The current findings may be due to presence of *S. aureus* and *Streptococcus* species as normal flora on the skin and oropharynx which may be flourished and causing diseases as a result of bad hygienic measures or environmental and managemental stresses or any factors weakening the host's immune system and/or damage the lining of the respiratory tract to such an extent that these pathogens are able to progress deeper into the respiratory tract and cause disease (Kabeta *et al*., 2015 an Lopez, 2001).

Finally, results of current investigation are on the same line of that recorded by Radostits *et al.,* (2007). They found that *P. multocida* causes an acute fibrinous bronchopneumonia in calves, these pathogens have adapted to parasitic life in the upper respiratory tract epithelia of apparently healthy animals.

## 5. Conclusion

It was concluded that respiratory manifestations are a very important phenomena in bovine calves. *P. multocida* and *M. haemolytica* are the most common bacteria causing infection as the rate of infection with *P. multocida* and *M. haemolytica* were higher in bovine calves affections.

## References

Abera, D.; Sisay, T. and Birhanu, T. (2014). Isolation and identification of *Mannheimia* and *Pasteurella* species from pneumonic and apparently healthy cattle and their antibiogram susceptibility pattern in Bedelle District, Western Ethiopia. J. Bacteriol. Res., 6(5):32-41.

Adamu, J. Y. and Ameh, J. A. (2007). Pasteurellosis to Mannheimiosis: Taxonomic Changes. Nigerian Vet. J., 28(1):75-79.

Ahmed, W. A.; Al-Rubaei, E. M. and Majeed, S. A. (2015). Prevalence of *Pasteurella* spp. in apparently healthy cattle and buffaloes herd in Baghdad governorate, Iraq. Al-Anbar J. Vet. Sci., 8(1):1-5.

Aly, A.; Mahmoud, E. and Osama, A. (1990). Some studies on respiratory troubles in fattening buffalo steers. Zagaig Vet. J., 18 (4):151-164.

Asaye, M.; Biyazen, H. and Bezie, M. (2015). Isolation and characterization of respiratory tract bacterial species from domestic animals with

pneumonic lungs from Elphora Abattoir, Ethiopia. Inter. J. Microbiological Res., 6(1):13-19.

Collee, J. G., Fraser, A. G., Marmion, B. P. and Simmons, A. (1996). Practical Medical Microbiology. 14th Ed.

Ellis, J.A. (2009). Update on viral pathogenesis in BRD. Animal Health Research Reviews, 10(2):149- 153.

El-Jakee, J. K.; Ali, S. S.; El-Shafii, S. A.; Hessain, A. M.; Al-Arfaj, A. A. and Mohamed, M. I. (2016). Comparative studies for serodiagnosis of haemorrhagic septicaemia in cattle sera. Saudi J. Biological Sci., 23(1):48-53.

Garoia, M.; Sandu, I.; Istrate, N. and Farvr, C. (1982). Haemophilus like bacteria; isolated from calves and lambs. Revistade Cresterea Animaleleler, 32(3):50-55.

Härtel, H.; Nikunen, S.; Neuvonen, E.; Tanskanen, R.; Kivelä, S-L.; Aho, P.; Soveri, T. and Saloniemi, H. (2004). Viral and bacterial pathogens in bovine respiratory disease in Finland. Acta Veterinaria Scandinavica, 45(3-4):193-200.

Kabeta, T.; Fikadu, T.; Zenebe, T. and Kebede, G. (2015). Review on the Pneumonic Pasteurellosis of Cattle Acad. J. Anim. Dis., 4(3): 177-184, 2015

Kreig, N. R. and Holt, J. G. (1984). Bergey's Manual of Systematic Bacteriology. Vol.1. Williams and Wilkins. Baltimore.

Lasisi, O. T.; Obiekwe, U. C. and Amosun, E. A. (2016). The prevalence of pneumonic pasteurellosis-causing microbes: *Pasteurella multocida* and *Mannheimia haemolytica* in abattoir samples in Nigeria. Nat. Sci., 14(7):66-71.

Lopez, A. (2001). Respiratory system, thoracic cavity and pleura. In: Thomson's Special Veterinary Pathology, 3rd Ed., eds M. D.

Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. J. C., Leonard, F. C. and Maguire, D., (2002). Veterinary Microbiology and Microbial Disease. Published by Blackwell. PP. 113-116.

Radostits, O. M.; Gay, C. C.; Hinchcliff, K. W. and Constable, P. D. (2007). Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th Ed. Saunders Ltd, Philadelphia, USA.

Snowder, G. D.; Van Vleck, L. D.; Cundiff, L. V.; Bennett, G. L. (2006)."Bovine respiratory disease in feedlot cattle: Environmental, genetic, and economic factors". J. Anim. Sci., 84 (8): 1999–2008.

Taylor, J. D.; Fulton, R. W.; Lehenbauer, T. W.; Step, D. L. and Confer, A. W. (2010). The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors. The Canadian Veterinary Journal, 51(10):1095–1102.

Zaki, E. R.; Tanios, A. I.; Hafez, N. M. and Yanni, A. A. (2002). Studies on *Pasteurella* species in buffalo calves. J. Egy. Vet. Med. Ass., 62(6A):111-118.