Haemorrhagic septicaemia (septicaemic pasteurellosis) in cattle in Baden-Wuerttemberg (Germany)

Haemorrhagische Septikämie (Wild- und Rinderseuche) bei Rindern in Baden-Württemberg (Deutschland)

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Summary

Haemorrhagic septicaemia (septicaemic pasteurellosis) caused by Pasteurella (P.) multocida capsular type B has been diagnosed in cattle in the federal state of Baden-Wuerttemberg (Germany) for the first time in June 2019 since its last official report in Germany in 1986 and its reoccurrence in 2010. A total of 13 cattle succumbed to sudden death on four farms located in the northern part of the Black Forest between June and November 2019. Post-mortem examinations were carried out on seven of these animals and all displayed marked hyperaemia, haemorrhages and oedema in the subcutis and in several inner organs. The pathological-anatomical findings were confirmed by histopathological analyses. Cultivation of P. multocida was successful in five of seven cattle with high bacterial loads in internal organs, and poor growth in two animals. All isolates were clearly identified as P. multocida by MALDI-TOF mass spectrometry and were compared with isolates originating from an HS outbreak in eastern Germany in summer 2010 using Fourier transform infrared (FT-IR) spectroscopy. Molecular capsular and LPS genotyping assigned six of the isolates to the genotypes B:L2 and one to B:L6, respectively. All isolates belonged to the RIRDC MLST genotype ST122. Antimicrobial testing of P. multocida isolates originating from each farm based on the determination of minimal inhibitory concentration (MIC) revealed susceptibility to ampicillin, ceftiofur, enrofloxacin, florfenicol, penicillin G, tetracycline, and tulathromycin. Resistance was determined for spectinomycin.

Keywords: Septicaemic pasteurellosis, Pasteurella multocida capsular type B, MALDI-TOF MS, FT-IR, LPS genotyping, MLST

Zusammenfassung


Schlüsselwörter: Wild- und Rinderseuche, Pasteurella multocida Kapseltyp B, MALDI-TOF MS, FT-IR, LPS-Genotyp, MLST

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Introduction

Haemorrhagic septicaemia (HS) is a serious disease in cattle and other bovines. It is characterised by an acute and septicaemic course resulting in high mortality rates (OIE 2013, Shivachandra et al. 2011). HS in cattle is a disease listed by the OIE (OIE 2020). However, small ruminants and pigs, and horses and donkeys to a much lower extent, are also susceptible to this disease (De Alwis 1999). HS has also been recorded in wild ungulates (Carrigan et al. 1991, De Alwis 1999), and a devastating HS outbreak led to a mass extinction of about 200,000 critically endangered saiga antelopes in Kazakhstan in 2015 (Fereidouni et al. 2019).

The causative agent of HS is Pasteurella (P.) multocida, a gram negative, facultative anaerobic, non-motile bacterium. P. multocida isolates can be classified by capsular serotyping or by DNA-based capsular typing into the five groups A, B, D, E and F (Townsend et al. 2001) and further subtyped based on eight LPS genotypes (L1–L8) (Harper et al. 2015) comprising 16 serovars (Brogden et al. 1978, Hedleston et al. 1972). However, the occurrence of capsular non-typeable P. multocida isolates has to be considered (Arumugam et al. 2011). Usually, the capsular type B is found in HS cases in Asia and Europe (Asian capsular type) whereas type E is encountered in Africa (African capsular type) (De Alwis 1992). The occurrence of HS is usually climate-related and areas regularly hit are Asia, sub-Saharan Africa and southern Europe (Shivachandra et al. 2011). However, HS has also been reported in cattle, pigs and wildlife within the last decade in countries located in central Europe in temperate climate zones such as Hungary (Magyar et al. 2017, Ujvari et al. 2015), Spain (Risco et al. 2013), Denmark (Eriksen et al. 1999) and Germany (Falkenberg 2016, Kutzer et al. 2019, LUA 2019, Müller and Locher 2017, Rohkohl et al. 2015, Soike et al. 2012, TSK Sachsen 2018, Völker et al. 2014). In Germany HS has reoccurred in 2010 (Soike et al. 2012) since the last officially reported cases in 1986 (OIE 2016, WAHIS 2019).

