

Bovine Respiratory Disease Complex: Prevalence of the Main Respiratory Viruses Involved in Pneumonia in Spain

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Abstract

The most common cause of disease among cattle is Bovine Respiratory Disease, and its control has a great impact on the profitability of cattle farms. It is a multifactorial and polymicrobial complex, involving both bacteria and viruses.

The objective of this study was to assess the prevalence of the most relevant bovine respiratory viruses (bovine viral diarrhoea, bovine herpesvirus type 1, and bovine respiratory syncytial viruses) due to their clinical relevance, and also their course over time to facilitate decision-making when designing vaccination programmes based on scientific evidence.

Samples from animals with early symptoms of respiratory disease were taken using the BOVIRESPCHECK kit, enabling nasal swabs to be taken from four different animals within the same farm. The samples were analysed separately for each animal (not pooled) for the detection of the three viruses by real time polymerase chain reaction.

Between 2016 and 2020, a total of 964 reports were created, from a total of 661 cattle farms distributed all over Spain. Each report corresponds to a farm that had an outbreak or respiratory problem at the time.

The most frequently detected virus was bovine respiratory syncytial virus, present in 31.5% of the reports, followed by bovine viral diarrhoea virus detected in 23.94% of them. Both viruses were found in combination in 15.96% of cases, showing a significant positive correlation between them. The bovine herpesvirus type 1 was detected in 17.2% of reports; however, the prevalence reached a peak at 28.11% in 2020 with the restriction in use of multivalent (non-marker) vaccines in the country.

These results underline the significance of implementing complete vaccination programmes that cover all the most important respiratory viruses. Vaccination against bovine respiratory syncytial and bovine viral diarrhoea viruses seems to be justifiable not only because of the high incidence of both pathogens, but also because of the positive correlation between them.

The use of infectious bovine rhinotracheitis monovalent marker vaccines should be added to the above-mentioned vaccines, as their exclusion could be partly responsible for the increased incidence of the bovine herpesvirus type 1 and its associated pathology in 2020. There is a need for a multivalent vaccine including the most relevant viruses with an IBR-marker component to control BRD.

Keywords

Respiratory infection; Cattle; Prevalence; Bovine respiratory syncytial virus; Bovine viral diarrhoea; Infectious bovine rhinotracheitis; Vaccines

Abbreviations

Bovine Respiratory Disease (BRD); Bovine Viral Diarrhoea Virus (BVDV); Bovine Respiratory Syncytial Virus (BRSV); Bovine Herpesvirus Type 1 (BoHV-1); Infectious Bovine Rhinotracheitis (IBR); Parainfluenza 3 Virus (PI3V); European Union (EU); Real Time Polymerase Chain Reaction (RT-PCR)

Introduction

The most common cause of disease among cattle is bovine respiratory disease (BRD), which means that the profitability of a farm largely depends on it being controlled [1]. It is a multifactorial and polymicrobial complex, which primarily involves both bacteria and viruses, besides from environmental components, host [2], and management factors [3].

Various viruses play a role in the onset of BRD. These include bovine adenovirus, bovine coronavirus, bovine rhinitis virus (A and B), influenza D virus, bovine parainfluenza-3 virus, bovine viral diarrhoea virus (BVDV), bovine herpesvirus type 1 (BoHV-1), and bovine respiratory syncytial virus (BRSV). The latter three are the most important because of their clinical relevance [4].

BRSV, BoHV-1 and BVDV are primary pathogens, i.e. their

presence induces respiratory damage and inflammation. In addition to damaging the respiratory tract, they facilitate the entry of secondary or opportunistic pathogens [4]. There are no treatments for these viruses, as the use of antibiotics that are useful in BRD is only directed at treating bacteria. Prevention is key to avoiding the problems associated with respiratory viruses.

Moreover, the new legal framework should be taken into account. New European regulations restricting the use of veterinary medicinal products and even more so the use of antibiotics (Regulation (EU) 2019/6 and (EU) 2019/4) [5,6] came into force in January 2022. With this in mind, it seems reasonable to think that preventive medicine will become more relevant. On the other hand, in 2019 Spanish law changed, prohibiting the use of non-marker vaccines against IBR (Royal Decree 554/2019) [7]. This enforced a change in certain vaccination protocols that had been very widespread until then in Spain and may, in some ways, have affected the prevalence of different viruses and the incidence of respiratory problems. Whilst traditional multivalent vaccines containing BRSV, BoHV-1, BVDV and PI3V were routinely used for BRD prevention, and taking into account that currently there is not any multivalent vaccine with marker IBR, new prevention strategies require more than one vaccine for covering the main BRD viruses. This constitutes a higher cost and added management.

