



An outbreak of hemorrhagic septicemia in a vaccinated herd of domestic water buffalo in Thi Qar province, Iraq: Clinical and pathological observations

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ARTICLE INFO

Received: 15.06.2014

Revised: 20. 07.2014

Accepted: 29.09.2014

Publish online: 12. 10.2014

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Abstract

An outbreak of hemorrhagic septicemia (HS) with a 100% morbidity and 27.5% mortality was reported in a herd of domestic water buffalo (*Bubalus bubalis*) at

Thi Qar / the south west of Iraq. This herd was vaccinated against the disease 45 days prior to transportation into Thi Qar province. The disease was diagnosed based on clinical signs (fever, nasal and ocular mucus discharges, profuse salivation, dyspnea, abnormal respiratory sounds “rales” and restlessness). *Pasteurella multocida* was isolated from the lungs of dead animals. The postmortem examination revealed edematous swelling of the neck, brisket and sub-mandibular regions; frothy exudate in congested trachea; widely distributed petechial hemorrhages; blood-tinged fluid in the thoracic and abdominal cavities, in addition, to enlargement and hyperemia of kidney. Histopathologically, there were distention of alveolar spaces and inter-alveolar connective tissue septa by inflammatory exudate consisting mainly of fibrin, edematous fluid, RBCs and inflammatory cells particularly polymorphonuclear cells (PMNs). In addition, the bronchial and bronchiolar lumens were filled with mucinous exudate and inflammatory cells. Thickening of pleura was also observed due to the pleuritis as indicated by the presence of sub-mesothelial fibrinous exudate, inflammatory cells and blood vessels congestion.

To cite this article: Waffa. A. Ahmed; Nagham. M Al-Gburi; Hamoudi. S. Rassol . (2014). An outbreak of hemorrhagic septicemia in a vaccinated herd of domestic water buffalo in Thi Qar province, Iraq: Clinical and pathological observations. MRVSA. 3 (2), 36-43. DOI: [10.22428/mrvsa.2307-8073.2014.00325.x](https://doi.org/10.22428/mrvsa.2307-8073.2014.00325.x)

Keywords: Buffalo (*Bubalus bubalis*), HS, *Pasteurella multocida*, Thi Qar/ Iraq.

Introduction

Domestic water buffalo (*Bubalus bubalis*) is known as the world’s second most important milch animal as it shares more than 95% of the milk produced in South Asia (Javaid *et al* 2009). There are about 285,537 head of buffaloes in Iraq; out of these, 39.33 % were vaccinated with alum precipitated vaccine against hemorrhagic septicemia (National survey, 2008).

The importance of Hemorrhagic septicemia (HS) to buffaloes rearing in Iraq is highlighted by a survey, which indicated its prevalence up to 38.74 % (National survey, 2008). The disease causes many outbreaks and infections in cattle, buffaloes and sheep in Basra (Al-Hamed, 2010), Al-Qadisyia (Salah, 2012) and Baghdad (Al-Shemmari, 2013). Hemorrhagic septicemia is an acute and often fatal disease primarily occurring in water buffaloes and cattle, but occasionally other domesticated and wild mammals (De-Alwis, 1992; Ahmed, 1996). Buffaloes are more susceptible to HS than cattle (De Alwis, 1999). Hemorrhagic septicemia is characterized by a rapid course, fever, edematous swelling in the head-throat-brisket region, swollen and hemorrhagic lymph nodes and presence of numerous sub-serous petechial hemorrhages (Nas *et al.*, 2012), The submandibular edema and extended edema were produced following increase permeability due to endo-toxemia following infection (Radostits *et al.*, 2007). Trachea, is large enough in animals that travels along the neck, where acute inflammatory reaction takes place as a result of released inflammatory substances, which in return lead to development of submandibular edema (Harper and Adler, 2006; Shafarin *et al* 2009). Hemorrhagic septicemia is caused by the gram-negative bacterium *Pasteurella multocida* (Wijewardana, 1992; Tabatabaei *et al.*, 2007). So far, various serotypes (A, B, C, D and E) of *P. multocida* have been detected among the livestock population (Ghandrasekaran and Chuink, 1981; Borowska-opacka and Kedrack, 2003; Kedrack and Borowska-opacka, 2003; Kumar *et al.*, 2004). Serotypes B: 2 and E: 2 are two common serotypes of *P. multocida* associated with disease in animals in Asia and Africa, respectively (Benkirane and De ALwis, 2002). The disease has a major impact on the livestock industry in countries of South and Southeast Asia, where HS associated with serotype B: 2 is distributed widely (Dagleish *et al.*, 2007; Hajikolaei *et al.*, 2008; Ataei *et al.*, 2009). It results in severe economic losses and is ranked as the most important contagious disease of cattle and buffaloes (Benkirane and De ALwis, 2002). The disease is generally associated with stressful conditions such as wet and humid weather condition in the tropic, poor husbandry practices or rearing under stressful free-range system (De Alwis, 1990). Iraq, being a sub-tropical region of South west Asia has an early hot summer with dry environment and a late hot summer with humid environment especially in the south of Iraq, such conditions favor the explosive occurrence of HS (Papadakis, 1966; Ahmed 1996).

