Journal of Animal Science and Research
ISSN 2576-6457 | Open Access

RESEARCH ARTICLE

Volume 7 - Issue 1

Bovine Respiratory Disease Complex: Prevalence of the Different Bacteria Involved in Pneumonia in the Iberian Peninsula

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Received: 15 Nov, 2022 | **Accepted:** 01 Feb, 2023 | **Published:** 08 Feb, 2023

Citation: Santo Tomás H, Teixeira R, Chacón G, Lázaro S, Sánchez-Matamorors A, et al. (2023) Bovine Respiratory Disease Complex: Prevalence of the Different Bacteria Involved in Pneumonia in the Iberian Peninsula. J Anim Sci Res 7(1): dx.doi.org/10.16966/2576-6457.162

Abstract

Bovine Respiratory Disease is the most frequent cause of disease and the main cause of death in cattle, which results in financial losses.

The aim of this article is to present the prevalence of the different bacteria involved in Bovine Respiratory Disease in animals with respiratory symptoms in the Iberian Peninsula.

Information was collected from a private laboratory, selecting the data from the analyses of the bovine respiratory disease panel performed between January 2017 and January 2020, which included the major respiratory pathogens. Samples originated from all across Spain (mostly from the North and Northeast) and Portugal.

The type of sample was classified into four groups: organs, bronchoalveolar lavage, nasal and tracheal swabs.

A farm was considered positive if at least one of the tests performed was positive to either real time qPCR or culture. Four hundred and fourty-eight reports were created representing 370 cattle farms with animals that showed respiratory symptoms.

The observed prevalence of *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma bovis* in the period examined was 32.8% (95% CI: 28.5%-37.2%), 55.6% (95% CI: 51.0%-60.2%), 24.1% (95% CI: 20.2%-28.1%), and 53.6% (95% CI: 49.0%-58.2%), respectively.

Regarding the age of the animals in the reports examined, 7.4% (33) were samples from adult animals (beef or dairy cattle), 51.3% (230) were from fattening calves (feedlots), and 23.2% (104) were from veal calves. The age group or production system was not identifiable in 18.1% (81) of the remaining reports.

As for the types of samples, the majority (58.5%) were organs from animals suffering from Bovine Respiratory Disease (lungs), followed by bronchoalveolar lavage (29.7%), nasal swabs (6.5%), and tracheal swabs (1.6%). In 3.8% of cases, the farms sent various types of sample together.

In 88.3% of cases where a virus was detected, bacteria were also found. On the other hand, in 45.3% of all the reports, the only agents detected were bacteria. Bacteria were involved in at least 77.2% of outbreaks.

These results confirm the need for preventing bacterial pneumonia to reduce the use of antibiotics and improve the financial returns and animal welfare.

Keywords: Bovine Respiratory Disease; Cattle; Pathogens; Spain; Portugal

Abbreviations: Bovine Respiratory Disease (BRD); European Union EU; Polymerase Chain Reaction PCR; Bovine Respiratory Syncytial Virus (BRSV); Infectious Bovine Rhinotracheitis virus (IBR virus); Parainfluenza3 virus (PI3 virus); Bovine Viral Diarrhoea Virus (BVDV); *Mannheimia haemolytica (M. haemolytica), Pasteurella multocida (P. multocida), Histophilus somni (H. somni) and Mycoplasma bovis (M. bovis)*

Introduction

Consumers and society at large are becoming increasingly demanding with certain products, placing more importance on animal welfare and antibiotic reduction. The new regulations on veterinary medicinal products (EU 2019/6) [1] and on medicated feed (EU 2019/4) [2] that came into force on 28 January 2022 throughout the European Union are proof of this.

