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Long-term IBR/IPV eradication programs in dairy herds using marker vaccines in four regions of Poland

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Summary

The study investigated IBR/IPV control in four Polish dairy herds using marker vaccines and DIVA-compliant protocols. Four farms (A-D) were monitored over three years, with seroprevalence trends analyzed across different lactation groups. Key outcomes included: successful reduction in seroprevalence on Farms A and C, attributed to vaccination (MLV/inactivated) and biosecurity adherence, zero seroprevalence on Farm B, which had no prior virus circulation; increased seroprevalence on Farm D due to poor biosecurity during herd expansion, despite vaccination. The research emphasizes that marker vaccines are safe and effective but require robust herd management to prevent outbreaks. The EU's flexible C+D+E classification for IBR/IPV allows tailored eradication programs, though high-prevalence regions like Poland face challenges. The study advocates for integrated approaches – combining vaccination, testing, and biosecurity – to achieve disease-free status, mirroring successes in other EU regions. Limitations include the small sample size, urging broader research to optimize protocols for large-scale implementation.

Keywords: IBR/IPV marker vaccines, DIVA, seroprevalence, Poland

Bovine herpesvirus type 1 (BoHV-1, BoAHV-1, Varicellovirus bovinealpha1), also referred to as infectious bovine rhinotracheitis virus (IBRV) or infectious pustular vulvovaginitis virus, is an enveloped double-stranded DNA virus belonging to the genus *Varicellovirus* within the subfamily *Alphaherpesvirinae* (family *Orthoherpesviridae*) (14, 15). BoHV-1 is a highly contagious pathogen that poses significant economic challenges to cattle farming worldwide. It is associated with respiratory disorders (one of group factors causing bovine respiratory disease complex – BRDC), reproductive failures (e.g., abortion, infertility) and systemic infections in neonatal calves, collectively contributing to reduced herd productivity through diminished milk yield and weight loss (1, 13, 21, 22, 35, 37, 43). Following primary infection, BoHV-1 establishes latency in sensory ganglia, particularly the trigeminal ganglion, and periodically reactivates, facilitating viral shedding and transmission. This latency mechanism ensures the virus's persistence within cattle populations, complicating eradication efforts (8, 23, 28).

Historically, BoHV-1 manifestations varied geographically: in Europe, the virus primarily caused infectious pustular vulvovaginitis (IPV) and balanoposthitis (IPB), whereas in North America, it emerged as infectious bovine rhinotracheitis (IBR) in the mid-20th century (21, 22). Due to its economic impact, IBR/IPV is listed as a notifiable disease by the World Organisation for Animal Health (WOAH), prompting stringent control measures (https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases/).

Decision 2004/558/CE implementing Council Directive 64/432/EEC (No longer in force, Date of end of validity: 20/04/2021) defined the requirements to be achieved in order to obtain approval for an IBR eradication program. Several countries within the European Union have successfully eradicated IBR/IPV (11), other countries implemented national or regional, compulsory or voluntary eradication programs. Eradication strategies often combine serological testing, culling of seropositive animals, and vaccination. Several vaccination protocols associated with diagnostic methods have been developed in order to reduce clinical consequences of the disease but also restrictions imposed in the cattle market. A preliminary step towards the design of control/eradication schemes consisted in the investigation of the infection and its level in respective countries, regions or herds, but in the case of BoHV-1 vaccination with marker vaccines constitutes the primary method of control and eradication in high prevalence regions (21, 40). Notably, marker vaccines enable differentiation between infected and vaccinated animals (DIVA principle). These vaccines, available as modified-live (MLV) or inactivated formulations, are widely adopted to mitigate clinical disease and facilitate trade compliance (8, 21, 25, 28, 31, 40, 42).

This study aimed to evaluate the efficacy of different BoHV-1 eradication proto-cols based on regular vaccination with marker vaccines (modified-live and/or inactivated), DIVA-compliant serological testing, and the elimination of infected animals (as a part of the regular culling process). Additionally, investigation of the critical role of herd management practices and biosecurity measures in the success of IBR/IPV control programs was performed.