The current report deals with an outbreak of HS that took place in a herd of domestic water buffalo. It describes the history, clinical manifestations, gross and histopathological changes and isolation of *P.multocida* from the lung of dead animals.

Material and methods

Study area

The current report describes an outbreak of HS that took place in Thi Qar governorate, a large city at the south of Iraq. It is the second governorate after Basra in the population of buffaloes (National survey, 2008). It has a long summer episode that extends from May to September. In summer, ambient temperature ranges from 30 to 48°C and even sometimes may go to 50°C. A severe hot and humid (humidity 45.38±13.11%) period begins from mid-July and extended up to mid-September (Tara, 2011).

Case history

The outbreak took place in a herd of domestic water buffaloes recently transported from some north and middle Iraqi governorates (Kirkuk, Salah Al Din and Baghdad) to Thi Qar in search for water and food. The affected herd was consisting of 40 animals (with an age range from 1-12 year) vaccinated against HS 45 days prior to transportation to Thi Qar.

Postmortem and histopathological examination

Postmortem examination was performed on 3 out of the total 11 dead animal. Tissue samples were collected from the necropsied animals and preserved in 10% neutral buffered formalin and processed for histopathological examination. Sections of 5 µm thickness were stained with hematoxyline and eosin for microscopic examination (Luna, 1968).

Bacterial isolation and identification

Other tissues samples (lung, pharynx and liver) were also collected and transported in a cool box to the Zoonotic diseases unit in the College of Veterinary Medicine, Baghdad University. For bacterial isolation and identification, the samples were cultured under aerobic condition on Blood agar (oxid), MacConky agar, brain heart infusion broth, and brain heart infusion agar. Biochemical tests were carried out on isolated bacteria (Quinn *et al.*, 2006).

Results

Clinical signs

All animals in the herd were affected (100% morbidity) and 11 died out of the total 40 (27.5% mortality). The animals showed variable clinical signs including elevated body temperature (41°C), depression, restlessness, and respiratory sounds “rales”. Other clinical signs included profuse salivation, dyspnea, mucinous nasal discharge, bloat, recumbence and death within 4 days. These signs were observed in most dead animals, while others died suddenly without showing signs of illness.

Bacterial isolation and identification

The bacterial isolates were recovered from the lungs, it appeared as gram negative cocobacilli, glucose and sucrose fermenter but lactose non-fermenter on MacConkey agar. They were positive to the oxidase, catalase, and indol tests and negative to the urea test.

Pathological examination

The gross pathological examination revealed edematous accumulation consisting of coagulated sero-fibrinous exudate within the neck, brisket and sub-mandibular regions. The trachea was congested and contained frothy fluid. Blood-tinged fluid was found in the thoracic and abdominal cavities. Sub-serosal petechial hemorrhages were observed on visceral organs of the abdominal cavity. Petechial hemorrhages were also noted on the epicardial surface of the heart and the sub-capsular surface of lymph nodes particularly

the pharyngeal and cervical lymph nodes which were enlarged. The kidneys were enlarged and hyperemic, however, the liver appeared to be normal. Histopathological examination of the lungs of the necropsied animals revealed distention of alveolar spaces and inter-alveolar connective tissue septa by inflammatory exudates consisting mainly of fibrin, edematous fluid, RBCs and inflammatory cells particularly PMNs, however, lymphocytes, and macrophages were also seen. The bronchi showed focal epithelial sloughing associated with infiltration of inflammatory cells (Figure 1). Other lung sections showed areas of congestion and hemorrhage and some of the alveolar spaces were filled with fibrino-purulent exudate consisting of fibrin, PMNs and macrophages. The bronchial and bronchiolar lumens were filled with mucinous exudate and inflammatory cells (Figure 2). In some other sections, there was severe destruction of pulmonary tissue associated with inflammatory cell infiltration particularly PMNs, lymphocytes and macrophages within the lung parenchyma and around blood vessels (Figure 3). Inflammatory exudate was also apparent in the bronchial and bronchiolar lumens. Infiltration of inflammatory cells particularly PMNs, macrophages and plasma cells was observed within the interstitial tissue, alveolar spaces and around bronchi associated with emphysema (Figure 4). Thickening of pleura was also observed due to pleuritis as indicated by presence of sub-mesothelial fibrinous exudate, inflammatory cells and blood vessels congestion. HS outbreaks usually occur as disastrous epizootics in many Asian and African countries resulting in high mortality and morbidity (Bain *et al.*, 1982; De- Alwis, 1992). Outbreaks of HS has been recorded in South Asia, Middle East and Africa (FAO, 1989) , Zimbabwe (Lane *et al.*, 1992), Pakistan (Khan *et al* 2006; Khan *et al.*, 211). A wide range of mortality rate (5 to 90%) has been reported in different outbreaks in India (Saini *et al.*, 1991) and Philippines (Molina *et al.*, 1994). In the present study, 100% morbidity and 27.5% mortality due to HS has been recorded in this out break in the buffaloes herd. This high mortality can be attributed to the acute and sometimes per acute clinical nature of HS particularly in buffaloes which tend to be more susceptible to the disease than cattle (Quinn *et al.*, 2004). Vaccination is the major control measurement of HS (Benkirane. and De ALwis, 2002). In the present study, in spite of the vaccination of the animals by the HS alum precipitated vaccine. The disease took place in the herd in the form of an outbreak. The occurrence of the disease might be due to the fact that the vaccine administrated parenterally and need to be repeated, in addition, this vaccine appeared to be not sufficiently efficacious (Tabatabaei *et al.*, 2007). The vaccination might be failure because it was carried out only 45 days prior to the onset of the high-risk season (late summer with high temperature and humidity) , however, these period is not sufficient for the protection of the animals against HS. The vaccination procedure must be carried out preferably two to three months before the high-risk season (Benkirane and De ALwis., 2002). Hot and humid weather is a major predisposing factor in initiating the outbreak of HS because *P.multocida* favors the high environmental temperature for its growth (Hajikolaei *et al.*, 2008). Under field conditions, HS is usually diagnosed on the basis of clinical signs and symptoms (De Alwis, 1999; Khan *et al.*, 2006; Farooq *et al.*, 2007).