Material and Methods

General data on the cases

General data on the affected farms including the date of first registered HS cases, the herd localities and sizes, and the number, age and sex of the perished animals were collected. Post-mortem examinations were performed on seven cattle originating from four herds located in three municipalities from two districts (Table 1, Fig. 1). Data on the lowest and highest maximal outdoor temperatures per day for each month during the period in which the cases of HS occurred was accessed from the open access database (www.wetterkontor.de).

Pathological examinations

The carcasses were subjected to pathological-anatomical and histopathological examinations. Tissue samples from the organs showing gross lesions were submitted for histological analyses using haematoxylin-eosin staining.
Microbiological examinations

For cultivation of bacteria, aseptically obtained specimens from lung, liver, spleen, kidney, mesenteric lymph nodes and other visibly affected organs were streaked directly onto 5% sheep blood agar and water-blue metachrome-yellow lactose agar according to Gassner. In addition, specimens of the lungs were smeared on Pasteurella Selective Agar (Oxoid, Wesel, Germany). The agar plates were incubated at 37°C under aerobic conditions for at least 48 h. For further analyses, colonies indicative for Pasteurella species were sub-cultured on agar with 5% sheep blood to obtain pure cultures. The MALDI-TOF mass-spectra generated by the microflex LT System (Bruker Daltonik, Bremen, Germany) were analysed using the Bruker Biotyper software Version 3.1 and evaluated with the Bruker Taxonomy database (DB 8:468 entries, Bruker Daltonik). Analyses were extended with an in-house database, including a set of 13 reference spectra of known P. multocida capsule types (MUP 0924–0931, 0933, 0934, 0937, 0942, 1503), comprising representatives of capA, capD, capF, capsular non-typeable, and five reference spectra of P. multocida isolates from capsular type B or E (Rau et al. 2016a). The creation of new reference entries, so-called main spectra projections (MSP), followed the manufacturer’s instructions and standards (Pranada et al. 2016, Rau et al. 2016b).

FT-IR (Fourier transform infrared) spectroscopy analyses were carried out and evaluated with a Tensor 27 spectrometer equipped with an HXT module (Bruker Optics, Ettlingen, Germany) (Contzen et al. 2011). Cluster analysis (Ward’s algorithm) was performed using the second derivatives of the spectra in the spectral range 500–1450 cm⁻¹. The IR-spectra of the isolates were compared with a collection of P. multocida including several capsular type B isolates (Fig. 2).

For the identification of HS-causing P. multocida isolates we used the OIE-recommended HS-causing-type-B-specific PCR according to Townsend et al. (1998). LPS genotyping was carried out using the multiplex PCR described by Harper et al. (2015). Furthermore, all isolates were subjected to the RIRDC MLST scheme developed for P. multocida based on PCR amplification and sequencing of seven housekeeping genes according to Subaaharan et al. (2010). The sequence types were determined with help of the Pasteurella multocida MLST Databases of PUBMLST (https://pubmlst.org/pmultocida/) (Jolley et al. 2004). For this purpose, genomic DNA was extracted using the QIAGEN Genomic-tip 100/G kit (Qiagen, Hilden, Germany) and the Roche High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturers’ instructions from a single colony grown in LB medium supplemented with 1% glucose at 37°C.

Minimum inhibitory concentrations (MIC) of antimicrobial drugs for P. multocida isolates recovered from the cattle 2, 3, 4, and 5 (Table 1) were determined based on

![FIGURE 1: Localisations of the HS outbreaks in farm A, B, C and D in the districts of Calw (CW) and Freudenstadt (FDS) in the federal state of Baden-Wuerttemberg. The farms are located within a circle with a diameter of 40 km (dotted circle). Stuttgart, capital of the federal state of Baden-Wuerttemberg (Graphic: CVUA Stuttgart)](image)

