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ORIGINAL ARTICLE

First outbreak of bovine haemorrhagic septicaemia caused by *Pasteurella multocida* type B in Spain – Short communication

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ABSTRACT

This paper describes the first documented outbreak of haemorrhagic septicaemia (HS) caused by *Pasteurella multocida* type B in cattle in Spain. This acute, highly fatal septicaemia causes major economic losses in cattle and buffaloes in many areas of Asia and Africa. In other species and in European countries it is an infrequently reported disease. Acute septicaemic pasteurellosis occurred in a free-range farm of 150 cattle and 70 beef calves in Southern Spain. Twenty-one calves and one cow were affected, of which three calves and the adult cow died. Postmortem examination revealed characteristic oedema in the ventral area of the neck and the brisket region, and widespread haemorrhages in all organs. Pure cultures of *P. multocida* were obtained from all tissues and organs studied. The aetiological agent was further confirmed by molecular and biochemical analysis as *P. multocida* capsular type B, biovar 3. Although the source of infection could not be determined, wildlife may play an important role. The use of tulathromycin in the initial stage of the disease might be related to the low morbidity and mortality of this outbreak. After using an autogenous vaccine no more cases of HS were observed.

KEYWORDS

outbreak, haemorrhagic septicaemia, *Pasteurella multocida*, capsular type B, septicaemic pasteurellosis, bovine

Haemorrhagic septicaemia (HS) is an acute, highly fatal and septicaemic disease caused by the capsular types B or E of *Pasteurella multocida* (Shivachandra et al., 2011). This heterogeneous pathogen is classified into three subspecies (*multocida, septica* and *gallicida*), five capsular types (A, B, D, E and F), 16 somatic serotypes (1–16) and 13 biovars (1–10 and 12–14) (Harper et al., 2006). The age, host species, immunity status, husbandry methods or wildlife–livestock interface can influence the appearance of HS. Young animals are more susceptible and immunosuppression or humid weather conditions can facilitate the appearance of HS outbreaks (De Alwis, 1992; Shivachandra et al., 2011; Soike et al., 2012).

In pigs, sporadic outbreaks of HS have been reported from different continents such as Asia (Verma, 1988; Gamage et al., 1995; Townsend et al., 1998b) and Europe (Soike et al., 2012; Ujvári et al., 2015), including Spain (Cardoso-Toset et al., 2013). In cattle and buffaloes, in contrast, HS is considered an endemic disease in some areas of Asia and Africa, causing high morbidity, mortality and substantial economic losses (De Alwis, 1992). North America and Europe have also reported sporadic cases of HS in cattle and wild ruminants (Eriksen et al., 1999; Soike et al., 2012; Ujvári et al., 2015; Magyar et al., 2017). However, in Spain this disease has not been reported yet in cattle. This paper describes the first outbreak of bovine HS caused by *P. multocida* type B in Spain.

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An outbreak of acute septicaemia occurred on a free-range system farm of beef cattle in Southern Spain (February 2015). This herd contained 220 animals, including 150 adult cattle and 70 calves. Their feeding was pasture-based and supplemented with commercial feed. Drinking water was provided *ad libitum* from a natural source, also frequented by local wildlife like wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). All adult cattle were routinely vaccinated against *Clostridium perfringens*, while the calves did not receive any treatments.

The acute septicaemia affected 21 calves and one cow. The most characteristic findings in sick calves were a marked oedema of the ventral area of the neck, fever, respiratory distress and nasal discharge. Three of the affected calves died within a few hours and the adult cow died suddenly without evident clinical signs. The remaining sick calves recovered after emergency treatment with tulathromycin (Draxxin®, Zoetis), injected subcutaneously in the neck as a single dose of 2.5 mg/kg body weight, after the first detection of clinical signs. Dead animals were submitted to the Department of Animal Health and Pathological Anatomy of the Faculty of Veterinary Medicine, University of Córdoba for necropsy as well as histopathological and microbiological examination.

Postmortem examination was performed according to standard procedures. The ventral area of the neck and the cranial area of the thorax showed severe oedema and, when sectioned, showed also subcutaneous and intramuscular petechial haemorrhages. There was an accumulation of fluid in the pericardial sac (hydropericardium), chest (hydrothorax), and abdomen (ascites). Multiple haemorrhages appeared widespread in all organs (Fig. 1). Tissue samples from lung, liver, spleen, kidney, heart, thymus, retropharyngeal lymph node, joints and submandibular tissue from four animals were collected after necropsy for the histopathological and the microbiological study. The samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned into 4-μm sections, and stained with haematoxylin and eosin for the histopathological study. Histopathological examination revealed oedema in the subcutaneous tissue of the ventral area of the neck and bacterial foci in the dermis. The lung tissue showed hyperaemia, alveolar oedema, emphysema, severe congestion, and microthrombi. Diffuse oedema was present in the cardiac muscle fibres. The kidneys, lymph nodes, stomach and thymus revealed severe hyperaemia. Bacterial foci were present in the kidney and lymph nodes. The spleen showed lymphoid depletion with a small amount of blood and a few lymphocytes (Fig. 2).

