Histopathological and Molecular Identification and Characterization of Pneumonic Pasteurellosis in Sheep and Goats from Outbreaks at AS-Sharika Province, Egypt

Amira Saad Helal Hassenin and Akram Dossier Mohdi Khan

ABSTRACT

Pneumonic Pasteurellosis highly fatal septicemic disease in multi-animal species in bovine, ovine, Poultry and swine. The strain identified from ovine pneumonia were characterized by MLST (Multi-host and RIRDC databases) and virulence-associated gene (VAG) typing and compared with bovine isolates. Recording outbreak of pasteurellosis with high mortality & History of several deaths in different farm and homeowner cases of sheep and goats with severe respiratory illness after shipping them from long distance Elarish and Sinai in period to El-Sharqia province. Most cases that still live are treated with antibiotics after make sensitivity test on sampling from sick cases and most cases recovered and responded to treatments. Histopathological changes of lung tissue from recently dead cases showed the proliferation of pasterella multicide in the respiratory system. The presence of Pasteurella multocida in swap samples collected from per acute & acute cases of sheep & goats was detected by bacteriological examination and Culture.

Keywords: Lung, Pneumonia, Pasteurella, Shipping fever.

1. INTRODUCTION

Hemorrhagic septicemia is a bacterial disease affecting the respiratory of buffalo, and cattle while in sheep & goats diseases called pneumonic pasteurellosis [1]. This is caused by Gram-negative bacteria [2]. These need stress factors as predisposing factors to cause severe illness and often deaths in most cases depending on the severity of stress factors. Shipping animals under uncontrolled conditions is one of the most common deaths and or occurrences hemorrhagic septicemia in small ruminants’ sheep and goats and also affects transportation those small animals with large numbers most common stress and concurrent infection with internal parasites as a lower immune system for poor or no treatments to cases for precaution before transportation.

1.1. Clinical Signs

Most clinical signs of pasteurellosis are acute and show high mortality and fatality up to 48 hours. Apparent per acute cases showed septicemia, moist mouth with extra salivation, mucopurulent, for short incubation period may be noticed or not [3], [4]. An acute form of pneumonic pasteurellosis continues for 5 days with complicated symptoms of recumbency, laying in the ground with severe septicemia, petechial hemorrhage, congestion in mouth and extended edematous swelling from neck to belly often legs [5], [6].

Postmortem pneumonic pasteurellosis in dead carcass [7] showed edematous dark reddish fluid in muscle from the diaphragm, subcutaneous peritoneum up to all abdomen, flank region. Cyanosis is a bluish mucous membrane with severe fibrous tissue in the alveoli and lobes of the lung causing obstructive pneumonia and some cases observed with severe gastrointestinal inflammation with hemorrhagic diarrhea [8].

Accurate identification for detection of different types of Pasteurella species and serotypes using hyperimmune sera [9] from rabbits and mice in most world countries but depending on definitive characteristics of P. multocida by coagulation test, immunoelectrophoretic, molecular diagnosis combined with histo-pathological lesion [10].

Hygienic measures all over the world using vaccination protocols with the old vaccine which is available to be...
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used in young animals called inactive vaccine showed a short period of protection and needs to be administrated in two doses every year [11]. So, most world health organizations developed new protocols for attenuated vaccines from local strain-infected regions to be used for protection and increased longevity with prolonged immunity or even solid natural protection [12], [13].

2. Material & Methods

From November 2018 to October 2019, 11 lung samples out of 33 carcass cases were under aseptic condition for further lab processing from 55 bacteriological swap cases of sheep & goats from different farms showing clinical signs of hemorrhagic septicemia.

2.1. Morphological & Bacteriological Detection

Samples taken from infected cases were bacteriologically identified by cultivation on 3%–5% sheep blood agar (Oxoid Ltd., UK) and MacConkey agar (Oxoid Ltd., UK), followed by incubation at 37°C for 24 hours. The plates were then examined for growth. Next, the colonies were Gram stained and tested, then observed for the following characteristics: no growth on MacConkey agar, then biochemical identification by catalase and oxidase positivity, indole production, with characterization of nonmotility, non-hemolysis on SBA, and acid from glucose [14], [15].