### TABLE 2: Gross lesions of organs revealed by post-mortem examinations

<table>
<thead>
<tr>
<th>Cattle No.</th>
<th>Respiratory tract</th>
<th>Liver</th>
<th>Spleen</th>
<th>Further organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hyperaemia, haemorrhages, interstitial oedema, and alveolar emphysema</td>
<td>Severe hyperaemia, swollen</td>
<td>Haemorrhages, hyperplasia of the pulp</td>
<td>Hyperaemia and haemorrhages of the meninges, heart, thoracic and abdominal cavities and mesentery, oedema of the subcutis</td>
</tr>
<tr>
<td>2</td>
<td>Hyperaemia, haemorrhages, and alveolar emphysema</td>
<td>Swollen and tough consistency</td>
<td>Hyperplasia of the pulp</td>
<td>Severe hyperaemia of the udder and abomasum, haemorrhages in the thoracic cavity and heart, severe oedema of the subcutis, pericardial effusion</td>
</tr>
<tr>
<td>3</td>
<td>Unchanged</td>
<td>Unchanged</td>
<td>Depleted</td>
<td>Discrete haemorrhages in the thoracic cavity and pericardium, oedema of the subcutis, pericardial effusion</td>
</tr>
<tr>
<td>4</td>
<td>Unchanged</td>
<td>Swollen and brittle consistency</td>
<td>Depleted</td>
<td>Haemorrhages in the pericardium, on the epicardium and mucosa of urinary bladder, severe oedema of the subcutis of the head and neck</td>
</tr>
<tr>
<td>5</td>
<td>Fibrin deposits on the lung</td>
<td>Few, small telangiectasias</td>
<td>Fibrin deposits on the organ surface</td>
<td>Haemorrhages on the endocardium, focal haemorrhages in the wall of the abomasum and the small intestine, patchy haemorrhages in the mesentery</td>
</tr>
<tr>
<td>6</td>
<td>Hyperaemia and oedema</td>
<td>Swollen</td>
<td>Hyperplasia of the pulp</td>
<td>Severe hyperaemia of the brain, epicardium covered with confluent petechia, massive, acute fibrinous peritonitis, intestinal hyperaemia, haemorrhages in the Waldeyer’s tonsillar ring and the pleura costalis</td>
</tr>
<tr>
<td>7</td>
<td>Oedema</td>
<td>Unchanged</td>
<td>Haemorrhages</td>
<td>Haemorrhages on the endo- and epicardium, spleen, diaphragm, serosa of the stomachs, oedema of the subcutis of the head and neck</td>
</tr>
</tbody>
</table>
the microdilution method using the antibiotic micro-
dilution plate Micronaut-S Veterinary Large Animal
(MERLIN, Bornheim-Hersel, Germany). Antimicrobial
susceptibility testing was carried out according to the
manufacturer’s instructions and evaluated according to
the CLSI guidelines (CLSI 2018) (Table 3).

Results

General data on the cases
A total of 13 cattle succumbed to sudden death on
four farms in the federal state of Baden-Wuerttemberg
(Germany). The farms are located in the administrative
districts of Calw and Freudenstadt in the northern Black
Forest region (Fig. 1). The HS cases occurred within a
period of approx. four months (June/July to November
2019). Eleven cattle died on the farms’ pastures and two
cows in an open loose-housing barn. From all seven cat-
tle submitted for post-mortem examinations, P. multocida
could be cultivated (Table 1). Antibiotics were adminis-
tered only in farm A, starting with enrofloxacin for two
days and continued with penicillin for further three days.
The minimum distance between the farms is 2 km and
they are located within a circle with a diameter of 40 km
(Fig. 1). The animals on these farms had neither direct nor
indirect contact. The cattle grazed in groups of 30 (farm A),
14 (farm B), and 28 (farm C) individuals, respectively. In
farm D 164 cows lived in an open loose-housing barn. All
farms are located directly at forest borders of the eastern
side of the northern Black Forest and are separated by
large areas of forest and meadows which have been partly
visited by wild animals such as wild boars.

All animals submitted to post-mortem examinations
had been found dead from one day to the next without
previous clinical signs such as fever, recumbency, stupor,
malaise or dyspnoea. Details on the animals subjected to
post-mortem examinations, and details on animals that
died peracutely on these farms but were not examined
are listed in Table 1.

Analyses of the climate data for the northern Black
Forest (weather station Neubulach near Bad Teinach-Zavelstein, district of Calw) during the period of the HS
outbreaks revealed maximum temperatures per day, as
follows: June 15.6–33.8 °C, July 18.0–34.2 °C, August
16.8–31.0 °C, September 11.7–27.4 °C, October 6.3–24.4
°C, and November (until 15. November) 2.5–15.9 °C.

