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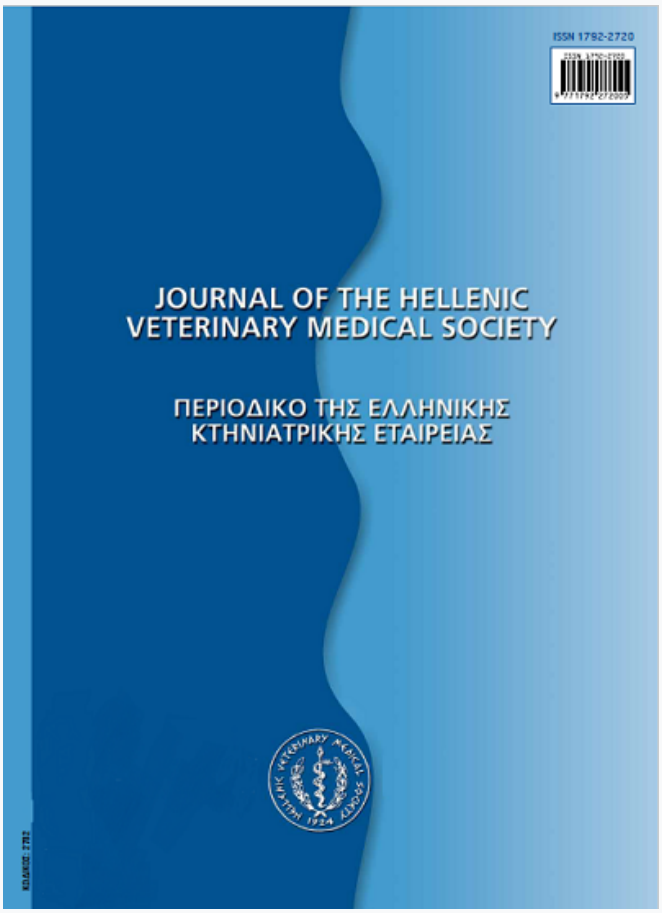

Mannheimia haemolytica and Bibersteinia trehalosi serotypes isolated from lambs with ovine respiratory complex in Spain

J. M. GONZALEZ, D. LACASTA, L. M. FERRER, L. FIGUERAS, G. ABADIE, M. DE LAS HERAS

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Research article
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Mannheimia haemolytica and *Bibersteinia trehalosi* serotypes isolated from lambs with ovine respiratory complex in Spain

González, J.M.[1], Lacasta D.[2], Ferrer L.M.[2], Figueras L.[1,2], Abadie G.[3], De las Heras M.[2]

[1]Gabinete Técnico Veterinario S.L., C/ Isla Conejera s/n., 50013 Zaragoza, Spain

[2] Departamento de Patologia Animal, Facultad de Veterinaria, Universidad de Zaragoza, Miguel Servet 177 50013 Zaragoza, Spain

[3] AFSSA Sophia Antipolis, Laboratoire d'etude et de recherche des petits ruminants et des abeilles, 105 route des Chappes, B.P. 111, 06902 Sophia Antipolis cedex. France

ABSTRACT. Ovine respiratory complex causes significant economic losses in ovine production systems around the world. *Mannhemia haemolytica* and *Bibersteinia trehalosi* are the two most frequently isolated bacterial species. In this study, bacterial isolates of *M. haemolytica* and *B. trehalosi* isolated from dead lambs were serotyped by means of indirect haemagglutination, with the general aim to improve information in the preparation for candidate strains for relevant vaccines. In total, 84 isolates were serotyped. Eight different *M. haemolytica* serotypes and four different *B. trehalosi* serotypes were identified. The most frequent *M. haemolytica* serotypes were A2 (29% of isolates), A12 (18%) and A1 (12%), while *B. trehalosi* were predominantly T4 (40% of isolates) and a group sharing characteristics of T3 and T15 serotypes (43%). Isolation of T4 serotype was associated with lack of consolidated lesions in lungs, whilst isolation of T3-T15 group was associated with lung consolidation. It is concluded that a potential vaccine to control the disease presented as a bacterin based on outer membrane proteins and leucotoxins of the organisms, it should contain the above, frequently implicated serotypes. Alternatively, vaccines based on iron-regulated proteins can be possible replacements.

Keywords: *Birbersteinia trehalosi*, lamb, *Mannheimia haemolytica*, ORC, ovine respiratory complex, pneumonia, serotyping.