Bovine Respiratory Disease (BRD) is the most frequent cause of disease and the main cause of death in cattle, particularly in certain feedlot systems [3,4]. This results in financial losses caused by the cost of treatments and mortality, reduced feed efficiency, and poorer quality carcasses [3]. It is called respiratory disease or complex because several agents trigger this multifactorial disease, involving both viruses and bacteria. Given the vast diversity of agents, attempts

at controlling the disease are sometimes made without a reliable diagnosis, using antibiotics which are only effective against bacteria and not against viruses.

The role played by viruses in BRD is clear: they initiate the disease in the majority of cases and are responsible for "explosive" outbreaks. However, bacteria can act in a primary or secondary manner, and are in both cases the principal cause of lung damage in animals suffering from BRD [5]. Lung lesions can affect both animals with respiratory symptoms, even if treated with antibiotics, and a high proportion of asymptomatic animals (subclinical disease) [6]. In addition, lung lesions have been directly associated with a reduction in short- and long-term performance due to a decrease in average daily gain [7] and, consequently, are one of the main causes of decreased profitability on farms.

It therefore seems reasonable to think that preventing bacterial pneumonia is key to reducing the use of antibiotics and improving the financial returns of cattle farms, while simultaneously improving animal welfare. It has been demonstrated that bacterial pneumonia vaccination is a cornerstone for the success of prevention plans [8-10]. Vaccination of the dam can improve colostrum quality against respiratory pathogens, which may be particularly useful in herds where BRD problems appear very early after birth. Excellent management of the colostrum must be ensured to achieve a good transfer of passive immunity for this vaccination strategy to be successful [4].

However, in order to define a proper vaccination programme for prevention based on scientific evidence, it is necessary to know the prevalence of the most relevant bacteria: *Mannheimia haemolytica (M. haemolytica), Pasteurella multocida (P. multocida), Histophilus somni (H. somni), and Mycoplasma bovis (M. bovis)* [3].

The aim of this article is, therefore, to present the prevalence of the different bacteria involved in BRD in animals with respiratory symptoms in the Iberian Peninsula, thereby helping veterinarians to select the appropriate vaccination programme, based on scientific evidence.

Materials and Methods

To conduct this study, information was collected from the private laboratory EXOPOL SL (San Mateo de Gállego, Zaragoza, Spain), selecting the data from the analyses of the bovine respiratory diseases panel performed between January 2017 and January 2020. The samples were obtained passively from farms with animals showing respiratory symptoms; the panel of respiratory diseases offered by EXOPOL included: Bovine Respiratory Syncytial Virus (BRSV), Infectious Bovine Rhinotracheitis virus (IBR virus), Bovine Parainfluenza 3 virus (PI3 virus), Bovine Viral Diarrhoea Virus (BVDV), *M. haemolytica, P. multocida, H. somni, and M. bovis* [11].

Following receipt of the samples, all the accompanying information was recorded (origin of the farm, age of the animals, number and type of samples). More specifically, the type of sample was classified into four groups: organs, bronchoalveolar lavage, nasal and tracheal swabs. All samples were individually tested by culture and pooled for qPCR as described below. It should be noted that for real time qPCR sample analysis, pools of up to five samples from the same farm were created.

For culture, the surface of the lung was sterilized using surgical material at high temperature. Afterwards, an incision was performed with a scalpel and a sterile loop was used to sample inside the lung. In the case of tracheobronchial lavages, samples were uniformly mixed, and 10 µl were streaked on the blood agar plate surface with a sterile loop. Finally, swab samples were surfaced onto the blood agar. For all the samples, sterile loops were surfaced onto blood agar (tryptic soy agar containing 5% sheep red blood cells) (BA) plates (Oxoid PB 5039A) with an incubation at 35-37° in aerobic conditions for 24-48 h. Identification of isolates were carried out by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany).