Being aware of this situation and considering vaccination to be a fundamental pillar of preventive medicine [3, 8], it is necessary to assess the possible level of involvement of the different viruses and any impact that the new regulations may have had. It is also essential to develop vaccination programmes accordingly.

The objective of this study was to assess the prevalence of the most relevant respiratory viruses (BRSV, BoHV-1 and BVDV) and their course over time to facilitate decision-making when designing vaccination programmes based on scientific evidence.

Materials and Methods

Samples from diseased animals were taken using BOVIRESPCHECK[®] kit (HIPRA Laboratories, Girona, Spain), an in vivo diagnostic tool that allows the identification of the main pathogens associated with BRD. A nasal swab is used as a sampling method and incorporates FTA card technology where the exudate is impregnated, allowing maximum stability and safety in the transport of the sample. Molecular diagnostic techniques (Real Time Multiplex PCR) are used for the specific amplification of the genetic material of the 4 viral agents. RT-PCR is used, which has high sensitivity and specificity. Briefly, the DNA and RNA are extracted from the FTA cards (QIAamp DNA and RNeasy minikits, Qiagen), and are used for specific gene amplification of BRSV, BoHV-1, and BVDV. The three viruses are detected using the three multiplex real time PCRs that have been previously published [9-11]. The samples are analysed separately for each animal (not pooled).

The BOVIRESPCHECK kit includes four Whatman nasal swabs and four FTA cards, enabling samples to be taken from four different animals. Although the diagnostic panel has included bacteria such as *Mannheimia haemolytica* and *Histophilus somni* since 2021, during the years covered in our study (2016–2020) it only showed results for the most relevant respiratory viruses: BRSV, BoHV-1 and BVDV.

Using this kit, a total of 964 reports were created between 2016 and 2020, from a total of 661 farms distributed all over Spain (Table 1).

Each report corresponds to a farm that had an outbreak or respiratory problem at the time. Samples were taken from animals with early symptoms of disease at these cattle farms, which included both dairy and beef units. Although the BOVIRESPCHECK kit includes

Table 1: Number of different farms, reports created, and individual samples by year, and total samples for the period 2016-2020.

	2016	2017	2018	2019	2020	Total
No. of farms	138	134	153	154	189	661
No. of reports	170	169	196	212	217	964
No. of samples	682	684	794	842	885	3887

material for sampling four different animals, the laboratory sometimes received samples from fewer animals, or two kits were sent relating to the same outbreak. In any case, 95.6% of reports referred to four samples from four animals; the remaining 4.4% included samples from between one and eight animals. If at least one sample tested positive for one of the three viruses, the report was considered positive. The number of samples refers to the number of animals sampled, which was 3,887 in total.

The autonomous regions that submitted the largest number of samples were Cataluña (27.5% of the total), Castilla y León (25.6%), Castilla la Mancha (10.6%), Galicia (8.9%), Aragón (6.2%), Murcia (5.8%), and Andalucía (3.6%); the remaining 11.8% came from the other Spanish regions.

Results and Discussion

Results

In all 965 reports created during 2016-2020, the most frequently detected virus was BRSV, present in 31.5% of cases. In all the years included in the study, BRSV was the most commonly detected virus, with positivity ranging from 35.02% in 2018 to 26.42% in 2019 (Table 2).

BVDV was detected in 23.94% of all reports, varying from a peak of 31.95% in 2017 to 18.43% in 2020 (Table 2).

Finally, BoHV-1 was detected in 17.2% of reports. Minimum positivity was detected in 2017 with 8.88% of positive reports; however, the prevalence reached a peak at 28.11% in 2020 (Table 2).

55.85% of reports were positive for at least one of the three agents analysed during the study, that is to say 44.15% of reports did not provide a diagnosis, although there were variations between the years (Table 2).

Diagram 1 shows the interactions between the different pathogens. Coinfection with the three viruses (BRSV, BoHV-1 and BVDV) was diagnosed during outbreaks in 3.34% of cases. BRSV was identified in coinfections with the BoHV-1 in only 6.86% of cases and in up to 12.62% of cases with BVDV. A significant correlation (p -value=0.03) of 0.07 was detected for an interaction between BRSV and BVDV. BoHV-1 and BVDV were found together in 3.9% of cases.

