Mannheimia haemolytica and *Bibersteinia trehalosi* Serotypes Isolated from Merino Breed Lambs in Extremadura (Southwestern Spain)

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Abstract Pneumonia caused by *Mannheimia haemolytica* is an important disease in ruminants. Because of its economic significance, several methods have been developed to study the pathogenicity and epidemiology of *M. haemolytica*. In this study, bacterial isolates of *M. haemolytica* and *Bibersteinia trehalosi* identified from the lungs of sheep were serotyped by means of indirect haemagglutination. Of the 598 lungs studied, 34 isolates were identified and serotyped. In decreasing order, *M. haemolytica* serotypes were: not typable (50 %), A1 (17.65 %), A7 (11.76 %), A6 (5.88 %), and A12, A2, A5 and A9 (each representing 2.94 %). The only *B. trehalosi* serotype was T4 (2.94 %). Serotypes A1, A6 and A7 of *M. haemolytica* were the most commonly isolated from pneumonic sheep producing greater changes in the lungs and having important implications for sheep production.

Keywords *Mannheimia haemolytica* · *Bibersteinia trehalosi* · Lambs · Serotypes · Lung lesion

Respiratory diseases are complex, occurring as a consequence of host interaction with various infectious agents, under the influence of environmental factors [1]. Pneumonia is responsible for important economic losses in the sheep industry in Spain, including Extremadura, where sheep herds are present in 94 % of the municipalities [2]. *Mannheimia haemolytica* is one of the most important respiratory pathogens of domestic ruminants causing serious outbreaks of acute pneumonia in neonatal, weaned and growing lambs [3].

Mannheimia haemolytica can be serotyped according to capsular antigens. A total of 17 capsular serotypes have been described, among which serotypes 3, 4, 10 and 15 are now classified as *Bibersteinia trehalosi*, with serotype 11 reclassified as *M. glusosida* [4].

The general aim of this study was to determine and identify *M. haemolytica* and *B. trehalosi* serotypes and the relationship of these microorganisms with the lung lesions that they produce.

This study was carried out in Extremadura in southwestern Spain. A total of 598 samples were collected from lambs with clinically healthy lungs (410) (Group A) and lambs with pneumonic lungs (188) (Group B). All samples were plated onto 10 % Sheep Blood Agar and incubated at 37 °C overnight. Complete identification of these isolates was based on biochemical profiles using the Phoenix (Becton–Dickinson Diagnostics) automatic identification system. Isolates were further characterised by an indirect haemagglutination technique. Antisera for each serotype were produced in rabbits using the reference strains and NCTC provided by Dr. Robert Davies (Scotland). Serotype A17 was provided by Dr. Laszlo Fodor (Hungary).

For macroscopic study, lungs were photographed and classified into two groups depending on the presence or

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absence of consolidation. Consolidated areas and total area were delimited using Adobe Photoshop cs5®. For histological study, samples were fixed in neutral buffered formalin (3.5 %, 0.1 M and pH 7.2), routinely processed and embedded in paraffin. Sections of 5 µm were stained with haematoxylin and eosin, Masson's Trichrome and van Gieson's stain for histological study [5]. Microphotographs were taken using a microscope (Eclipse 80i, Nikon®, Tokyo, Japan) with a digital video camera (DXMI200F, Nikon®).

The experiment was performed with the permission of the local bioethics committee (Approval No. 19/2014, date 13/02/2014).

Exploratory data analysis and descriptive statistics were performed by means of the Chi squared test. The results were considered significant when P values were <0.05 . The software R® was used for this task [6].

A total of 34 lungs presented isolates of *M. haemolytica* and *B. trehalosi*. Only 5 lungs did not show macroscopic lung lesions, however, 29 lungs were affected. In these lungs, only one isolate (1/34) was identified as *B. threalosi*, with the rest identified as (33/34) *M. haemolytica*.

Serotyping of isolates by capsular antigens showed a high degree of heterogeneity. The most frequent serotypes for *M. haemolytica* strains were A1 (6 strains), A7 (4 strains) and A6 (2 strains). The rest of the serotypes (A12, A2, A5 and A9) were found in only one strain and 17 strains were untypable. The serotype for the *B. trehalosi* strains was T4 (1 strain).

We could observe a statistically significant relation between prevalence of these pathogens and consolidation pulmonary ($P < 0.001$) lesions. Therefore, these data showed that when there exists a macroscopic lesion in lungs, 15.43 % (29/188) identified serotypes were identified as *M. haemolytica* or *B. trehalosi*. In lungs without macroscopic lesions, pathogens were isolated from only 1.22 % (5/410). Moreover, in Group A, serotype A12 (1 strain) and the untypable strains (4 strains) were *M. haemolytica*, but in Group B, all serotypes except for A12 were present including serotype T4 of *B. trehalosi*. However, there was no statistically significant relationship between serotypes of these pathogens and lung lesions in the studied lambs ($P = 0.293$).