Individual and pooled samples were processed and nucleic acid isolation was performed with the commercial kit MagMAX™ Pathogen RNA/DNA (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions and using an automated magnetic particle processor (KingFisher Flex; Thermo Fisher Scientific, Waltham, MA, USA). Nucleic acid specimens were analyzed by real-time PCR (qPCR) for *M. haemolytica, P. multocida, H. somni, M. Bovis* and Bovine Herpesvirus type 1 (IBR virus), and reverse transcription real-time PCR (RT-qPCR) for Pestivirus (BVD), PI3 virus and BRSV using EXOone commercial kits (EXOPOL S.L.U, San Mateo de Gállego, Spain) according to the manufacturer's protocol. qPCR and RT-qPCR reactions were carried out in a 7500 Fast Real-Time PCR thermal cycler (Applied Biosystems, Waltham, MA, USA), and results were analyzed with the respective software (7500 software v2.3, Foster, CA, USA). Samples with a threshold cycle below 38 were considered positive. The results for the different bacterial agents obtained in the laboratory, the presence or absence of viruses (without distinguishing the aetiological agent), and the associated information were collected and analysed using Microsoft Excel and WinEpi. When analysing the information and calculating the prevalence of the different agents, a farm was considered positive if at least one of the tests performed was positive. In addition, if a farm sent samples within a period of less than three months, these were considered as pertaining to the same outbreak. The differences in prevalence for bacteria in adult and fattening animals were calculated using logistic regression.

Results and Discussion

Results

Four hundred and forty-eight reports were created between January 2017 and January 2020, representing 370 cattle farms with animals that showed respiratory symptoms. Samples were received from all the autonomous regions of Spain, except for the Balearic Islands. The highest percentage of farms that sent samples were in Cataluña (28.1%), followed by Aragón (13.2%), Castilla y León (11.8%), and Navarra (10%). In addition, Portugal represented 6.7% of the farms analysed.

With regard to the age of the animals in the reports examined, 7.4% (33) were samples from adult animals (beef or dairy cattle), 51.3% (230) were from fattening calves (feedlots), and 23.2% (104) were from veal calves. The age group or production system to which they belonged was not identifiable in 18.1% (81) of the remaining reports.

As for the types of samples sent for diagnosis, the majority (58.5%) were organs from animals suffering from BRD (lungs), followed by bronchoalveolar lavage (29.7%), nasal swabs (6.5%), and tracheal swabs (1.6%). In 3.8% of cases, the farms sent various types of samples together.

Between January 2017 and January 2020, at least one infectious agent responsible for BRD was identified in 81.5% of reports (95% CI: 77.9%-85.1%). Of all the analyses performed, 20.5% were positive for only one agent, while 60.9% of samples had between two and four different agents. More specifically, the presence of a virus was detected in 36.2% of the reports studied, whereas 77.2% of reports were positive for bacteria.

On a more detailed level, only 4.2% of all the reports were positive solely for viruses; the remaining 32% of reports that were positive for viruses were also positive for bacteria. In other words, in 88.3% of cases where a virus was detected, bacteria were also found. On the other hand, in 45.3% of all the reports, the only agents detected were bacteria.

Breaking down this information by bacterial agents examined, the observed prevalence of *M. haemolytica, P. multocida, H. somni* and *M. bovis* in the period examined was 32.8% (95% CI: 28.5%-37.2%), 55.6% (95% CI: 51.0%-60.2%), 24.1% (95% CI: 20.2%-28.1%), and 53.6% (95% CI: 49.0%-58.2%), respectively (Table 1).

The prevalence is quite similar in the different years of the period studied, from 2017 to 2019 (Table 2).

Furthermore, if we evaluate the prevalence of bacterial agents by age group or production system, we can see that the highest percentages of the different bacterial agents were in fattening and veal calves, whereas the prevalence of all the agents in adult cattle was markedly lower (Table 3). In addition, the prevalence of *P. multocida, H. somni* and M. bovis found over the three years was slightly higher in feedlot calves than veal ones.

When comparing the groups, there are not significative differences between veal and feedlot calves; however, the differences are significative when fattening animals are compared to adults (Table 4). Prevalence of *M. haemolytica*, *M. bovis* and *P. multocida* are significantly higher in fattening calves than in adult cattle while a trend is also observed for *H. somni*.