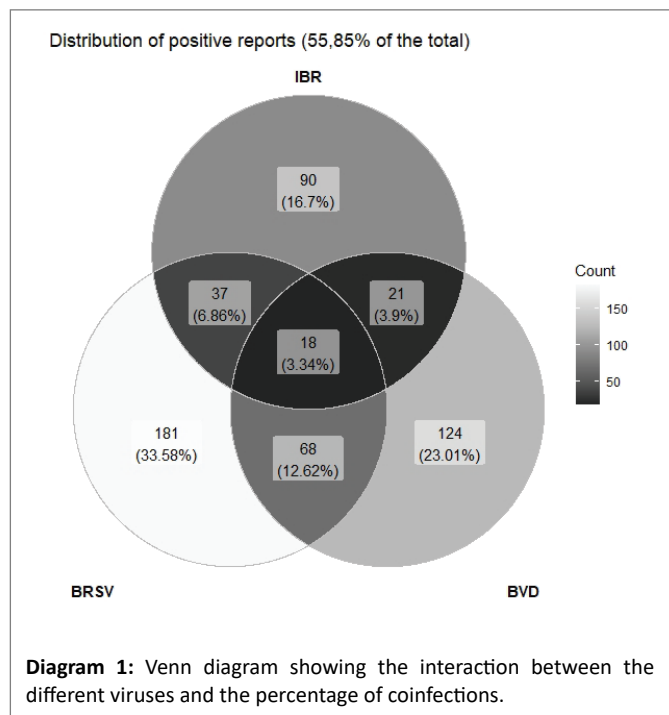
Discussion

The geographical distribution by autonomous region of the samples received is similar to that of the national herd [12]. Therefore, this study can be considered as representative of the reality in Spain, where the largest cattle populations are in Cataluña, Castilla y León, Castilla la Mancha, Galicia, and Aragón.

A positive result of a diagnostic test, such as PCR, involves detection of the agent (or its genetic material) at the time of sampling. Therefore, the results become more significant when the samples are taken during a respiratory disease outbreak or in herds with current respiratory problems. Sampling live animals makes it possible to reach an early

Table 2: Number of tests per year and positivity percentage for each agent. Data above the mean are shown in bold.

	2016	2017	2018	2019	2020	Total
Number of analyses	170	169	197	212	217	965
Positive to BoHV-1 (%)	17.06	8.88	19.80	10.38	28.11	17.20
Positive to BRSV (%)	32.94	33.14	35.05	26.42	30.88	31.50
Positive to BVDV (%)	20.59	31.95	28.93	21.23	18.43	23.94
Positive reports (%)	52.94	64.45	63.45	46.23	60.37	55.85



diagnosis of the infectious process. The utility of this tool depends greatly on selecting the appropriate animals and on the skill of the veterinarian when taking samples. Samples should be taken from animals in the first stages of disease (fever, depression) that have not been previously treated with antibiotics [4] and should not be taken from chronically ill animals. When sampling with nasal swabs (as when using the BOVIRESPCHECK kit), the swab should be scraped vigorously so that the samples contain epithelial tissue.

In any case, in terms of BRD, the final objective is to detect the disease on a group level rather than its prevalence. Taking samples from various animals in one batch or pen increases the probability of detecting a pathogen. Decisions must be made on a group level, not an individual one.

Viruses act as initiators of disease in many cases and are responsible for “explosive” outbreaks. BRSV, BoHV-1 and BVDV are all primary pathogens, i.e. they induce damage to the respiratory tract, which is usually followed by an inflammatory response [4]. A primary pathogen should not be present in normal conditions, so that when it is detected, it can be considered to be responsible for the clinical picture, either as a single agent or in combination with other agents [4]. This makes interpreting a positive result very simple: the virus is circulating in the population and is involved in the clinical picture.

On the other hand, a negative result for a particular pathogen does not automatically rule out the involvement of that virus in the disease

complex. A negative result should be interpreted with some caution, as it only indicates that the virus was not detected at the time of sampling. Interpretation of this result can mean that the virus in question is not present in the respiratory disease, but it can also mean that the virus is involved in the outbreak and has not been detected (false negative). In this scenario, there are two main reasons. The first one is that the proportion detected in different parts of the respiratory tract varies. For example, in the case of BRSV, its tropism is greater in the lower airways, so the sensitivity of the diagnosis increases if the samples are taken from the lower respiratory tract instead of the upper [13]. The second one is that the excretion of these viruses is very short-lived, starting in some cases two days after infection, and lasting a maximum of 14 days, at very low amounts. However, the clinical signs are only evident 5-7 days after infection [14].