Pathology

On first inspection, all animals were in good physical
and nutritional condition. Post-mortem examinations
revealed hyperaemia, haemorrhage or oedema in various
organs or in at least one organ with different severity.
The kidneys of all seven animals did not display vis-
able pathological changes. The pathological findings are
summarised in Table 2.

The pathological anatomical changes were corrobo-
rated by pathohistological investigations. Examination
of six cattle revealed hyperaemia, haemorrhages and
oedema in the lung (cattle 2, 3 and 7), liver (cattle 2),
kidney (cattle 5), meninges (cattle 1, 2, 6), udder (cattle
1 and 2), heart (cattle 1), skeletal muscles (cattle 1) or
subcutis (cattle 2) at different intensities. In addition,
necrosis of the renal tubules (cattle 2), lungs and muscles
(cattle 3) were found. In cattle 4, interstitial oedema and
necrotising vasculitis in the tissue between oesopha-
gus, trachea and buccal mucosa was apparent. Cattle 5
showed scarring and interstitial fibrosis of the lung.

Microbiological examinations

High bacterial load of P. multocida was detected in lung,
liver, spleen, kidney and lymph nodes in animals 1 to 4
and 7 after cultivation on blood agar for 24 h. Due to the
pathological findings, swabs were also taken from the
pleura, urinary bladder, udder and muscle (cattle 1) and
from the brain, heart, subcutis and udder (cattle 2), from
muscle (cattle 3), and from blood and subcutis (cattle 7),
respectively. These specimens also showed very strong
P. multocida bacterial growth.

In cow 5, only two colonies of P. multocida could be
cultivated from the liver and the spleen, respectively and
in cattle 6, cultivation of P. multocida was only successful
from the spleen and the pleura with poor and extensive
bacterial growth, respectively.

TABLE 3: Antimicrobial susceptibility testing of P. multocida
isolates from animal 2, 3, 4 and 5 determining the minimum
inhibitory concentrations (MIC)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (μg/ml)</th>
<th>Interpretative categories and MIC breakpoints</th>
<th>S (μg/ml)</th>
<th>I (μg/ml)</th>
<th>R (μg/ml)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>0.025</td>
<td>&lt;0.03</td>
<td>0.06–0.12</td>
<td>&gt;0.25</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤0.125</td>
<td>&lt;2</td>
<td>4</td>
<td>&gt;8</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>0.0625</td>
<td>&lt;0.25</td>
<td>0.5–1</td>
<td>&gt;2</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Florfenicol</td>
<td>≤1–2</td>
<td>&lt;2</td>
<td>4</td>
<td>&gt;8</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Pencillin G</td>
<td>0.125</td>
<td>&lt;0.25</td>
<td>0.5–1</td>
<td>&gt;1</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>&gt; 64</td>
<td>&lt;32</td>
<td>64</td>
<td>≥128</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5–2</td>
<td>&lt;2</td>
<td>4</td>
<td>&gt;8</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Toluathromyc</td>
<td>≤1–4</td>
<td>&lt;16</td>
<td>32</td>
<td>≥64</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

S = sensitive, I = intermediate, R = resistant

FIGURE 2: Cluster analysis of infrared spectra of P. multocida isolates was performed using the second derivatives of the spectra in the spectral range of 500 to 1450 cm⁻¹. Ward’s algorithm was applied. The seven isolates from farm A, B, C, and E are indicated with the cattle number in bold letters, according to Table 1. More details on all P. multocida strains used for comparison are provided on https://maldi-up.ua-bw.de. (Graphic: CVUA Stuttgart)
In antimicrobial susceptibility testing, *P. multocida* isolates originating from all four farms were sensitive to ampicillin, cefotiof, enrofloxacine, florfenicol, penicillin G, tetracycline, and tulathromycin and resistant to spectinomycin (Table 3).

**MALDI-TOF MS**

The putative *P. multocida* isolates were confirmed at species level by MALDI-TOF MS with the commercial Bruker database (score values 2.315 ± 0.051). The application of the database, which had been extended by 13 additional reference entries for capsular type defined strains, resulted in an increase of the score values to 2.619 ± 0.065. The hit lists for the isolates from the HS cases were headed by matches of *P. multocida* from both HS causing capsule types B and E.