Material and methods

Farms and animals. The study was carried out at four dairy farms (A-D) located in the 4 voivodeships of central, south-west and south Poland: Opolskie (A), Lower Silesia (B), Great Poland (C) and Silesia (D), over a period of 3 years (Fig. 1). Infections with rotavirus, coronavirus and *Cryptosporidium parvum* in calves and *Streptococcus uberis* in adult cows had been previously detected in the herds. These herds had been involved in a voluntary IBR/IPV control program. The immune status of all animals was verified using a commercial gB IBR ELISA (IDEXX IBR gB X3, IDEXX Switzerland GmbH, Liebefeld-Bern, Switzerland). The sensitivity and specificity of this assay was previously estimated at 99% and 99.7%, respectively (19). The test provides a specificity and sensitivity of 97.1% and 96.7%, respectively, compared with the virus neutralization test (VNT) (34). All tests were performed at the Diagnostic Laboratory EPI-VET of the Faculty of Veterinary Medicine, Wroclaw, Poland. No animals without quarantine or seropositive for BoHV-1 were introduced to the farms (A-C) during the study, however, the biosecurity rules and proper herd management were not fully respected on farm D. The study was field research.

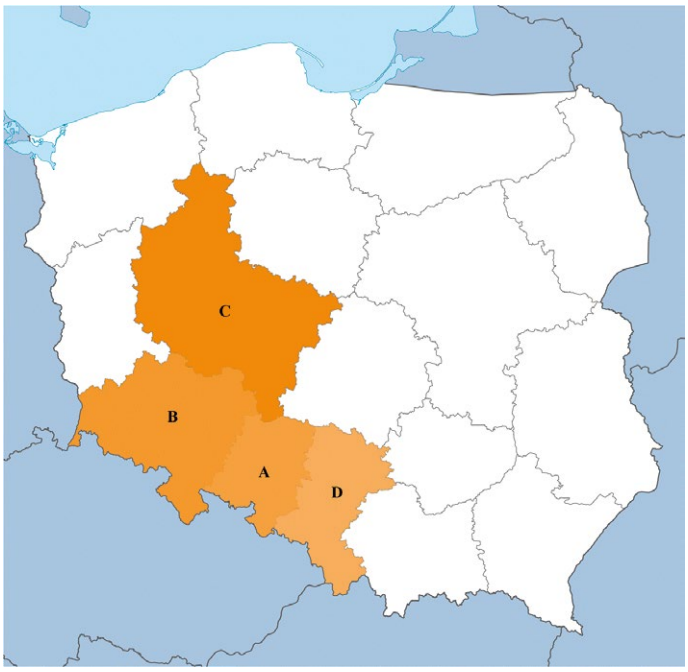

Fig. 1. Examined geographical regions of Poland

The study subjects included healthy cattle ageing 2 months to approximately 10 years. Physical examination to confirm the animals' suitability for inclusion in study was done by the Examining Veterinarian prior to first vaccination. Health status was observed shortly before the vaccination and approximately one hour after vaccination. All individuals were eligible for vaccination.

Vaccination and vaccination schedules. The monovalent commercial a marker modified-live vaccine (MLV) and a marker in-activated vaccine were used. The MLV contained live-attenuated BoHV-1, strain Difivac (gE-negative) in minimum titer 105.0 CCID50 and maximum titer 107.0 $CCID_{50}$ per 2 ml dose. The vaccine was administered either by intramuscular route or by intranasal route (on farm D only, when calves are younger than 3 months). The inactivated vaccine contained strain Difivac (gE-negative), which induces a geometric mean seroneutralising titre of at least 1 : 160 in cattle. This vaccine was administered as a 2 ml dose by subcutaneous route only. Schedules of vaccination in A to D farms are showed on Figure 2. All animals and their health status were monitored after vaccination by field veterinarians who worked on the farms.