Due to this latter point, it is essential for samples to be taken in the first stages of disease, to increase the probability of detection of the different viruses that can be involved in the disease [4]. In 55.85% of reports the results were positive for at least one of the three viruses, which is similar to what has been reported by other authors in Europe (58.6%) [15], although higher than in a previous report in Spain too (36.2%) [16].

Needless to say that within the present study, only the three main respiratory viruses are included, excluding not only other minor viruses but also bacteria. The same study reported that out of the 36.2% of positive reports to viruses, only 4.2% of a total of 448 reports created in Spain and Portugal 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 found concomitantly. Overall, more than 77% of all the reports were positive to bacteria [16]. One of the reasons for the discrepancy might be the type of sample. Samples for the present study originated from alive diseased animals (nasal swabs) while, as reported by Santo-Tomas et al.(2023), about 60% of the samples originated post-mortem (i.e., organs such as lungs), which is indicative of a more progressed evolution of the BRDC. This may partly explain the swift from viruses to bacteria as the respiratory disease evolves in the individuals.

In any case, BRSV was the most commonly identified virus in respiratory diseases during the study, appearing in over half the diagnosed outbreaks (56.4% of positive reports, Diagram 1). These results are in line with previous studies that showed that BRSV is the most common cause of lower respiratory tract infections [17]. Other studies have reported that its prevalence within BRD is 60% in dairy cattle [18] and up to 70% in beef cattle [19]. These results are not surprising, since infection with BRSV is the major cause of BRD in cattle worldwide, especially during their first year of life [20]. Due to the nature of the service, it is difficult to classify samples by production type, so the results obtained represent both systems, dairy and beef. Most likely, the greatest proportion of the samples belong to animals below one year of age, when rearing calves in dairy units are more susceptible to BRD and beef animals enter fattening units, the highest risk period in this system.

Calves between one and three months of age are most susceptible to BRSV, while maternal derived antibodies (MDAs) are still present. Intranasal BRSV vaccines have demonstrated to be effective in the presence of MDAs; however, the route of administration may not be the only factor related to the efficacy of these vaccines [20]. For instance, a commercial vaccine available in Europe that can be used from the first day of life had reduced efficacy against BRSV in calves vaccinated in the presence of MDAs -according to the summary of product characteristics [20, 21].

On the other hand, a monovalent BRSV vaccine demonstrated to significantly reduce viral shedding, clinical signs, lung lesions and mortality even in the presence of MDAs. Protection was shown until at least ten weeks of age, when an intramuscular booster would commonly be applied, for instance through a multivalent vaccine, especially if containing the same strain [20]. Some authors have suggested this heterologous prime-boost approach as a possible solution to overcome BRSV-related problems. Heterologous prime-boost may be achieved by exposing the immune system to different forms of antigen by different routes to modulate magnitude, quality and location of immune response [22] or by combining different delivery routes for priming and boosting [23]. This way, the period of major incidence of this virus can be covered, and consequently reduced [19].

The second most commonly detected virus was BVDV, present in at least one in four outbreaks (23.94%) over the five years included in the study, which is equivalent to 43% of cases in which a diagnosis was made (positive reports, Diagram 1). Its relevance in BRD is mainly due to its immunosuppressant role, as this facilitates coinfection with other pathogens; alone, it hardly inflicts any damage at all on the respiratory tract [14]. The respiratory clinical signs are mild if BVDV is not accompanied by superinfection [14]; however, immunosuppression facilitates the action of other pathogens that could be present, such as bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, etc.) or other viruses [24].

BVDV was found in combination with BRSV in 15.96% of cases (Diagram 1), also showing that there is a significant positive correlation between the two, while there was no correlation for the remaining possible combinations. This result is in line with other publications that demonstrate that BVDV worsens the severity of BRSV outbreaks [24] and facilitates or increases its replication [25]. Thus, vaccination protocols aiming to reduce BRD need to include BRSV in combination with BVDV, as long as a risk of BVDV circulation exists (i.e., no need in BVD-free countries).