**Capsular typing**

Fourier-transform infrared (FT-IR) spectroscopy with cluster analysis was carried out as described previously (Nagib et al. 2014). The available isolates from HS cases described in this study were compared with isolates from HS cases from eastern Germany in 2010/2011, as well as other *P. multocida* with defined capsule types (Soike et al. 2012). The cluster diagram shows the level of uniformity between the capB-type *P. multocida* from the 2019 event, and the similarity to isolates (R70, URF75) from the HS cases 2010/2011 from Germany (Fig. 2).

The type-B-specific PCR for HS-causing *P. multocida* bacteria consistently retrieved a positive result. Additional testing on the capsular type confirmed that all isolates belong to capsular type B.

LPS genotyping allocated the isolates obtained from the animals 1–4 and 6–8 to genotype L2, which includes the Heddleston serovars 10, 11, 12 and 15. LPS genotype L6 had been retested with the same result and was unequivocally associated with pathological anatomical findings typical for HS in cattle this finding remains unclear. However, all except for one isolate could be assigned to the genotype B:L2:ST122 and all isolates to the clonal MLST group ST122. This is in agreement with findings for isolates associated with HS in bovines originating from Europe (Hotchkiss et al. 2011, Soike et al. 2012) and for isolates which been collected in Asia and India (Hotchkiss et al. 2011, Peng et al. 2018, Petersen et al. 2014).

Testing of antimicrobial susceptibility revealed sensitivities to a broad spectrum of antibiotics, including ampicillin, cefotiof, enrofloxacine, florfenicol, penicillin G, tetracycline, and tulathromycin for all *P. multocida* isolates. These results are in agreement with published data (BVL 2019, OIE 2018, Rohkohl et al. 2015, Soike et al. 2012).

**Discussion**

**Clinical signs, pathology**

The cases of HS in cattle described in this report developed typical acute or peracute, predominantly sudden fatal outcomes of the disease without obvious or only subtle clinical signs the day before. Pathological changes were characterised by hyperaemia, haemorrhage and oedema in several organs. The major inner organs lung, liver, spleen and the serosa of the body cavities were most strongly affected by hyperaemia and haemorrhages. Additionally, the brain, udder and muscles were also visibly affected in some cases. Oedema were present in the lung and subcutis. These pathological findings were pronounced at different intensities and could be confirmed by histopathological examinations. Völker et al. (2014) also outline comparable pathological lesions and Soike et al. (2012) comprehensively described pathological findings in cattle, fallow dear and pigs that had succumbed to death due to recent outbreaks of HS. Severe organ lesions are caused by virulence factors of *P. multocida* capsule type B, especially endotoxins (LPS) triggering a sudden and immense secretion of proinflammatory cytokines provoking endotoxic shock symptoms and finally leading to death (Harper and Boyce 2017, Horadagoda et al. 2001).

**The pathogen**

In the cases reported here, HS led to a high bacterial load of *P. multocida* in the lung, liver, spleen and kidney in five of seven examined cattle. The affected organs, i.e. brain, udder, muscle, heart and the serosa of body cavities corresponded with those recently described by Völker et al. (2014) and Soike et al. (2012). In two cases, however, *P. multocida* was detected in two organs only (kidney and spleen, spleen and pleura), although the animals had not been treated with antibiotics. With the exception of the pleura, the bacterial load was low. This observation raises the question of the role of bacterial load, virulence associated genes and pathogenesis of *P. multocida* during HS (Verma et al. 2013).

*P. multocida* was identified unambiguously using MALDI-TOF MS and the capsular type was determined by FT-IR analyses and verified by capsular-PCR (Soike et al. 2012, Townsend et al. 2001).

The FT-IR cluster diagram shows a high level of uniformity between the seven capB-type *P. multocida* from the 2019 event, and a great similarity to isolates from the HS cases 2010/2011 from Germany (Fig. 2).