Using the standard method of bacteriological and morphological characterization of growing culture of suspected isolates using gram stain, electron microscope with identification of no hemolysis and biochemical test for Pasteurella spp [16].

2.2. Histo-Pathology

Tissue samples taken from the lung were stabilized using the regular method of tissue stained in hematoxylin with iodine after being immersed in formaldehyde with the buffered solution to help slice tissue into small sections of width 3–5 cm.

2.3. Immunohistochemical Staining

Slices of lung lobes, and alveoli that histopathology were immersed in wax and then removed wax from this section and should be truly moist for ready to be stained by avidin-biotin-peroxidase complex (ABC-P) technique (manufactured, USA). and stained by Antigen retrieval was recognized using laboratory-stained oven standard for sample heating up to 20 minutes or less fixed in concentrated buffer solution [17]. Followed by immersed in 0.3% hydrogen peroxide in methanol for 15 minutes, to stop further peroxide reaction should be incubated at 45°C for 15 minutes with regular sera of goat spp. Then at room temperature section of tissues was fixed in monoclonal and multi-clonal antibodies of Pasteurella species with different dilutions of hyperimmune serum as instructed. results of all used colored labelled ads directed by lab protocols for final indication of samples. Highly specialized electron microscope used by histo-technician for observation and recorded results. All Histo-pathology tissued are examined at 500X resolution using an ultra-light electron microscope at Zagazig University, Faculty of Veterinary, Egypt.

3. Results

3.1. Growth and Hemolysis

Suspected colonies showed growth on Sheep Blood Agar (SBA) and with clear no hemolytic activity. This means that the bacteria did not cause any significant breakdown of red blood cells, resulting in a lack of clearing or discoloration around the colonies on the agar (Fig. 1a). Characteristics identification of bacterial isolates of Pasteurella Multocida in a lab are as follows:

1. Gram Staining and Cell Structure: The bacterial cells were Gram-negative bacilli observed not to keep purple color or blue and instead appeared pink/red under electron microscope. They had a coccoid bipolar structure, which means they were spherical in shape with two distinct ends (Fig. 1b).

2. Additional Characteristics: The isolates were non-motile, meaning they did not exhibit movement using flagella. They tested positive for catalase and oxidase, indicating the presence of specific enzymes. They were also indole-positive, which means they produced indole when tested. Furthermore, the isolates were able to use glucose for acid production, a characteristic commonly observed in Pasteurella multocida.

3. API® 20NE Test: Eight isolates did not grow on MacConkey agar, and all isolates positively known as Pasteurella multocida using the API® 20NE test.

![Fig. 1. (a) Sheep Blood Agar (SBA) showed non-hemolysis and (b) Gram-negative coccoid under the electron microscope.](image)

![Fig. 2. Molecular identification of Pasteurella multocida isolates with specific primers.](image)
Fig. 3. (a–c) Interstitial pneumonia is characterized by the filtration of interstitial tissue with lymphoid cells and mononuclear cells, smooth destructive muscle cells along fibroid.

This identification method uses a commercial system that determines bacterial identification based on specific biochemical reactions. The numerical profile obtained for these isolates was 3000004, with a percent identification (% ID) of 96%.

4. Unusual Isolates: more than three cultures exhibited the red color of isolates on specialized plate growth, suggesting the ability to ferment lactose. Surprisingly, the API® 20NE test other mixed infections, respectively, with different numerical profiles and per cent identifications. However, subsequent polyclonal using molecular diagnosis with primers, in fact, *Pasteurella multocida* (Fig. 2).

These findings highlight the importance of confirming bacterial identification through molecular techniques like PCR, as they provide more accurate and reliable results compared to solely relying on biochemical tests.

3.2. Histopathology

Gross eye examination and macroscopically found that the right cranial lobe & left cranial lobe highly affected the medial right lobe respectively. Infected tissue and lobes highly showed solidification with severe inflamed enlarged hardy to be manipulated and in texture.