Samples collection. Calves, heifers and cows from every farm were randomly selected for the study using simple random method. The number of blood samples were calculated with the Epi-scope 2.0 software to ensure at least 95% certainty (level of confidence) that at least one seronegative cow would have been detected if less than 50% of vaccinated cows had seroconverted after vaccination. The following formula was used for the calculation of the samples size (38):

$$n = \{1-(1-P)^{(1/d)}\} \times (N-d/2) + 1$$

where:

n – required sample size

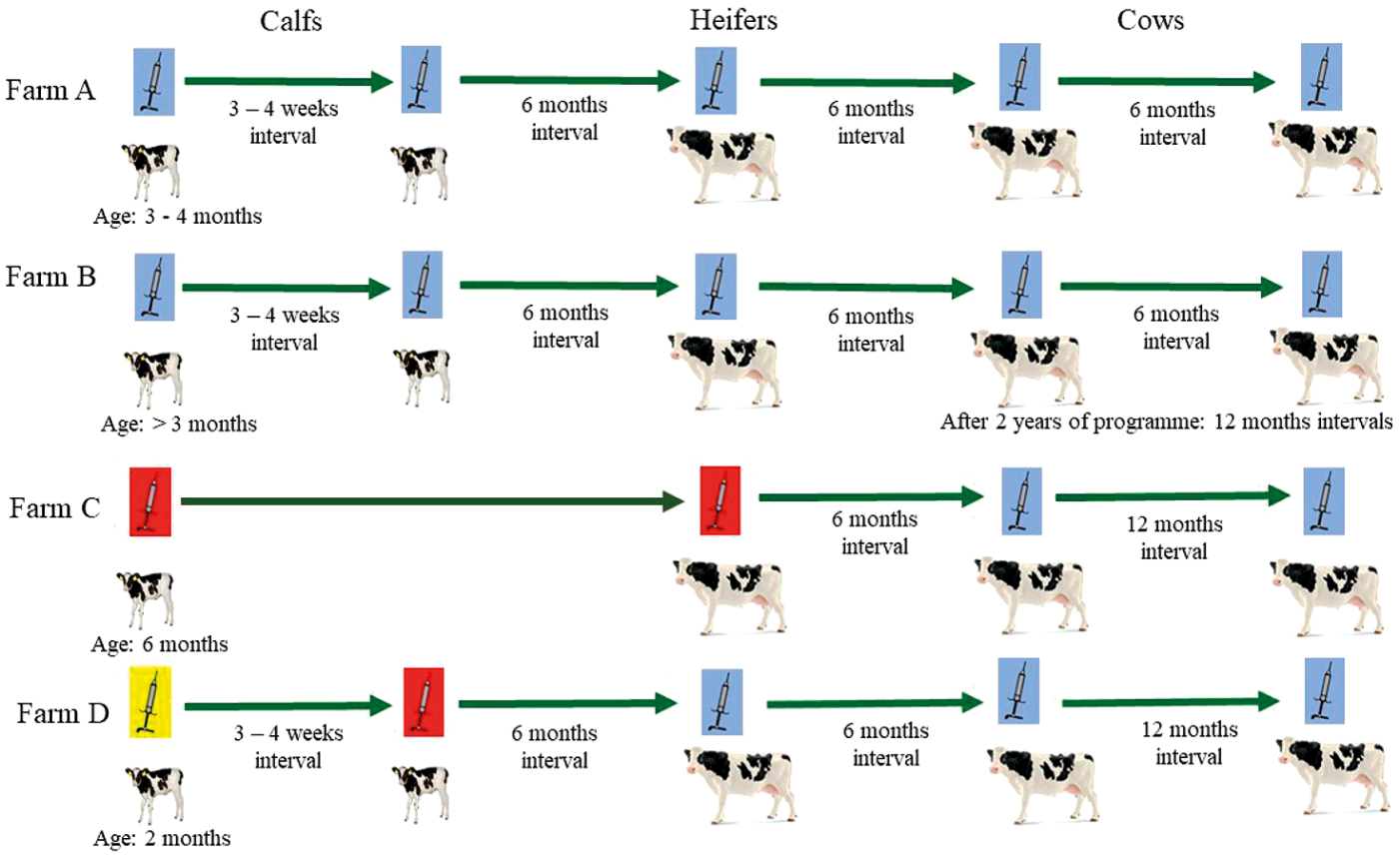

Fig. 2. Schedules of vaccination in A to D farms

Explanations: inactivated vaccine, subcutaneous administration; MLV, intramuscular administration; MLV, intranasal administration