Table 1: Number of total samples, number of positive and negative samples and total prevalence of the different bacterial agents examined in the whole study period.

Pathogen	No. of samples	Positive	Negative	Prevalence
Histophilus somni	448	108	340	24.10%
Mannheimia haemolytica	448	147	301	32.80%
Mycoplasma bovis	448	240	208	53.60%
Pasteurella multocida	448	249	199	55.60%

Table 2: Total prevalence of the different bacterial agents examined in the whole study period, broken down by years.

Pathogens	Complete period	2017	2018	2019
M.haemolytica	32.8%	32.3%	33.1%	34.3%
P. multocida	55.6%	57.3%	53.2%	57.7%
H. somni	24.1%	22.9%	18.0%	28.9%
M. bovis	53.6%	45.8%	62.6%	52.7%

Table 3: Total prevalence of the different bacterial agents examined, broken down by age of the animals and production system in the study period.

Pathogens	Adults	Feedlot	Veal
M. haemolytica	9.1%	35.2%	35.6%
P. multocida	33.3%	61.7%	50.0%
H. somni	12.1%	27.8%	21.2%
M. bovis	24.2%	62.6%	49.0%

Table 4: Prevalence (%) of different bacteria in samples from adult cattle and fattening calves. Prevalence is higher ($p<0.05$) for *M. haemolytica, M. bovis* and *P. multocida* in fattening calves than in adult cattle and a trend is also observed for *H. somni*.

Pathogen	Prevalence in adults (%)	Prevalence in fattening cattle (%)	p-value
Histophilus somni	12.12	25.75	0.09
Mannheimia haemolytica	9.09	35.33	0.005**
Mycoplasma bovis	24.24	58.38	<0.001***
Pasteurella multocida	33.33	58.08	0.008**

The data shown herein belongs to positive reports to at least one of the two techniques used, culture or qPCR. It is worth mentioning that all the samples that were positive to culture were also positive to qPCR. However, some samples showed a positive result to qPCR while they were negative in culture [data not shown]. Therefore, these results directly reflect the positivity of qPCR.

Interestingly, the highest discrepancy of positiveness was observed with *H. somni*. When both techniques were compared, bacterial culture detected positive results for *H. somni* in 2.7% of the samples, whereas 24.1% gave positive results by qPCR. Hence, culture failed to detect 88.8% of the positive samples identified by qPCR.

Discussion

The geographical distribution of the samples received expressed as a percentage is similar to the distribution of the fattening herds across Spain. Therefore, this study can be considered as representative of the reality within the fattening system in the Iberian Peninsula, where the largest fattening cattle populations are in Cataluña, Aragón, and Castilla y León. It is worth noting that 96% of all annual entries into the veal and feedlot systems include calves of up to twelve months of age [12]. Notwithstanding, adult dairy and beef cattle represent 46.6% of the cattle census [13]; thus, this segment of the bovine population is likely underrepresented within the present study.

Moreover, the results obtained show that suspicion of respiratory disease in animals on the farm is usually linked to a positive diagnosis of the possible agents involved, with no pathogens identified in only 18.5% of cases. However, given the significant number of agents involved in the respiratory complex, it is essential to perform this aetiological diagnosis [14].

The prevalence of viruses (36.2%) is lower than that of bacteria (77.2%). This is because viruses are only detectable in infected animals for a short period of time and, although they may have been involved in an outbreak, they are no longer detectable at the time of sampling. Results are most relevant when the samples are taken in an outbreak or herds with ongoing BRD problems [14]. Therefore, the usefulness of the tool greatly depends on selection of appropriate animals to sample and on the practitioner's skills to sample. In order to increase diagnostic reliability, animals in the first days of disease, with no previous treatment and no severe symptoms, should be the first option [14].