Correspondence: D. Lacasta,
Departamento de Patologia Animal, Facultad de Veterinaria,
Universidad de Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain.
E-mail: dlacasta@unizar.es

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INTRODUCTION

In sheep, respiratory diseases cause important economic losses, particularly in intensive production systems. Respiratory diseases are complex problems, occurring as a result of host interaction with various infectious agents, under the influence of environmental factors (Brogden et al., 1998; Lacasta et al., 2008). The disorder is often termed 'Ovine respiratory complex' (ORC) (Lacasta et al., 2008), which has three main clinical presentations: (i) septicaemic, (ii) acute respiratory form and (iii) chronic respiratory form. The septicaemic and acute respiratory form usually result in death of affected animals, although, occasionally, some animals may survive the acute stage and recover or become chronically affected. In addition, many lambs show long-standing respiratory lesions that remain in a subclinical form and are only detected at slaughter. The most frequent aetiological agents of the disorder are *Mannheimia haemolytica* and *Bibesteinia trehalosi*, of which 13 and 4 serotypes, respectively, have been described. Control programs of the disorder include application of zootehcnical emasures (e.g., in relation to ventilation of housing premises), as well as vaccination of animals at risk to develop the disease.

In the Spanish sheep industry, 'ovine respiratory complex' is a significant cause of deaths, carcass rejections in abattoirs, suboptimal growth rates, decreased carcass quality and increased veterinary expenses (González et al., 2001; Lacasta et al., 2004). Objective of the present study was to identify serotypes of *M. haemolytica* and *B. trehalosi* strains isolated from cases of ovine respiratory complex in Spain, with the general aim to improve information available for selection of vaccine candidate strains that can subsequently be employed for updating the antigenic content of vaccines available to control the disease.

MATERIALS AND METHODS

Dead lambs (age at death: 1-150 days), considered on clinical grounds, to have died from ovine respiratory complex were examined in detail; relevant tissue samples from the respiratory tract were collected. Animals were allocated in one of two groups, according to lung lesions observed at post-mortem examination: lambs without (group A) or with (group B) lung consolidation.

In total, 127 lambs were examined and sampled. Of these, 32 originated from breeding farms (age at death: 1-90 days) and 95 originated from feedlots (age at death: 45-150 days). Of the lambs studied, 22 were included in group A (age at death: 62-143 days) and 105 were included in group B (age at death: 1-150 days).

Tissue samples were processed by standard bacteriological techniques and, in total, 82 *M. haemolytica* and 45 *B. trehalosi* isolates were obtained in pure culture. For the record, identity was confirmed as isolates were found to be Gram negative, non-motile, oxidase producers, catalase producers, acid producers by fermentation of glucose and indole negative. Complete species identification of the isolates was based on biochemical profiles by using standard bacteriological techniques. Then, they were further characterised by using API micro standardised strips (API Zym, API 20NE, API 20E, API 50 CH; Biomerieux, France).

Samples from lambs in group A yielded mainly *B. trehalosi* strains (17/22, 77%) and samples from lambs in group B yielded mainly *M. haemolytica* strains (77/105, 73%). It is noteworthy that *B. trehalosi* strains were isolated only from lambs that died in feedlots (Table 1).

All isolates were lyophilized and forwarded to the microbiology laboratory of AFSSA Sophia Antipolis,

Table 1. Origin characteristics and frequency of bacterial isolates from dead lambs with respiratory disease in Spain.

Type of farming establishment	Bacterial species isolated	Lung lesions		Total
		Lambs without consolidation (group A)	Lambs with consolidation (group B)	
Breeding farms	*M. haemolytica*	0	32	32
Feedlots	*M. haemolytica*	5	45	50
	B. trehalosi	17	28	45
		22	105	127

Laboratoire d'etude et de recherche des petits ruminants et des abeilles, in France, for serotyping. Isolates were reconstituted and serotyped using indirect haemagglutination with bovine red blood cells and specific BT and MH serotypes antisera (Sanchis et al., 1988).

Categorical analyses between type of lung lesion, type of origin farm and serotypes were performed by means of the Chi squared test, at SPSS 18.0 software (IBM, Chicago, USA).

RESULTS

Successful reconstitution of bacterial strains was achieved in 84 of the 127 lyophilized isolates sent to the laboratory (66%). Of these, 23 of them were isolated from lambs that died in breeding farms and 61 were isolated from lambs that died in feedlots. Moreover, 19 strains were from samples from group A lambs and 65 from samples from group B lambs (Table 2). In general, it was possible to reconstitute more strains from group B (86%) than from group A (62%) samples ($P = 0.026$).

Of the strains that were reconstituted, 70 (83%) were found to be typeable; of these, 35 were *M. haemolytica* and 35 were *B. trehalosi*. All 14 untypable strains were *M. haemolytica* strains, which could not be assigned into any of the known serotypes, whilst, in contrast, all *B. trehalosi* could be typed.