N – herd size
d – minimum expected number of animals which did not seroconvert after vaccination
P – probability of finding at least one seronegative animal in the sample (level of confidence = 95%)

Blood samples were taken from the jugular vein (calves and heifers) or the coccygeal vein (milking cows) right before the vaccination (day 0) and then a minimum 4 weeks after the second and next vaccinations. Blood was left at room temperature for 8-12 h after collection, and centrifuged. Next, serum samples were frozen at –80°C and transported directly to the Diagnostic Laboratory EPI-VET of the Faculty of Veterinary Medicine, Wroclaw, Poland which implements a quality management system (ISO/IEC 17025:2005 + API:2007 + AC:2007).

ELISA test. Serum samples were tested with blocking enzyme-linked immunosorbent assay (ELI-SA) (IDEXX IBR gE Ab Test, IDEXX Switzerland GmbH, Liebefeld-Bern, Switzerland). The presence of anti-gE BoHV-1 antibodies indicated a previous exposure to a field strain of BoHV-1. The absence of antibody to the gE antigen was determined by calculating the S/N value of each serum sample. Values higher than 0.70 were classified as negative, those with values less than or equal to 0.60 were classified as positive and samples with values ranging from 0.60 to 0.70 were considered inconclusive. The ELISA was performed according to the manufacturer's manual at an absorbance of 650 nm wavelength.

Statistical analysis. The 95% confidence intervals (CI 95%) for seroprevalence were calculated using the Wilson score method (2). The within-herd seroprevalence was compared between years using the maximum likelihood G test and the trend was evaluated with the chi-square test for trends (41). All tests were two-tailed and a significance level (α) was set at 0.05. The analysis was performed in TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA).

Results and discussion

All animals included in the studies were healthy at the physical examination before the administration of the vaccine. There were no mortalities or serious local or systemic reaction at any time after any of the vaccinations. In our study blood was taken from a total of 2,615 calves (from 2 to 6 months of age), heifers (from 6 to 18 months of age) and cows (from 18 months of age to approximately 10 years of age) were enrolled (Tab. 1). Figure 3 shows the evolution of the seroprevalence at all farms levels over the years. Within-herd seroprevalence on farms was calculated for 4 different production groups (before lactation; 1st; 2nd-3rd; > 3rd lactation).

Farm A. In the 1st year, seroprevalence was the highest in the group > 3rd lactation, significantly lower in the group 2nd-3rd lactation ($p < 0.001$), again significantly

Tab. 1. Number of animals in individual farms (A-D) from which blood was collected for ELISA tests in subsequent years

Farm	Virus circulation	1st year	2nd year	3rd year	Total
A	Yes	125	296	183	604
B	No	298	196	501	995
C	Yes	418	82	181	681
D	Yes	44	213	78	335
Total		885	787	943	2615

lower in the group 1st lactation ($p < 0.001$), and the lowest in the group before lactation ($p = 0.008$). In the group before lactation, seroprevalence did not change significantly during the study ($p = 0.057$). In the group of 1st lactation, seroprevalence significantly decreased between the 1st and the 3rd year ($p = 0.010$). In the group of 2nd-3rd lactation, seroprevalence significantly decreased between the 1st and the 2nd year ($p < 0.001$), and between the 2nd and the 3rd year ($p = 0.010$). In the group of > 3rd lactation, seroprevalence significantly decreased between the 1st and the 2nd year ($p < 0.001$), and then significantly increased between the 2nd and the 3rd year ($p = 0.011$). Within-herd seroprevalence on farm A is showed in Figure 4.

Farm B. Seroprevalence remained at 0% in every group of animals for the entire study. Within-herd seroprevalence on farm B is showed in Figure 5.

Farm C. In the 1st year, seroprevalence did not differ significantly between groups ($p = 0.191$). In the group before lactation, seroprevalence significantly decreased (to 0%) between the 1st and the 2nd year ($p = 0.003$) and remained at the same level in the 3rd year. In the group of 1st lactation, seroprevalence did not change significantly during the study ($p = 0.947$). In the group of 2nd-3rd lactation, seroprevalence significantly increased between the 1st and the 3rd year ($p = 0.006$). In the 2nd year there were only 4 cows (2 seropositive) – too few to assess. In the group of > 3rd lactation, seroprevalence significantly increased between the 1st and the 2nd year ($p < 0.001$), and then significantly decreased between the 2nd and the 3rd year ($p < 0.001$). Within-herd seroprevalence on farm C is showed in Figure 6.