Vaccination against common BRD viruses (BRS, IBR, BVD and PI3 viruses) is very widespread, which can also contribute significantly to

controlling their prevalence. These viruses are primary pathogens, so when they are identified in a laboratory, it is possible to determine whether they are the sole agents responsible for the problem or in combination with other agents [14]. In the case of bacteria, a positive result should be interpreted differently depending on the agent, as different bacteria can act as either primary and/or secondary pathogens.

A primary pathogen, *per se*, induces damage and inflammation into the respiratory tract. This infection may result in clinical disease (or not) depending on the infectious dose and host immunity. On the other hand, a secondary or opportunistic pathogen can be present in healthy animals as part of the normal respiratory microbiome, not producing an inflammatory response. Secondary pathogens commonly need a prior breach of the innate immunity to induce inflammation and clinical signs [14]. This fact makes the interpretation of secondary pathogens more difficult.

In any case, the odds of identifying any of the aforementioned bacteria increase when samples are taken from diseased animals [3], which is indicative of the identified bacteria to be part of the BRD complex.

M. haemolytica and *H. somni* can act as primary or secondary pathogens [14], that is to say, they can initiate the disease by themselves or as a consequence of predisposing factors, such as regrouping animals, weaning, changes in feed, or the presence of other pathogens, such as viruses [15]. Their presence is an indication of whether they are responsible for the outbreak as initiators or in combination with other pathogens [14].

The observed prevalence of *M. haemolytica* is consistent, with slight variations between years, from 32.3% in 2017 to 34.3% in 2019. *M. haemolytica* is identified as being part of the respiratory complex in at least one in three outbreaks associated with respiratory disease. When the prevalence is compared by production system, the prevalence is significantly higher in fattening cattle, where feedlots and veal calves showed 35.2% and 35.6%, respectively. These are similar to those found in the United Kingdom (34%) [16] and slightly lower than those detected in Belgium (42.1%) [5].

A wide range of vaccines is available on the market with the different combinations of viral agents involved in BRD, and their use is widespread. There are slightly fewer options when it comes to bacterial agents. The majority of vaccines include *M. haemolytica* in their composition, which can contribute to reducing the percentage of positives for this *Pasteurellaceae* in terms of prevalence. However, it is necessary to note the different efficacy of these vaccines depending on their composition, bearing in mind that more modern vaccines with leukotoxoid (Lkt) have shown greater efficacy than the older ones that only included bacterins [17].

High efficacy of anti-leukotoxin vaccines is also a result of confirmed, universal cross-protection between different Lkt-types produced by *M. haemolytica* serotypes other than most common A1 (A5,A6,A8,A9 or A12). [17]. This cross-protection has also been shown with other *Pasteurellaceae* species producing similar virulence factors, such as *Mannheimia varigena* [18] or *Bibersteinia trehalosi* [19].

On the other hand, although *H. somni* has been detected in Spain and Portugal on several occasions, to the authors' knowledge, this is the first time that its prevalence has been reported in both countries. The use of PCR techniques for detecting this bacterium improves the diagnosis compared with bacterial culture [16], as it increases the sensitivity of the technique. The difference in detection of *Histophilus somni* between culture (2.7%) and qPCR (24.1%) is likely associated with the limitation of growing these fastidious bacteria and common practices implemented in the field such as the use of therapeutic antibiotics [5,16]. The results of this study confirm those previously published by Bell et al. [16] regarding the use of qPCR assays for improving the diagnosis of bacteria.

Therefore, the prevalences detected by PCR in this study are more precise than those published previously using the bacterial culture method [20], given the different sensitivities of the two techniques. The prevalence of *H. somni* over the three years included in the study was 24.1%, with a minimum prevalence of 18% in 2018 and a maximum prevalence of 28.9% in 2019. It should be noted that *H. somni* is identified as part of the respiratory complex in at least one in four outbreaks of BRD in Spain and Portugal.

