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EVALUATION OF THE IMMUNE RESPONSE IN CALVES VACCINATED WITH COMMERCIAL VACCINE AGAINST PI3 AND BRSV ON A FARM IN WESTERN ROMANIA

EVALUAREA RĂSPUNSULUI IMUN LA VIȚEII VACCINAȚI CU VACCINURI COMERCIALE ÎMPOTRIVA PI3 ȘI BRSV ÎNTR-O FERMĂ DIN VESTUL ROMÂNIEI

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ABSTRACT | REZUMAT

Respiratory diseases in calves are of significant importance, both globally and locally.This study aimed to compare two calf vaccination protocols on a dairy farm in Western Romania, a protocol already used on the farm and a new one, to determine the optimal time for calf vaccination and the effectiveness of the vaccination. To assess the immune response in calves vaccinated against *Bovine Respiratory Syncytial Virus (B RSV)* and *Bovine Parainfluenza Virus (PI3)*, we measured the post-vaccination antibody titre using the indirect ELISA method. The values of serum proteins in the first 24 hours in the calves from the two groups had values above 5 g/100 ml, which demonstrates a good passive transfer of maternal antibodies as a result of colostrum. High levels of maternal antibodies, which persisted during the study period, interfered with post-vaccination antibodies and led to decreased anti-*BRSV* antibody titres in both protocols.

Keywords: calves, bovine respiratory syncytial virus BRSV, Bovine Parainfluenza virus PI-3

Bolile respiratorii la viței au o importanță semnificativă, atât la nivel global, cât și local. Acest studiu și-a propus să compare două protocoale de vaccinare a viței-lor într-o fermă vaci de lapte din vestul României, un protocol deja folosit în fermă și unul nou, pentru a determina momentul optim pentru vaccinarea vițeilor și eficacitatea vaccinării. Evaluarea răspunsului imun la viței vaccinați împotriva *Virusului Respirator Sincițial Bovin* (*BRSV*) și împotriva *Virusului Parainfluenței Bovine (PI3)* s-a făcut pe baza determinării titrului de anticorpi post-vaccinare prin metoda ELISA indirectă. Valorile proteinelor serice în primele 24 h la viței, din cele două loturi, au avut valori peste 5g/100ml, ceea ce demonstrează un bun transfer pasiv al anticorpilor materni ca urmare a colostrului. Nivelurile ridicate de anticorpi materni, care au persistat în perioada cercetării, au interferat cu anticorpii post-vaccinare și au condus la scăderea titrului de anticorpi anti-BRSV în ambele protocoale.

Cuvinte cheie: viței, Virusul respirator sincițial bovin VRSB, Virusul parainfluenței bovine PI-3

The increased incidence of respiratory diseases is determined by the polyfactorial aetiology on the one hand and the high susceptibility of cattle to respiratory diseases on the other (1, 5). Abiotic and biotic factors intervene in the aetiology of these conditions, which act interdependently (3). Abiotic factors, represented by stress, overcrowding, poor hygiene and food conditions, and deviations from growth technology, cause a decrease in the general resistance of the body and the appearance of microlesions at the level of the mucous membrane of the respiratory system, which represents entry gates for biotic factors. Biotic factors are represented by viruses, bacteria, and parasites, which act directly on the respiratory system or complicate the initial lesions produced by abiotic factors, producing respiratory conditions with variable clinical symptoms and lesions (5, 8, 11). Of all the microorganisms implicated in the aetiology of respiratory diseases in calves, viruses have a major role, being assigned the greatest importance. Viral infections are subtle at first, going unnoticed, but become apparent after several passes from one calf to another, when the more resistant calves also become ill.

The bacteria that intervene in the etiopathogenesis of respiratory diseases, in general, are epiphytes of the respiratory tracts; there are always carrier and eliminator animals in cattle herds. *Pasteurella* and *Mannheimia* bacteria can frequently be isolated from clinically healthy calves but also from diseased ones (6, 7). These bacteria can be isolated as primary pathogens or grafted onto existing lesions, which they aggravate. As a result of the polyfactorial aetiology, respiratory diseases of calves are difficult to prevent, and at the same time, difficult to combat. Prevention measures are complex and are based on the avoidance of contributing factors and the application of immunoprophylaxis (8, 9, 13).

Specific prophylaxis for respiratory diseases in

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calves is based on the use of antiviral and antibacterial vaccines.Considering the importance of respiratory diseases in calves, there are concerns about elucidating the causes involved in the aetiology of respiratory diseases in calves.