For the microbiological study, samples of all organs were plated on blood agar, MacConkey agar, Tryptone Sulphite Neomycin agar (TSN agar) and Rappaport-Vassiliadis medium (Oxoid), and incubated at 37 °C for 24–48 h under microaerobic conditions. A total of 18 isolates were obtained from the lung (n = 4), liver (n = 3), spleen (n = 2), kidney (n = 1), heart (n = 1), thymus (n = 1), retropharyngeal lymph node (n = 1), joints (n = 3) and submandibular tissue (n = 2). Based on colony morphology, Gram staining, oxidase and catalase tests (Gram-negative coccobacilli, catalase and oxidase positive), a first hypothetical identification as *P. multocida* was accomplished. The identification was further confirmed by detection of the *KMT1* gene using a species-specific PCR assay (Townsend et al., 1998a). The subspecies was determined based on sorbitol and dulcitol fermentation (Mutters et al., 1985), biovar on the production of the enzyme ornithine decarboxylase, urease activity and fermentation of seven carbohydrates: dulcitol, sorbitol, lactose, maltose, arabinose, xylose, and trehalose (Blackall et al., 1997), and capsular type by a multiplex PCR (Townsend et al., 2001). The isolates were phenotypically and molecularly characterised as *P. multocida* subsp. *multocida* capsular type B and biovar 3. The isolated strains were tested by the disc diffusion test for susceptibility to amoxicillin, gentamicin, ceftiofur, florfenicol, tulathromycin, and oxytetracycline. All strains proved to be susceptible to all antibiotics tested.

A month after the first outbreak, six new calves became affected by HS. To control this second outbreak, the affected animals received the same emergency treatment with tulathromycin as the affected animals in the first outbreak. When the causative strains were isolated, an autovaccine containing 1×10^8 cfu/ml of *P. multocida* type B was produced at the Department of Animal Health at the University of Córdoba (unpublished data). Briefly, *P. multocida* type B isolates were cultivated on blood agar (Oxoid) at 37 °C for 24 h and inactivated with 0.3% formaldehyde (30–40% w/v, Panreac, Spain). The suspension was centrifuged at 720 *g* for 15 min at 4 °C and the pellets were resuspended in an isotonic saline solution until the pattern 0.5 on the McFarland scale was obtained. The vaccination scheme consisted of a first subcutaneous dose of 4 ml for adult cattle and 3 ml for calves, followed by revaccination one month later and subsequent doses every year. No more animals fell ill or died due to *P. multocida* type B infection after treatment with the autovaccine.

In Europe, HS is an extremely uncommon disease that was detected for the first time in free-range pigs in Spain in 2009 (Borge et al., 2011). Since then, *P. multocida* type B has also been detected in HS outbreaks in pigs (Cardoso-Toset et al., 2013) and wild boar (Risco et al., 2013) in this country. To our knowledge, this disease has not been previously reported in the bovine species in Spain, so this is the first study describing the characteristics of an outbreak of HS in cattle in this country. This disease can cause major economic losses unless it is quickly recognised and treated.

Although the source of infection could not be accurately determined, it may be related to contact with pigs or wildlife. The affected animals were raised under extensive housing conditions, which facilitate contact among wild and domestic species. Other authors described some previous outbreaks of HS involving cattle, pigs and fallow deer in the same area of Germany (Soike et al., 2012; Volker et al., 2014). In fact, the role of fallow deer and red deer as healthy carriers has been previously reported (Soike et al., 2012). However, the lack of data from neighbouring areas made it impossible to establish a certain epizootiological

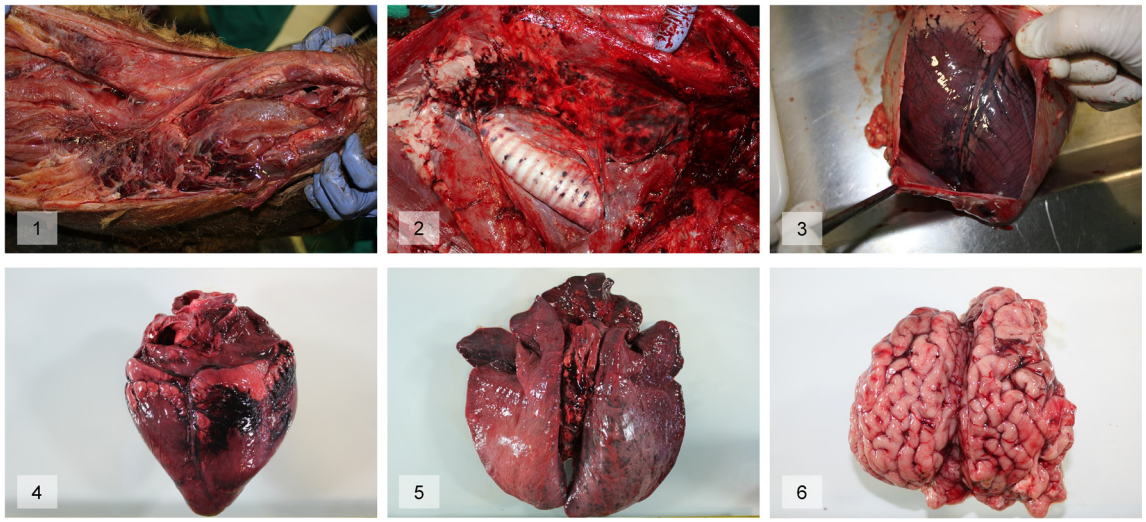

Figure 1. Gross lesions: Oedema and haemorrhages in the ventral area of the neck (1) and cranial area of the thorax (2), hydropericardium (3), haemorrhages in the heart (4) and in the cranial as well as caudal lobes of the lung (5), and congestion of the central nervous system (6)