Farm D. In the 1st year, seroprevalence was the lowest (0%) in the group before lactation ($p < 0.001$), and it did not differ significantly between the 3 remaining groups ($p = 0.168$): in all 3 groups seroprevalence was high. In the group before lactation, seroprevalence remained at 0% for the entire study. In the group of 1st lactation, seroprevalence significantly decreased between the 1st and the 2nd year ($p < 0.001$) and then significantly increased between the 2nd and the 3rd year ($p < 0.001$) – eventually seroprevalence did not significantly differ between the 1st and the 3rd year ($p = 0.423$). In the group of 2nd-3rd lactation, seroprevalence did not significantly differ between the 1st and the 2nd year

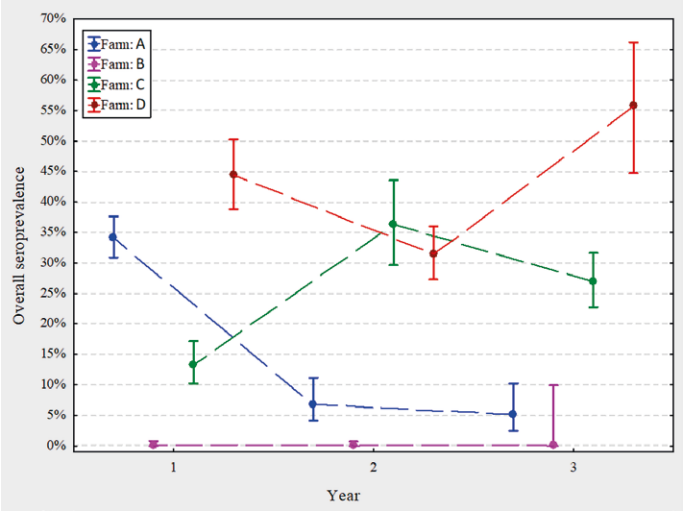

Fig. 3. Overall within-herd seroprevalence (Farms A-D)
Explanation: Whiskers indicate 95% confidence intervals (CI 95%)

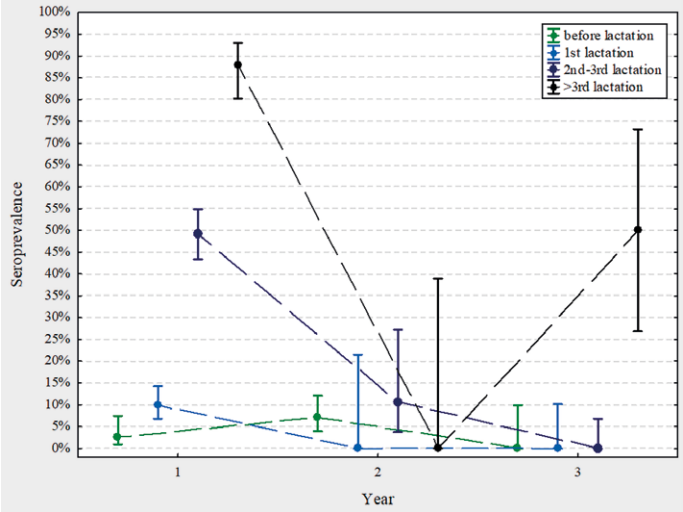

Fig. 4. The change of within-herd seroprevalence in 4 production groups (before lactation; 1st; 2nd-3rd; > 3rd lactation) on farm A
Explanation: Whiskers indicate 95% confidence intervals (CI 95%)