MATERIALS AND METHODS

The research was carried out on two batches of Holstein Frisian calves from a dairy farm in Western Romania, with a herd of 2000 dairy cows. Respiratory disorders are a frequent problem of cattle and are influenced by the microclimate, especially by air currents and temperature variations.

Calves were divided into two groups: 50 calves per protocol, they were vaccinated against bovine parainfluenza and bovine respiratory syncytial virus. In both groups, the evolution of the post-vaccination antibody titre and total serum proteins was followed comparatively throughout the studied period.

The vaccination protocol was drawn up according to the vaccine manufacturer's instructions, which are mentioned in the product leaflet (animal age and method of vaccine administration). A comparative study was conducted between the two vaccination protocols named "Protocol A" and "Protocol B".

To test the effectiveness of the established immunity against *Mannheimia haemolytica*, the collection of nasal secretions on AMIES transport medium was performed.

In **protocol A**, the first vaccination of calves was done with the intranasal vaccine at 10 days of age in calves containing the live modified *Bovine parainfluenza virus type 3* strain Bio 23/A and the live modified *Bovine respiratory syncytial virus* strain Bio24/A, followed by a subcutaneous vaccination with the vaccine, which contains inactivated *Bovine respiratory syncytial virus*, strain Bio 24, inactivated *Bovine parainfluenza virus* type 3, strain Bio 23, and inactivated *Mannheimia haemolytica*, serotype A1, strain DSM 5283, at 20 days of age and booster after 21 days. 10 days after the intranasal vaccination, the first R1 blood collection was performed from the jugular vein of each vaccinated calf, and the second R2 collection was performed 21 days after the second administration of the systemic vaccine.

The intranasal vaccination of the calves in protocol A was administered with the help of an intranasal applicator that makes a fine spray inside the nostril. The solution particles varying in size between 30 and 100 µm and 1 ml were administered in each nostril.

To quantify total serum proteins at the time of intranasal vaccination, blood was collected again in vacutainers with a coagulation activator, repeating the serum testing by the refractometric method.

Protocol B consisted of vaccinating calves with an intranasal vaccine at 9 days of age containing live modified *Bovine parainfluenza virus type 3*, strain RLB 103, and live modified *Bovine respiratory syncytial virus*, strain 375 (23), followed by subcutaneous vaccination with a vaccine containing the EV908 strain of the inactivated *bovine respiratory syncytial virus*, inactivated *Parainfluenza virus-3 strain SF-4,* and inactivated *Mannheimia haemolytica* A1 strain M-4/1, on days 19 and 47 of life. The vaccine was administered 1 ml into each nostril using a nasal applicator that creates a fine spray inside the nostril with particles of 30 to 100µm in size.

Installation of the protective immunity, according to the manufacturer's leaflet, occurs 10 days after intranasal vaccination. At this age, the blood in sterile vacutainers containing a coagulation activator was transported to the analysis laboratory in the shortest possible time.

The blood samples in protocol B were collected from the jugular vein 10 days after the intranasal R1 vaccination, and the second R2 was collected 21 days after the second systemic vaccination. Also, at the time of local immunisation of the nasal mucosa, blood was collected to quantify the amount of total serum proteins by the refractometric method.

At the average age of 20 days before the vaccination, blood was collected again to determine the titre of maternal antibodies in the blood serum tested by the refractometric method. After blood collection, the first active immunization was performed with an inactivated vaccine, containing the inactivated *Bovine respiratory syncytial virus strain EV908*, the inactivated *Parainfluenza virus-3 strain SF-4*, and the inactivated *Mannheimia haemolytica* A1 strain M-4/1, in a dose of 5 ml in the lateral region of the neck, according to the manufacturer's leaflet (24). After 28 days from the first subcutaneous vaccination, the booster was performed with the same vaccine, according to the instructions in the product leaflet, where it is recommended to repeat the vaccination.

According to the manufacturer, the humoral immune response against *Bovine respiratory syncytial virus* and *Parainfluenza virus-3* is at its highest level 14 days after completion of the booster. To test this, blood was collected and transported at a temperature of 8°C to the laboratory for the antibody titre by the ELISA method.

The calves were sheltered in identical microclimate conditions, with no discernible variations in the impact of environmental factors that could have influenced a particular protocol positively or negatively. We monitored the total serum protein count in the blood serum of calves, along with the antibody titres resulting from vaccinations. The purpose of this was to monitor the development of immunity in both the nasal mucosa and systemic immunity.