Serotyping of isolates by capsular antigens showed a high degree of heterogeneity. The most frequent serotypes for *M. haemolytica* strains were A2 (14 strains), A12 (9 strains) and A1 (6 strains). The most frequent serotype for *B. trehalosi* strains was T4 (14 strains), although there was a group of isolates sharing characteristics of T3 and T15 (15 strains) and another group with characteristics of T4-T10 (3 strains) (Fig. 1).

M. haemolytica strains from breeding farms showed higher heterogeneity than those from feedlots (8 serotypes in strains from farms *versus* 4 serotypes in strains from feedlots). Serotypes A6, A7, A8 and A14 were recognised only from breeding farm samples, while serotypes A1, A2, A9 and A12 were recognised from both origin types. All *B. trehalosi* serotypes were in strains from feedlots. Serotype A2 was isolated more often in samples from feedlots (56%) than from breeding farms (24%) ($P = 0.086$).

With reference to lung lesions, from group A lambs serotype A2 was the only *M. haemolytica* typed, although the most frequently recorded was serotype T4 (10 strains). Additionally in group A, serotypes T10, T3-T15 group and T4-T10 group were found. Serotypes T4 and T3-T15 group were found in both groups (A and B), but with contrasting patterns. T4 serotype was associated with group A ($P = 0.041$), whilst T3-T15 group was associated with group B ($P = 0.041$). In fact, T4 serotype had a 5-fold greater probability of isolation from group A, whilst serotype group T3-T15 had 5.11-fold greater probability to be isolated from group B (Table 3).

DISCUSSION

This is the first description of *M. haemolytica* and *B. trehalosi* serotypes in Spain. A noteworthy feature of the study was the increased proportion of isolates

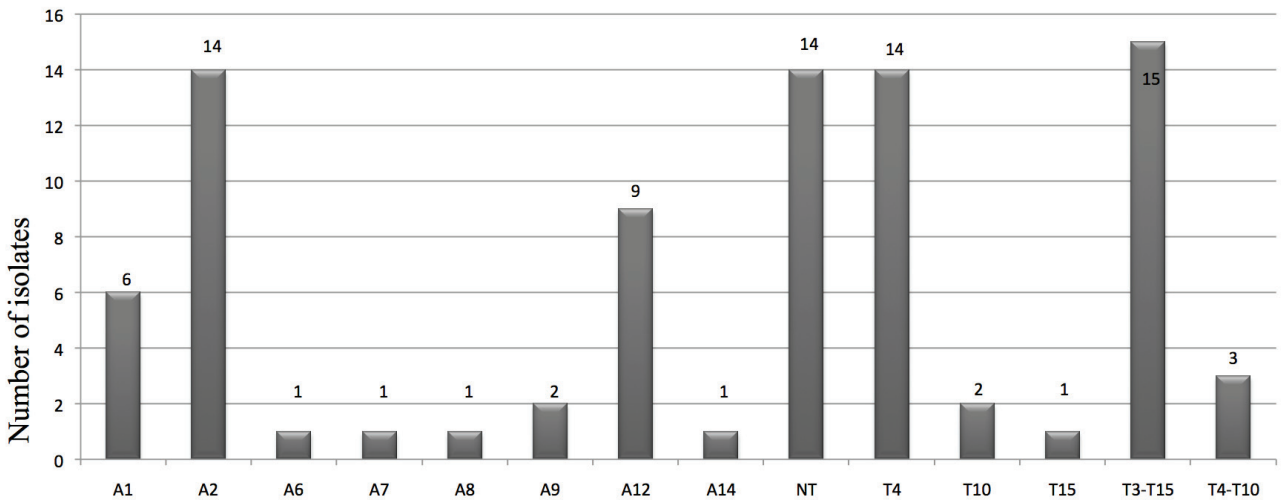

Fig. 1. Distribution of serotypes of M. haemolytica and B. trehalosi isolated from dead lambs with respiratory disease in Spain.

Table 2. Origin characteristics of M. haemolytica and B. trehalosi isolates that were successfully reconstituted for serotyping.

Type of farming establishment	Lung lesions		Total
	Lambs without consolidation (group A)	Lambs with consolidation (group B)	
Breeding farms	0	23	23
Feedlots	19	42	61
	19	65	84

Table 3. Frequency of serotypes of M. haemolytica and B. trehalosi isolates that were successfully reconstituted for serotyping.