The prevalences also vary depending on the age group or production system of the animals. *H. somni* is part of the respiratory complex in 12.1% of cases in adult cows, while in feedlots and veal calves the prevalence is much higher at 27.8% and 21.2%, respectively, differences indicate a trend (p=0.09) to higher positivity in fattening cattle than in adults. These prevalences are similar to those found in other European countries, such as the United Kingdom (23.3%) [16], although slightly lower than in Belgium (36.4%) [5].

With regard to vaccination against *H. somni,* although on the American markets and in other parts of the world there are several alternatives, in Europe there is only one registered vaccine, in combination with *M. haemolytica.* This vaccine is quite widely used in fattening calves, and has demonstrated high efficacy, with a 50% reduction in respiratory symptoms and lung lesions caused by both pathogens [21,22], and over 80% reduction in antibiotic use [23]. In addition to confirming the reduced use of antibiotics and mortality associated with BRD in fattening conditions in Spain, a more recent publication also demonstrated an improvement in the average daily gain in vaccinated animals of around 200 g/day [24].

On the other hand, *M. bovis* behaves like a primary pathogen and is typically associated with chronic infections [14]. The prevalence detected during this study was 53.6%, ranging from 45.8% in 2017 up to 62.6% in 2018. The prevalence seems numerically higher in feedlots (62.6%) than in veal units (49%) and significantly higher in both segments than in adult animals. The results obtained showed higher prevalence in the Iberian Peninsula than previously reported in the United Kingdom (35.3%) [16] or Belgium (33.3%) [5]. There are no licensed vaccines for this pathogen in Europe. Its impact can be reduced with the use of certain management measures in some situations, or by using autogenous vaccines prepared from cultures isolated at the farm.

The case of *P. multocida* is different in that it behaves exclusively as an opportunistic pathogen, so that prior infection by another agent or other predisposing factors are necessary for the disease to develop [13]. *P. multocida* is commonly part of the resident flora in cattle [13,15]. Therefore, detection of this pathogen does not count as evidence that it is directly involved in a respiratory condition [13]. It is only when detection is linked to clinical signs that a role in the BRD complex can be suspected.

In any case, in the three years analysed in the study, the prevalence found was 55.6%, ranging from 50% in veal calves to 60% in feedlot

calves, respectively. There are no major variations between the different years included. When comparing the prevalence of *P. multocida* detected in this study with that published in other countries, it is lower than in Belgium (89.1%) [5], but higher than in the United Kingdom (33.3%) [16]. There are licensed vaccines against *P. multocida*, but their efficacy is dubious as they have at times shown adverse effects or no differences compared with unvaccinated animals [25].

In view of the results, the presence of bacteria has been demonstrated in at least three in four (77.2%) outbreaks. Furthermore, in 88.3% of cases where viruses were detected, bacteria were also detected concomitantly, similar to the data published by Pardon (2020) [5] in Belgium. These data support the idea that the preventive strategy of farms should include the use of broad spectrum and highly effective bacterial vaccines.

Conclusion

Bacteria are involved in at least 77.2% of outbreaks. The prevalences of *M. haemolytica*, *H. somni*, *P. multocida* and *M.bovis* observed between 2017 and 2019 were 32.8%, 24.1%, 55.6%, and 53.6%, respectively. These results confirm the need for greater control of bacterial pneumonia on farms. These results highlight the importance of including measures for bacterial pneumonia prevention on cattle farms such as vaccination programmes, together with measures against other pathogens such as viruses, to minimise the impact of BRD, reduce the use of antibiotics and improving the profitability of farms and animal welfare.

Acknowledgments

The authors would like to thank Pere Ordis, Ivan Mato, Margarita Barreto and Martijn Seelie for their intellectual collaboration and advice when creating this article and Daniel Angelats for the help with the statistical analysis..

Conflict of Interest

Author and collaborators declare that there is no conflict of interest regarding the materials used or the conclusions extracted from this paper.

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