Histo-pathologically, the section taken from lobes of infected lungs and bronchi showed catarrhal and patches of reddish spots mixed with fibrous fluid. Mononuclear cellular infiltration was noted in the bronchioles (Fig. 3).

Microscopically, lobular and obstructive-pneumonia with fibrinous exudate (n = 11) and catarrhal purulent (n = 22) infiltration of alveoli, lung lobes and internal connective tissue with squamous cell and fibrous mixed by many leukocytes, neutrophil as shown (Figs. 4a–4c).

The lumen of alveoli is filled with necrotic cells with fibrous tissue adhered to a number of syncytial cells in spindle shape infiltrated with many neutrophils (Fig. 5). Parts of lung lesion showed necrotic coagulation with exudative catarrhal mucous. In certain sections of stained alveoli, the lumen was identified as widespread necrotic, mucopurulent pneumonia with mixed fibrous tissue and monocytes. more than 11 solidified sections of the pulmonary section showed high perforation with neutrophil, fibroid cells, and sero-edematous fluid with adeno filtrations. On the other hand, more than 5 sections showed interstitial pneumonia characterized by filtration of interstitial tissue with lymphoid cells and mononuclear cells, smooth destructive muscle cells along with fibroid (Figs. 3a–3c).

16 lung tissue sections, interstitial pneumonia was characterized as mononuclear cell infiltration in interstitial areas (n = 16). In 4 lung tissue sections, pulmonary adenomatosis was seen. The immunohistochemical and microbiological results are shown. At the end of the immunohistochemical stainings, pneumonic pasteurellosis was found in 65 of 110 animals. The immunopositive areas were localized in the bronchi, bronchiole alveolar lumen and epithelial.

4. Discussion

Hemorrhagic septicemia is one of the most important causes of losses in shipping animals and the most affected animal in Sharqia province in Egypt. New research and studies have detected big concerns and important infectious disease issues in sheep and goats [18]–[21].

Most affected villages and regions in south climatic areas and Sharqia province identified pneumonic pasteurellosis caused by *Pasteurella multocida* [22]–[25] compared to other studies Pasteurella.

Pneumonic pasteurellosis in alveoli and lobules was narcotic, fibrinous and mucolytic with obstructive bronchi that make an explanation for severe symptoms of respiratory illness and loss in many sheep and goat flocks. more cases showed in histopathology more severe lobar pneumonia than lobular with severe infection.

The rapid spread of disease through close animal contact spreads mucous of coughing infected cases and mucous in feeding parts and watering supplies makes the situation of *Pasteurella* get worse. Easy growth and contamination of other respiratory bacteria as suppressed immunity of
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Fig. 4. (a) Infiltration of alveoli, (b) lung lobes and (c) internal connective tissue with squamous cells and fibrous mixed by many leukocytes, and neutrophils.

Fig. 5. Necrotic coagulation with exudative catarrhal mucous infiltrated with neutrophilic, mononuclear cells in and around the bronchus and bronchioles.

ill cases and shown mucopurulent bronchopneumonia in the most pathological lesion [26]. The mixed bacterial infection gets conferred using molecular identification to *Pasteurella multocida* causes of *pneumonic pasteurellosis* by PCR [27]. Bacteriological isolation with the microscopic examination was completed and confirmed with highly sensitive and more accurate using histochemical for histopathological identification of *pasteurellosis* [28]–[30]. Using hygienic measures and precautions to control and prevent infection is an important way before shipping or moving animals for long distances using specific antibiotics is a good way to standardize protocol.

Sever losses in herd flocks of sheep and goats and deaths fatalities in many cases caused by hemorrhagic septicemia with the most virulent respiratory bacteria called *Pasteurella multica* will affect animal production and breeding [31]. *Pneumonic pasteurellosis* causes outbreaks in many regions all over the world need together close attention and take all precautions using the most accurate method for diagnosis and control.

Hygienic measures, good animal sanitation during transmission for long distances through vaccination, and good treatment are the best ways to control diseases in our region.

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**Conflict of Interest**

Authors declare that they do not have any conflict of interest.

**References**


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