Nevertheless, there are concerns about the use of modified live viral BVDV vaccines. Different studies have shown a negative effect of live BVDV vaccines, increasing mortality rates in feedlots, causing leukopenia and decreasing neutrophils and lymphocytes, or causing immunosuppression. The use of this type of vaccines may enhance BRD, while inactivated BVDV vaccines are safer [26].

The third most commonly detected virus during this period was the BoHV-1, identified in 17.2% of cases, which is equivalent to 31% of occasions on which a diagnosis was made (Diagram 1). However, the most interesting aspect is that it became more relevant in the last year of the study (2020), being detected on 28.11% of occasions.

Although there are fluctuations over the study period, this greater incidence in 2020 could largely be due to the change in Spanish law relating to BoHV-1 vaccination implemented at the end of 2019 (Royal Decree 554/2019) [7]. The Royal Decree only permits BoHV-1 vaccination with gE-negative marker vaccines, so the multivalent vaccines (BRSV, BoHV-1, BVDV, and PI3V) that had been used traditionally are no longer used as their IBR component was not marker (gE+).

When multivalent vaccines have continued to be administered, these have always included BRSV and PI3V in their composition, most often together with BVDV too. The inclusion of BoHV-1 in the vaccination programme means including monovalent marker vaccines. That is to say, the situation has moved from using a single multivalent vaccine (BRSV, BoHV-1, BVDV and PI3V) to the need to

prescribe at least two vaccines in order to cover all the most relevant respiratory viruses. This is relevant when considering that two or more viruses were detected simultaneously (coinfections) in 26.72% of positive reports. On a field level, this means that in some cases only one vaccine has been administered because of the cost factor (one vaccine instead of two); in other cases, this has led to failures in the vaccination protocols as it has been necessary to adapt to different products. In line, it has been previously reported that incorrect use, storage and administration of cattle vaccines is common and can lead to vaccine failure [27]; changing products may exacerbate these failures.

Together with reducing clinical signs of IBR, BoHV-1 vaccination with marker vaccines has demonstrated to reduce culling rates due to respiratory disease in vaccinated dairy herds [28]. Modified live viral gE-marker vaccines have demonstrated to also be more effective to reduce seroconversion of not infected animals [29]. These live gE-marker vaccines have not shown the negative effects observed with the non-marker modified live viral vaccines available outside Europe, such as abortions, oophoritis or reduced fertility [3,30].

The trend observed in 2020, with an almost three-fold increase in the incidence of BoHV-1 compared with the previous year, emphasizes the importance of implementing complete vaccination programmes, covering the most significant respiratory viruses. Altogether, the results highlight the need of multivalent vaccines containing gE-negative marker IBR to ease correct application of protocols and cover the main respiratory viruses.

Despite Northern European countries having reported a certain seasonality for BRD between September and February [25], or more specifically for BRSV in the winter months [15], no seasonality for the three viruses was observed in the present study. The fact that no seasonality was observed could be due partly to the weather conditions and latitude of Spain, although the number of fattening cattle present in the country compared with the Northern nations could also be an important factor. In the case of fattening calves, whether they are in feedlots or veal units, the highest risk period is the first two months after entering the feed-yards, which occurs more or less regularly throughout the year in Spain and can help to dilute the seasonality effect.

During the five-year period presented in this study, BRSV was invariably the most commonly detected virus in cases of respiratory problems. It was present in 31.5% of reports, which is equivalent to 56.4% of reports with a positive diagnosis. In almost every year; BVDV was the second most commonly detected virus, present in one in four cases, and with some correlation to BRSV. As for BoHV-1, its incidence seems to have increased substantially in respiratory outbreaks over the course of 2020, with 28.11% of cases being detected in this year. This marked increase in the incidence could be a direct consequence of the legislative changes regarding the use of non-marker IBR vaccines (including polyvalent vaccines), meaning that vaccination against this pathogen decreased during this period.

Conclusion

These results underline the significance of implementing complete vaccination programmes that cover all the most important respiratory viruses. BRSV and BVDV vaccination seems to be justifiable not only because of the high incidence of both viruses, but also because of the positive correlation between them. The use of BoHV-1 monovalent marker vaccines should be added to the above-mentioned vaccines, as their exclusion could be partly responsible for the increased incidence of the BoHV-1 and its associated pathology in 2020. There is a need for a multivalent vaccine including the most relevant viruses with an IBR-

marker component to control BRD in countries where regulations do not allow the use of non-marker vaccines.

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