Further LPS genotyping revealed HS isolates of the genotype L2 (comprising serovars 2, 5) in all farms. However, a *P. multocida* isolate of the LPS genotype L6 (comprising the serovars 10–12, 15) was isolated from an animal on farm A and a day later an isolate with the LPS genotype L2 from another animal on the same farm. It has been reported that HS in cattle and buffalo is exclusively caused by the LPS genotype 2 (Harper and Boyce 2017). Even though, *P. multocida* belonging to the LPS genotype 6 had been retested with the same result and was unequivocally associated with pathological anatomical findings typical for HS in cattle this finding remains unclear. However, all except for one isolate could be assigned to the genotype B:L2:ST122 and all isolates to the clonal MLST group ST122. This is in agreement with findings for isolates associated with HS in bovines originating from Europe (Hotchkiss et al. 2011, Soike et al. 2012) and for isolates which been collected in Asia and India (Hotchkiss et al. 2011, Peng et al. 2018, Petersen et al. 2014).

**Epidemiology**

In Germany, the last cases of HS were officially reported in 1986 (OIE 2016, WAHIS 2019). However, the re-emergence of HS in Germany has been confirmed by several outbreaks over the past decade. Cases have been reported in Brandenburg and Saxony-Anhalt (2010) (Soike et al. 2012), Lower Saxony (2013/2014) (Rohkohl et al. 2015, Völker et al. 2014), Mecklenburg-Western Pomerania (2014) (Falkenberg 2016), and recently in Bavaria (Müller and Locher 2017), Saxony (TSK Sachsen 2018), Thuringia (Kutzer et al. 2019), Rhineland-Palatinate (LUA 2019), and in Baden-Württemberg (present study). All outbreaks in cattle have in common that animal deaths occurred mainly during the hot season
in association with grazing. In the present report, the temperature exceeded 30°C in June. The first HS cases occurred within one week and further sporadic cases followed in the subsequent three months. The last case of HS was in late November when the temperatures had already dropped below 16°C. HS outbreaks regardless of the season have been reported in subtropical (India) (Kumar et al. 2004) and temperate regions (Germany) (Rohkohl et al. 2015). The occurrence of HS at various locations within a 50 km diameter and a time period of only one week has been reported by Rohkohl et al. (2015) and Soike et al. (2012). Rohkohl et al. (2015) described the onset of an HS outbreak in the warm season and recurrence in November due to latently infected cows returning into the cowshed. This phenomenon can be explained by persistence of the pathogen in the crypts of tonsils and low infectious doses of virulent bacteria (Shivachnadra et al. 2011, Soike et al. 2012). What causes the pathological findings despite low bacterial load in the inner organs is still unknown, as are the pathomechanisms and pathogenesis of septicaemic *P. multocida* infections.

To date, the source of HS and the pathogen spread over long distances, especially to southwestern Germany, remains unresolved. Possible explanations for these outbreaks are direct or indirect contact with wildlife, which could function as carrier (Kutzer et al. 2019, Soike et al. 2012). However, the proof of the source, spread and transmission of infections to livestock is also still pending for the cases reported for Germany (Rohkohl et al. 2015, Soike et al. 2012, Völker et al. 2014).

**Conclusion**

HS has re-emerged in Germany in the past decade. The source of infection, the reservoir, the mode of transmission and the pathomechanisms are still poorly understood. Farmers, veterinarians and hunters should be alerted by sudden cases of deaths in domestic and feral even-toed ungulates. In cases of HS outbreaks, immediate removal of dead animals, implementation of the principles of ‘stand still’, and metaphylactic administration of antibiotics in the short term and vaccination with adequate LPS serotypes in the long term represent effective measures. To prevent outbreaks of HS, it is essential to know the extent and spread of this disease and the sources of infection. Further studies are urgently needed to increase knowledge about HS in central Europe.

The exchange of reference spectra of capsule type defined *P. multocida* isolates is facilitated by the MALDI-user platform (https://maldi-up.ua-bw.de).

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**Ethical approval**

Since this is a study on fatal cases of cattle and no living animals were involved, no ethical vote was necessary. The authors declare that no research interventions or experiment with animals or human beings, no clinical trials or clinical research were conducted in the context of this study.

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**Authors contribution**

RS: Project idea, planning and conception of this study, literature research, writing and revision of the manuscript, data analysis. BB, IS and AKS: Performance of the pathological-anatomical examinations and the histopathological analysis. SM: Collection and analysis of the field data. LDS: Performance and analysis of the molecular investigations. JR: Performance and evaluation of the MALDI-TOF MS and FT-IR studies. All authors contributed to this manuscript by writing and revision and approved the final version.

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