Serotype	Lung lesions		Total
	Lambs without consolidation (group A)	Lambs with consolidation (group B)	
A1	0	6	6
A2	2	12	14
A6	0	1	1
A7	0	1	1
A8	0	1	1
A9	0	2	2
A12	0	9	9
A14	0	1	1
T3-T15 group	4	11	15
T4	10	4	14
T4-T10 group	1	2	3
T10	2	0	2
T15	0	1	1
Not typable	0	14	14
	19	65	84

that were typable in the present study (83%), which is much higher than that reported previously (Angen et al., 1999a;b). However, there were differences between the two bacterial species; all *B. trehalosi* strains were typable, whilst of the *M. haemolytica* strains 29% were not typeable.

Serotypes A2, A12 and A1 were the most frequently identified in *M. haemolytica* strains, which agrees with the results of other authors from various parts of the world (Fodor et al., 1999; Donachie, 2000; Odugbo et al., 2003; Villard et al., 2006; Vougidou et al., 2012). All *M. haemolytica* serotypes, bar A5, A16 and A17, were detected during the study. Serotypes T4 and group T3-T15 were the most frequently identified in *B. trehalosi* strains, findings which are different than other ones previously reported. Donachie et al. (2000) and Villard et al. (2006) have reported that that serotype T15 was the most frequently isolated in Scotland and serotype T3 was the most frequently isolated in France, respectively.

We isolated *M. haemolytica* serotypes considered to be of reduced pathogenicity (A6, A7, A8, A14), only from breeding farms, whilst serotypes considered of higher pathogenicity (A2, A1) were isolated from both breeding farms and feedlots. As

mentioned above, serotype A2 strains, considered to be of increased pathogenicity among *M. haemolytica*, was isolated more often in samples from feedlots than from breeding farms.

Lambs in breeding farms died younger than lambs in feedlots. Age of the animals may be a factor determining the *B. trehalosi* isolates involved, as these usually isolated from older lambs (Hajtos et al., 1985), as found in the present study. Increased livestock density, mixed origin of animals and continuous movement of animals, which normally happen in feedlots may be supporting the increased frequency of isolation of *B. trehalosi* strains from lambs in feedlots. The generally stressful conditions in those establishments could possibly increase the probability of transmission of pathogens, e.g. of *Parainfluenza III virus* (Cutlip et al., 1993), of adenoviruses (Cutlip et al., 1996), of *Respiratory Syncytial Virus* (Al-Darraji et al., 1982), of herpesviruses (Scott et al., 1990) and of *Peste des petits ruminants Virus* (Aktas et al., 2011), all of which can predispose to bacterial respiratory infections in lambs.

Strains of serotype group T3-T15 were isolated from clinical forms, although more often from group B lambs, which is in agreement with the findings of Odendaal and Henton (1995), who reported that T15 serotype was more prevalent in cases of respiratory disease than in cases of septicaemia in sheep. T4 serotype was associated to lack of respiratory lesions and indicates, possibly, an increase pathogenicity, which can lead to septicaemic infection and rapid death of the affected lambs.

Data summarized in this study may have important implications for vaccines selection in Spain. The most important immunogens of *M. haemolytica* and *B. trehalosi* are outer membrane proteins, leucotoxin and iron-regulated proteins (Confer, 1993); all these have been used in vaccines currently licenced in various countries of the world. Vaccines licenced in Spain are of the type of bacterins based on bacterial outer membrane proteins protection. Vaccines based on outer membrane proteins or leucotoxin should contain all relevant serotypes, because these immunogens have a poor cross-serotype protection (Ackermann and Brogden, 2000; Sabri et al., 2000). Additionally, some of these vaccines have, in principle, been designed for cattle, where the most prevalent *M. haemolytica* serotype is A1 (Odendaal and Henton, 1995), thus they confer reduced protection against serotypes of the organisms of pathogenic significance in sheep (Purdy et al., 1998). Vaccines manufactured with a more recent technology, based on iron-regulated proteins (Gilmour et al., 1991; Murray et al., 1992) can offer better protection and can afford full protection independently of serotype.

CONCLUDING REMARKS

The results from the present work can be of use in vaccine development in Spain. If the option is a bacterin based on outer-membrane proteins or leucotoxin, it should contain, at least, serotypes A1, A2, A9, A12, T3, T4, T10 and T15. Alternatively, vaccines based on iron-regulated proteins may be developed, which offer protection independently of serotype involved. Nevertheless, further investigations are required to obtain more information about *M. haemolytica* and *B. trehalosi* serotypes prevalent in sheep farms, in order to design optimized control programmes for the disease.

CONFLICT OF INTEREST STATEMENT

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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