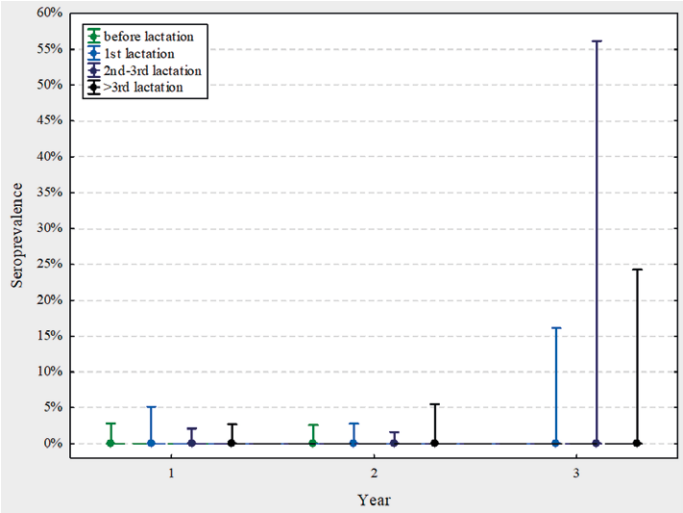

Fig. 5. The change of within-herd seroprevalence in 4 production groups (before lactation; 1st; 2nd-3rd; > 3rd lactation) on farm B
Explanation: Whiskers indicate 95% confidence intervals (CI 95%)

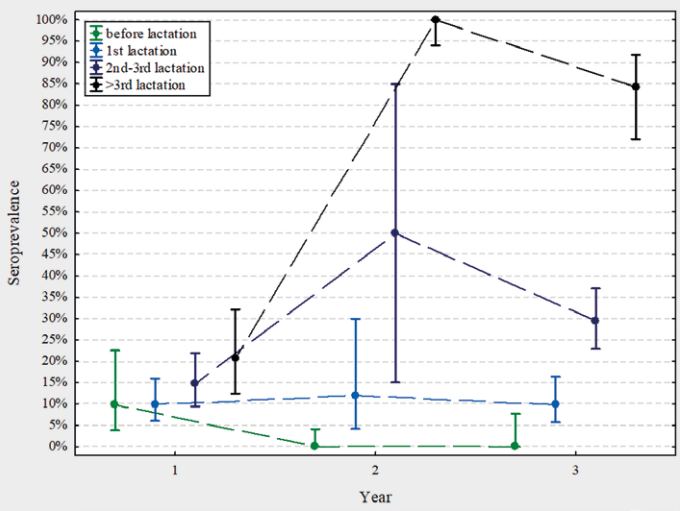

Fig. 6. The change of within-herd seroprevalence in 4 production groups (before lactation; 1st; 2nd-3rd; > 3rd lactation) on farm C
Explanation: Whiskers indicate 95% confidence intervals (CI 95%)

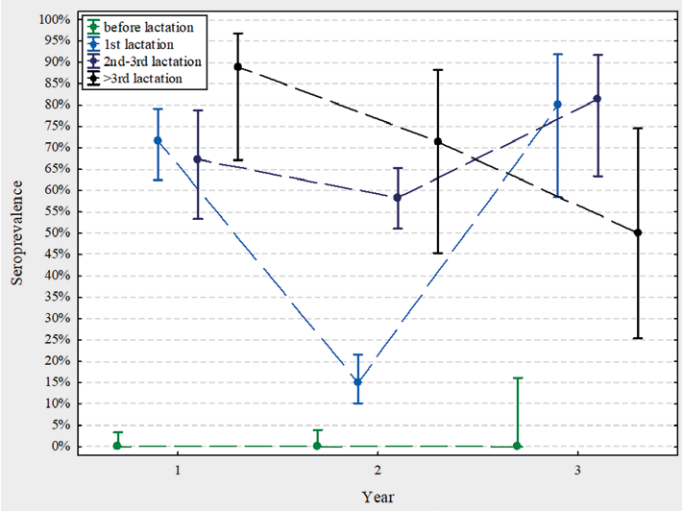

Fig. 7. The change of within-herd seroprevalence in 4 production groups (before lactation; 1st; 2nd-3rd; > 3rd lactation) on farm D
Explanation: Whiskers indicate 95% confidence intervals (CI 95%)

($p = 0.249$) and then significantly increased between the 2nd and the 3rd year ($p = 0.016$). However, the difference between the 1st and the 3rd year was not significant ($p = 0.178$). In the group of > 3rd lactation, seroprevalence was gradually decreasing between years and eventually it was significantly lower in the 3rd year compared to the 1st year ($p = 0.018$). Within-herd seroprevalence on farm D is showed in Figure 7.