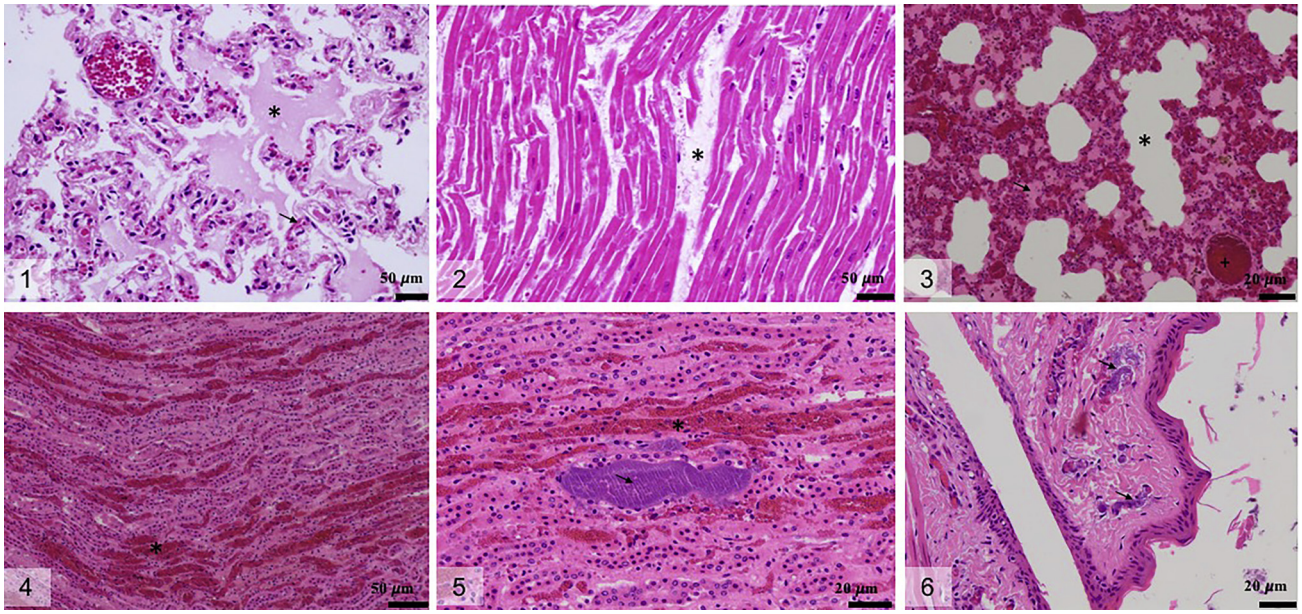

Figure 2. Histopathological lesions: Alveolar oedema (*) and microhaemorrhages (arrow) in the lung (1). Oedema (*) between the cardiac muscle fibres (2). Oedema (arrow), emphysema (*) and severe congestion (+) in the lung tissue (3). Severe hyperaemia (*) and bacterial foci (arrow) in the kidney (4, 5). Groups of bacteria (arrow) in the dermis (6)

relationship. As regards the factors predisposing to the emergence of HS in another study (Shivachandra et al., 2011), the most affected animals were young calves and the average relative humidity recorded in the month of disease onset was 72.9%.

Although mortality is nearly 100% when HS is first introduced in an area (De Alwis, 1992), in this study both morbidity and mortality were low (10% and 18%, respectively). This controversy has also been found in the literature. While low morbidity and mortality were reported in wild boar (Risco et al., 2013), swine (Ujvári et al., 2015) and cattle (Magyar et al., 2017; Ujvári et al., 2018), Cardoso-Toset et al. (2013) described high morbidity and mortality rates in swine (70% and 95%, respectively). This difference might be attributable to the timing of the treatment administered. High recovery rates have been reported when antibiotics were used at an early stage of the disease (Shivachandra et al., 2011). Interestingly, in the outbreaks with a low morbidity and mortality, as in our case, treatment was carried out right after the onset of the disease (Risco et al., 2013; Ujvári et al., 2015; Magyar et al., 2017), while Cardoso-Toset et al. (2013) did not provide any information about treatment. Our data also suggest that an early antibiotic treatment can effectively minimise the losses. In addition, further studies on the antimicrobial susceptibility of emerging pathogens, such as *P. multocida* type B in European countries, should be performed because of the increase of antibiotic resistance (Ujvári et al., 2018). In accordance

with the recommendation to use killed vaccines for preventing new cases of HS (Shivachandra et al., 2011), treatment with an autovaccine seems to prevent further outbreaks on farm level.

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