As part of the study, the calves' health status was evaluated by collecting blood samples within the first 24 hours of their life. The level of total serum proteins was measured using a refractometer. These immunoglobulins correspond to the immunological status of each calf obtained by passive immunization through maternal colostrum. In the specialised literature, it is mentioned that the incidence of diseases is proportional to the level of immunoglobulins held after birth, and there is a correlation between total serum proteins and immunoglobulins, the ratio being 0.71 (19). We tested serum samples from calves in both protocols for antibody titres using the indirect variant of the ELISA technique. The value of the serum titre obtained by the indirect ELISA method ≤ 20 is considered negative and non-protective, and the titre > 20 is considered protective, both for PI 3 and BRSV. This type of ELISA test is useful for diagnosis, monitoring immunity, and ensuring vaccine quality control (6).

RESULTS AND DISCUSSION

During the first 24 hours of life of calves in **protocol A**, the values of total serum proteins were between 5.4 and 7.6 g/100 ml, which means that colostrum administration was performed on time and passive transmission of maternal antibodies was at a good level. The geometric means of the titres after the first administration of the intranasal vaccine by the indirect ELISA method were high for both the PI 3 virus and bovine respiratory syncytial virus. The geometric mean of the titres for PI 3 was 119.44 serum positive, and in the case of BRSV, it was 68.86 serum positive.

The vaccine administered at the age of 20 days was an inactivated vaccine administered subcutaneously in a dose of 2 ml. Before the vaccination, blood was collected again to determine the titre of maternal antibodies in the blood serum tested by the refractometric method. After the first subcutaneous vaccination, the vaccine manufacturer recommends a repeat vaccination 21 days after the first vaccination to increase the antibody titre. According to the manufacturer, the humoral immune response against *Bovine Respiratory Syncytial Virus* and *Parainfluenza Virus-3* is at its highest level 21 days after completion of the booster. The results of the serological examination at the second collection showed positive, protective titres obtained as a result of vaccination against PI 3. Instead of against *Bovine respiratory syncytial virus*, the titres were positive and protective for 40 % of the calves studied.

The geometric mean of the titre of the positive sera at the second collection was protective (Fig. 1.), only for the *PI3 virus,* respectively at 108.73, and for *BRSV,* the geometric mean of the titre was non-protective at 9.63. The results obtained from the nasal secretions revealed that no strains of *Pasteurella* and other symbiotic strains of the nasal cavity, such as *Mannheimia haemolytica*, were isolated.

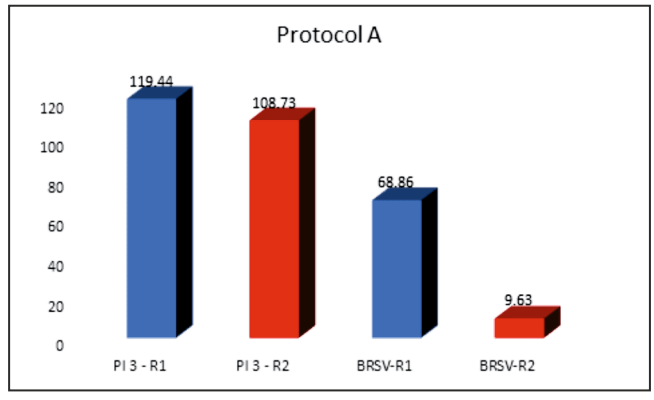

Fig. 1. Evolution of PI 3 and BRSV geometric mean of the titres – Protocol A

Protocol B was performed on 50 calves that were similar in age to those in Protocol A. During the first 24 hours of life of calves in protocol B, serum total protein values were between 5.0 and 7.0 g/100 ml, which means that colostrum administration was performed on time and passive transmission of maternal antibodies was at an average to good level.

The data obtained following the serological examination at the first R1 blood collection in the calves from protocol B revealed positive titres protective against PI 3 in all calves in the 100% batch; instead, against the BRSV virus, positive titres were obtained at 80% from calves. The geometric means of positive sera titres were 85.15 for PI3 and 44.68 for BRSV.