On 9 March 2016, Regulation (EU) 2016/429 of the European Parliament and of the Council on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law') (12) entered into force, which reorganized, updated and unified the veterinary rules in force in the European Community. The Regulation contains comprehensive guidelines for monitoring, eliminating and maintaining the status free from the transmissible animal diseases listed therein. Key aspects of the new Regulation are the emphasis on animal traceability, biosecurity practices and control of wildlife pathogens, promotion of sustainable agriculture and reducing the impact of diseases on public and animal health and the environment. Having regard to the aforementioned Regulation, and in particular Article 8 (1) thereof, 2 and Article 9 (2), Commission Implementing Regulation 2018/1882 (10) introduced the classification of transmissible diseases into five categories (A-E), each with specific responses ranging from mandatory eradication to optional eradication or surveillance. These categories are defined as follows:

A: "(…) disease that does not normally occur in the Union and for which immediate eradication measures must be taken as soon as it is detected (…)"

B: "(…) diseases which must be controlled in all member states with the goal of eradicating them throughout the Union (…)"

C: "(…) diseases which are of relevance to some member states and for which measures are needed to prevent them from spreading to parts of the Union that are officially disease-free or that have eradication programs for the listed disease concerned (…)"

D: "(…) diseases for which measures are needed to prevent them from spreading on account of their entry into the Union or movements between member states (…)"

E: "(…) diseases for which there is a need for surveillance within the Union (…)"

According to this regulation, IBR/IPV in cattle is classified as a transmissible disease category C + D + E under control, eradication is not obligatory, but disease outbreaks are subject to mandatory reporting, if they occur in IBR/IPV-free Member States or in IBR/IPV-free zones in EU countries. Commission Delegated Regulation (EU) 2020/689 indicates the justification for introducing optional eradication programs to reduce the economic losses associated with IBR/IPV (9), although approved eradication programs for IBR/IPV are not yet consistently applied in all EU Member States (16, 20). In accordance with Commission Implementing Regulation (EU) 2021/620 (11), Member States or zones thereof that are free from IBR/IPV disease are: Austria, Czechia, Denmark, Finland, Germany, Sweden (Whole territory) and Italy (Regione Valle d'Aosta and Regione Trentino – Alto Adige: Provincia Autonoma di Bolzano – Alto Adige). These countries or regions are free from IBR/IPV by gradually culling all seropositive animals and replacing them with seronegative cattle (1). This goal was possible because the BoHV-1 seroprevalence was relatively low and the financial resources of the country allowed for compensating the differences between the slaughter value and the breeding value of cattle.

In countries with high BoHV-1 seroprevalence, IBR/IPV control programs are mainly based on vaccination with gE marker vaccines together with the removal of gE seropositive animals from the herd (28).

In the 1970s, BoHV-1 was first isolated from cattle in Poland (3, 18). BoHV-1 seroprevalence was 20.6% in 1996-1998 and 37.7% in 2004-2005 (30). In another study, BoHV-1 seroprevalence at the herd level in unvaccinated dairy cattle herds with clinical respiratory symptoms was estimated at 53% in all studied herds, 11.1% in small herds (≤ 100 cows) and 73.2% in large herds (> 100 cows) (34). In 2010-2016, in order to control the occurrence of IBR/IPV in the territory of Poland monitoring studies were carried out based on the regulation of the Minister of Agriculture and Rural Development (32). Approximately 0.3% of herds were tested each year. The results of the control tests showed that the percentage of seropositive herds ranged from 3.5 (2011) to 5.3 (2010), and the percentage of seropositive animals from 2.1 (2011) to 4.7 (2010). The tests, commissioned by a dairy cooperative from north-eastern Poland, in 2011-2014 showed that infection with the BoHV-1 field virus was found in 31.8% of dairy cows. Considering the herd status, 24.2% of farms were free from IBR/IPV. These studies covered 2,532 cattle from 29 dairy farms, suppliers of milk to this dairy (32).