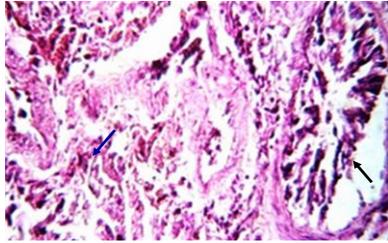


Figure 1: Shows inflammatory cells in lung parenchyma (→), sloughing of bronchial epithelia (←) (H&Ex40)

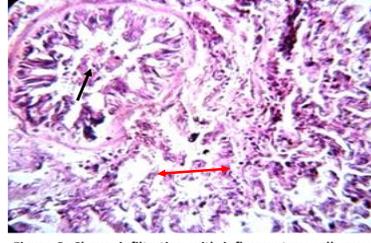


Figure 2: Shows infiltration with inflammatory cell cause thickening of interalveolar septa (→), mucin and inflammatory cell within bronchial lumen (←) (H&Ex40)

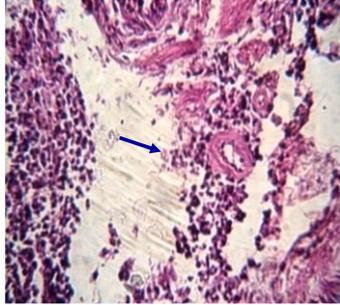


Figure 3. Shows peribronchiolar aggregation of mononuclear inflammatory cells (→) (H&Ex40)

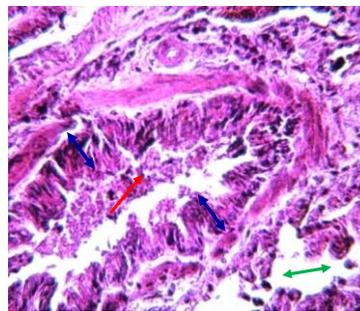


Figure 4. Shows inflammatory exudates in bronchial lumen (suppurative bronchiolitis →), hyperplasia of bronchial epithelia (←), emphysema (↔) .(H&Ex40)

The clinical signs were observed in the present study such as fever, reduced appetite, profuse salivation, mucous nasal discharge, dyspnea and restlessness along with distinctive respiratory sound “rales” and typical swellings in the submandibular region, were appeared on most of the dead animals. These clinical signs are compatible with clinical signs reported in HS cases reported worldwide (Al-Shemmari, 2013; De Alwis, 1999; Farooq *et al.*, 2007). Edematous swelling of the neck, brisket and sub-mandibular regions; frothy exudate in congested trachea; widely distributed petechial hemorrhages were the predominant postmortem changes observed in the dead animals, in addition to hyperemia and enlargement of the kidney in the current outbreak. These finding are in constant with those reported by previous studies (Al-Shemmari, 2013; De Alwis, 1999; Benkirane and De ALwis, 2002; Farooq *et al.*, 2007; Sheikh *et al.*, 1996). *P. multocida* was recovered from the lung, while it was not recovered from other samples because it is fastidious pathogen and the samples were deteriorated (Quinn *et al.*, 2004). This result is in agreement with the results reported by (Al-Humam, 2004; Townsen *et al.*, 1998). It can be concluded from this study that HS is highly fatal disease, can provisionally be diagnosed on a combination of clinical signs, gross and histopathological examination and environmental seasonal consideration such as temperature and humidity. In addition, vaccination gainst HS before transportation consider as important factors in the initiation of the disease also.

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