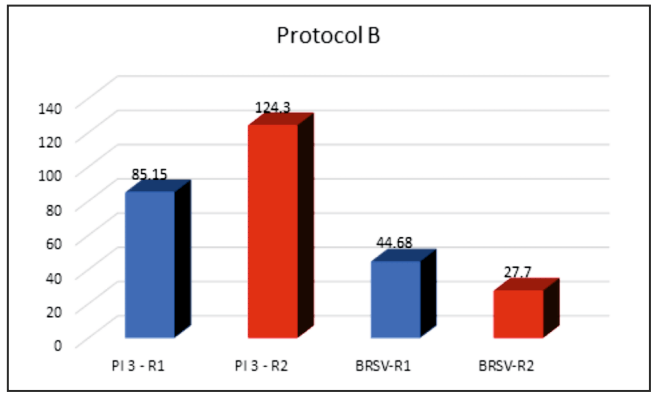

Fig. 2. Evolution of PI 3 and BRSV geometric mean of the titres – Protocol B

The geometric mean of the titres of the positive sera, obtained following the serological examination, at the second R 2 collection (Fig. 2.) indicated positive protective values against PI 3, respectively 124.3, and in the case of the BRSV virus, the geometric mean of

the titres of the positive sera was 27.7. In protocol B, 4% of the calves had titres with negative values that were below the positivity limit.

In the case of protocol B, at the second collection, an increase in the geometric mean of the titres was found in the case of the PI3 virus, from 85.15 at the first collection to 124.3 at the second collection. On the other hand, in the case of the BRSV virus, the geometric mean titres decreased from 44.68 at the first sampling to 27.7 at the second sampling. Also, in protocol B, nasal secretions were collected, and the results were negative for *Mannheimia heamolytica*.

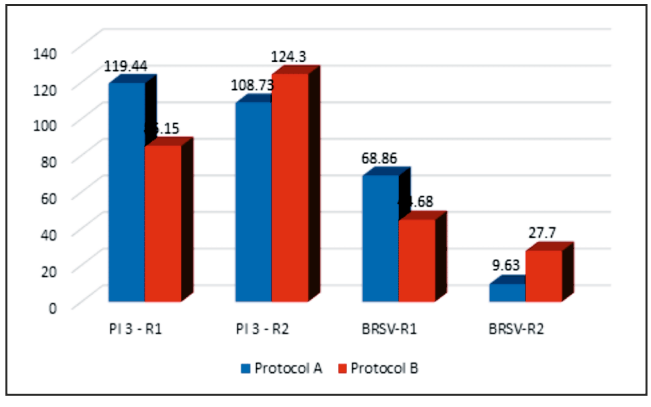

Fig. 3. Comparative evolution of *PI 3* and *BRSV* geometric mean of the titers

Comparing the two vaccination protocols (Fig. 3), it can be seen that:

- In the case of Protocol A, the geometric means of the titres were protective in the case of the PI 3 virus at the first collection, after which they decreased at the second collection, carried out 21 days after the second systemic vaccination, but still had protective values;
- In the case of the BRSV virus, after the application of vaccination Protocol A, the geometric mean of the titres had protective values after the first sampling but decreased after the second sampling to a geometric mean of the titres with non-protective values;
- In the case of Protocol B, the PI3 antibody titre values had protective values at both blood collection and at the second sampling the titre values increased compared to the first sampling;
- In the case of BRSV, the values of the titres in the case of protocol B, although they decreased comparatively from the first sampling to the second sampling, still had positive values.

The decrease in antibody titres in the case of BRSV appeared as a result of interference between maternal antibodies and post-vaccinal antibodies, although the package insert of the vaccine used in protocol A states that post-vaccinal antibodies, which appear after intranasal administration, do not interfere with maternal antibodies.

The data obtained by us are similar to those in the specialised literature, where it is mentioned that at the time of primary BRSV vaccination in calves, at 14 days of age, there was no increase in the level of post-vaccination antibody titers in the blood of the calves. This may be due to the presence and interference of maternal antibodies transmitted through colostrum and the immaturity of the immune system at this early age (2, 4, 12, 16). Although primary or secondary vaccination did not increase antibody levels in the blood of calves, a cell-mediated response likely occurred after vaccination (15).

Vaccination against BRSV is a challenging issue in calves, as maternal antibodies are often present in calves for 1 to 6 months. This can hurt vaccination schedules as these antibodies do not decline until approximately 40 days of age (17).

The results obtained by other authors indicate that a single vaccination with an inactivated BRSV vaccine can interact with maternal antibodies and induce partial protection in very young calves. The level and duration of protection will improve after the administration of the second dose of the inactivated vaccine. A vaccination schedule of two basic doses is recommended under field conditions (18).

Other authors mention that maternal antibodies remain in the calves' blood for up to 4-6 months, during which time they block the active response to infection with this virus. To avoid this shortcoming, more calves must be tested, applying other methods apart from serological ones (2, 21).

Our results are similar to those in the literature, with no response to BRSV vaccination in the case of protocol A but with protective titres, although lower, in the case of protocol B.