Serotypes A1 and A2 are the most prevalent worldwide. These serotypes colonize the upper respiratory tract of healthy sheep and are generally species-specific in their ability to cause pneumonia [7]. In this study, serotypes A1, A7 and A6 were the most prevalent, all of which were isolated in lungs with consolidation. Some serotypes (A8, A13, A14, A16 and A17) were not found in these lambs.

In our study, only one animal presented *B. trehalosi* T4. Our results are similar to studies carried out in other

Fig. 1 Bronchioalveolar pneumonia

Fig. 2 Mixed changes between bronchioalveolar pneumonia and purulent bronchopneumonia

geographic regions. Donachie [8] reported that serotype T15 was the most frequently isolated in Scotland and serotype T3 was the most frequently isolated in France according to Villard [9].

In the early stages of the respiratory process, *M. haemolytica* produces cranioventral bronchioalveolar pneumonia characterised by a moderate thickening of the alveolar wall due to hyperplasia of type II pneumocytes [10]. In our study, 15 % of animals with *M. haemolytica* presented these changes in their lungs, highlighting hyperplasia of type II pneumocytes (Fig. 1). The relationship between this pathogen and lung lesions was also indicated by a higher percentage of *M. haemolytica* in affected lungs (15.43 %) than in healthy lungs (1.22 %).

Fig. 3 Fibrinopurulent bronchopneumonia

On the other hand, 35 % of animals presented mixed changes between bronchointerstitial pneumonia and purulent bronchopneumonia (Fig. 2). The subacute to chronic cases appeared as fibrinopurulent bronchopneumonia, and sequelae included abscesses and pleural fibrous adhesions [10]. In our case, this was the most common amongst the animals affected with *M. haemolytica*.

In conclusion, this study indicates the importance of *M. haemolytica* in lambs from Extremadura. We show that serotypes A1, A6 and A7 are the most frequently isolated from pneumonic lungs, and that the fibrinopurulent bronchopneumonia lesions are the more common lesion type. This presents important implications for sheep production in the region (Fig. 3).

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Compliance with Ethical Standards

Conflict of interest None of the authors have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

1. Lacasta D, Ferrer LM, Ramos JJ, González JM, Heras M (2008) Influence of climatic factor son the development of pneumonia in lambs. *Small Rumin Res* 80:28–32. doi:[10.1016/j.smallrumres.2008.08.004](https://doi.org/10.1016/j.smallrumres.2008.08.004)
2. López Gallego F (2003) El sector ovino en Extremadura: Producción y comercialización. In: La agricultura y la ganadería Extremeñas en 2002. Caja Badajoz, Spain, pp 145–173
3. Ackermann M, Brodgen K (2000) Response of the ruminant respiratory tract to *Mannheimia (Pasteurella) haemolytica*. *Microbes Infect* 2:1079–1088. doi:[10.1016/S1286-4579\(00\)01262-4](https://doi.org/10.1016/S1286-4579(00)01262-4)
4. Angen O, Muters R, Caugant DA, Olsen JE, Bisgaard M (1999) Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluated by DNA–DNA hybridizations and 16S rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia glucosida* sp. nov., *Mannheimia ruminantis* sp. nov. and *Mannheimia varigena* sp. nov. *Int J Syst Bacteriol* 49:67–86. doi:[10.1099/00207713-49-1-67](https://doi.org/10.1099/00207713-49-1-67)
5. Galapero J, Fernández S, Pérez CJ, Calle-Alonso F, Rey J, Gómez L (2016) Identifying risk factors for ovine respiratory processes by using Bayesian networks. *Small Rumin Res* 136:120–133. doi:[10.1016/j.smallrumres.2016.01.017](https://doi.org/10.1016/j.smallrumres.2016.01.017)
6. R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>
7. Zecchinon L, Fett T, Desmecht D (2005) How *Mannheimia haemolytica* defeats host defense through a kiss of death mechanism. *Vet Res* 2:133–156. doi:[10.1051/vetres:2004065](https://doi.org/10.1051/vetres:2004065)
8. Donachie W (2000) Pasteurellosis. In: Acribia (ed) *Enfermedades de la oveja*, 2nd edn. Zaragoza, pp 230–238. ISBN:9788420009872
9. Villard L, Gauthier D, Lacheretz A, Abadie G, Game Y, Maurin F, Richard Y, Borges E, Kodjo A (2006) Serological and molecular comparison of *Mannheimia haemolytica* and *Pasteurella trehalosi* strains isolated from wild and domestic ruminants in the French Alps. *Vet J* 171:545–550. doi:[10.1016/j.tvjl.2005.02.018](https://doi.org/10.1016/j.tvjl.2005.02.018)
10. McGavin D, Zachary J (2007) Pathologic basis of veterinary disease. In: Elsevier (ed) *Pathology of organ system*, 4th edn. St. Louis, pp 490–495. ISBN:9780323075336