In Poland, in accordance with the Act of March 11, 2004 on the protection of animal health and the control of infectious diseases of animals (39), IBR/IPV is a disease subject to mandatory registration. Based on art. 57d of the aforementioned Act, a program for the control of infectious bovine rhinotracheitis/pustular vulvovaginitis was introduced in the territory of the Republic of Poland on January 1, 2018 (32). All farms participating in this study voluntarily joined the disease eradication program in accordance with the provisions of the aforementioned regulation. One of the elements of the program was the introduction of vaccination with a marker-deletion vaccine.

The ability to differentiate infected from vaccinated cattle was crucial for preventing trade restrictions in Europe. This differentiation strategy is known as DIVA (Differentiating Infected from Vaccinated Animals). BoHV-1 DIVA vaccines (syn. marker vaccines) are based on the absence of one of the non-essential glycoproteins (gC, gE, gG, gI, or gM). However, only the gE-null BoHV-1 mutant is immunogenic, exhibits very low residual virulence, is expressed in a large subset of field BoHV-1 strains, and induces gE-specific antibodies detectable via diagnostic tests (40). Marker vaccines based on the gE-null mutant are widely used in Europe and globally, in both live and inactivated forms (5, 7, 16, 21, 24, 27, 28).

The above-mentioned study of a dairy cooperative from the north-eastern part of Poland showed that 12.3% of the tested animals were vaccinated with deletion vaccines against IBR/IPV. Of the 27.6% of farms where the deletion vaccine was used, 10.3% had a low BoHV-1 infection rate (< 10%), 10.3% had a moderate BoHV-1 infection rate (> 10% and < 30%), and the remaining 27.6% had a high BoHV1 infection rate (> 30%) (32).

During the three years of the study, no positively reacting individual was recorded in herd B (without virus circulation). Furthermore, the study results showed that seroprevalence within the herd decreased significantly in dairy farms where the virus circulated (A and C), regardless of the vaccination scheme used. The exception is farm D, where seroprevalence increased despite the vaccination scheme used, which should in theory generate a better immune response. The MLV vaccines used there twice should induce a strong humoral and cellular immune response due to the weakened virus replication (29). The marker vaccines used in this study were effective and safe. Moreover, Kaashoek et al. (17) reported that double vaccination with an inactivated gE-negative vaccine could prevent the development of clinical signs after challenge infection, and the peak of virus shedding was significantly reduced. The attenuated vaccine induced the best clinical protection, as evidenced by the complete absence of clinical signs and fever in cattle. In addition, the attenuated vaccine reduced the shedding of the challenge virus much more than the inactivated vaccines (4). The authors attribute the increase in seroprevalence on farm D to the situation of the farm. During the project, the farm was expanding and animal identification and biosecurity procedures failed. Biosecurity is a key point to a successful farm, but it all starts with the correct management of the farm: proper disinfection of people, cars and materials getting into the farm, and reducing the effect of natural factors on the farm (6). The authors do not rule out the influence of Seronegative Latent Carriers, although it has not been proven so far that the appearance of these carriers is associated with the use of a deletion vaccine only in the gE gene (8, 26).

It is obvious that the results obtained on 4 farms cannot be treated as a general rule. However, after 3 years of successful application of our vaccination program, it is very likely that with the development and widespread use of marker vaccines and the possibility of distinguishing post-vaccination and post-infection antibodies, the vaccination program described here will be even more effective in combating IBR infections. The use of deletion vaccines may in the future, allow for the status of a region/country free from IBR/IPV. These findings have important implications for both EU policy and national implementation. While the current C+D+E classification provides a flexible framework, our results suggest that additional guidance may be needed for high-prevalence member states.

The success of regional eradication programs in Italy's Valle d'Aosta and Trentino-Alto Adige provinces demonstrates that targeted approaches can work, but require sustained commitment and resources.

In conclusion, our study confirms that IBR/IPV control under the EU regulatory framework is achievable through integrated strategies combining vaccination, biosecurity, and surveillance. The current Polish eradication program represents both a significant challenge and opportunity to refine these approaches in high-prevalence settings. Future research should focus on optimizing protocols for large herds, investigating potential viral reservoirs, and developing cost-effective implementation models that balance regulatory requirements with practical realities of dairy farming.

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