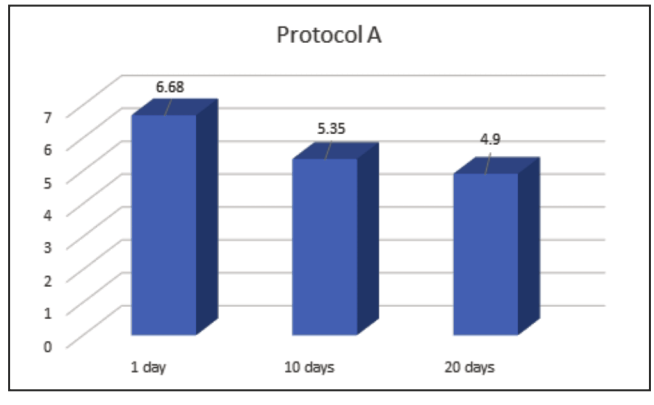

Fig. 4. Evolution of serum total protein mean values – Protocol A

Recently, the intranasal route has been preferred, which stimulates the development of antibodies not only locally but also in serum. The antibody titre obtained after vaccination is influenced by the amount of

antigen present in the vaccine dose. It is thus possible to appreciate the degree of immunisation achieved by determining anti-PI-3 antibodies in the nasal mucosa (10). The evolution of total serum protein values during the study had a downward trend, according to Figures 4 and 5.

In practice, it is recommended to apply a vaccination protocol, depending on the epidemiological situation on each farm.

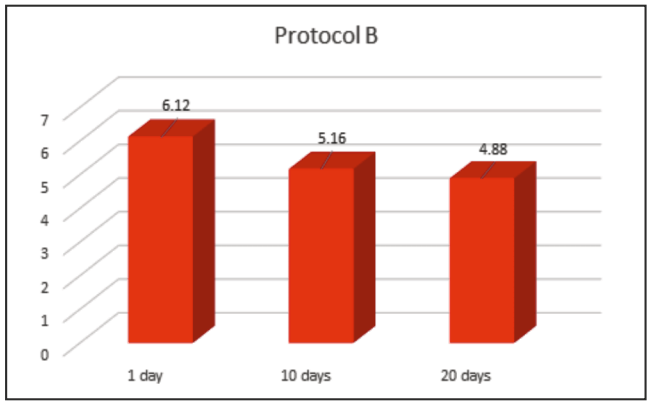

Fig. 5. Evolution of serum total protein mean values – Protocol B

In newborn calves, there is usually a close correlation between total serum protein and IgG in the blood because the largest protein present in colostrum is IgG. The correlation between total serum protein and IgG in calves at 24 hours of age is approximately 0.71. This means that approximately 50% of the variation in total protein in calf blood at 24 hours of age can be attributed to the IgG fraction (19).

Most researchers suggest the following data for estimating the level of passive transfer of total serum proteins at 24 hours of calf life:

> 5.5 g/100 mL is a good level of passive transfer;

5-5.4 g/100 mL average level of passive transfer;

<5g/100 mL passive transfer failure (19).

The age at which calf blood samples were collected may affect the relationship between total serum proteins and IgG. When we use the refractometer, we do not measure IgG directly but instead measure total serum proteins, which correlate with IgG on the first day of life. Correlations between serum total protein and IgG were adequate up to about 5 days of age; after the switch occurs, the relationship between the nature of total serum proteins and IgG is less reliable (18, 20).

In field conditions, a vaccination programme adapted to the epidemiological conditions on the farm and the determination of the maternal antibody titre before the first systemic vaccination are recommended.

CONCLUSIONS

In the case of Protocol A, the geometric mean BRSV antibody titres decreased from 68.86 at the first sampling to 9.63 at the second sampling, and the antibody titres in the case of PI3 also registered a decrease from 119.44 to 108.73 at two samplings.

In the case of protocol B, the geometric mean BRS V antibody titres registered a decrease from 44.68 in the first collection to 27.7 in the second collection; instead, the geometric mean titres of PI3 antibodies registered an increase from 85.15 to 108.73.

The high levels of maternal antibodies, which persisted during the research period, interfered with the post-vaccination antibodies and led to the decrease in the anti-BRSV antibody titre in both protocols.

The serum protein levels in the first 24 hours of the calves from both groups were above 5 g/100 ml, indicating a successful transfer of maternal antibodies through colostrum.

Comparing the two vaccination protocols, we recommend maintaining the current on-farm vaccination protocol.

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