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ADMINISTRATION OF HYPERIMMUNE PLASMA TO CALVES WITH NEONATAL DIARRHOEA AS AN ALTERNATIVE TO CONVENTIONAL THERAPY

ADMINISTRAREA PLASMEI HIPERIMUNE LA VIȚEII CU DIAREE NEONATALĂ CA ALTERNATIVĂ LA TERAPIA CONVENȚIONALĂ

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ABSTRACT | REZUMAT

Hyperimmune plasma has started to become an alternative to traditional treatments for neonatal diarrhoea in calves due to the alarming rate of antibiotic resistance emergence in the bovine sector. This study aimed to evaluate the efficacy of this hyperimmune product administered in digestive infectious disorders of neonatal calves while comparing this therapy with the conventional approach, which involves the use of antibiotics. For this purpose, the study enrolled 30 Holstein calves with digestive disorders, divided into two groups: the experimental group (n=20), which received hyperimmune plasma, and the control group (n=10), which was treated with antibiotics. To assess the efficacy of the therapy, the evolution of serum IgG titres was monitored, with antibody quantification performed before any form of therapy, at 1 hour and 24 hours after administering hyperimmune plasma and at 72 hours and 96 hours after initiating antibiotic therapy. In both cases, increases in serum IgG titres were noted; however, in the experimental group, the antibody titre increased from an average of 12.69 ± 2.39 mg/mL before plasma administration to an average of 123.30 ± 5.52 mg/mL just one hour after starting the therapy. In the control group, the serum antibody titre increased from an average of 13.53 ± 2.46 mg/mL to 74.46 ± 14.74 mg/mL at 96 hours after initiating antibiotic therapy. The results of this study encourage the use of hyperimmune plasma not only to improve the serum antibody titres of newborn calves but also as an alternative to antibiotic-based therapy for neonatal diarrhoea.

Keywords: neonatal diarrhoea, calves, serum IgG, hyperimmune plasma, reduced antibiotic consumption

Plasma hiperimună a început să reprezinte alternativă la tratamentele tradiționale utilizate în diareea neonatală a vițeilor, ca urmare a ritmului alarmant de emergență a antibiorezistenței din sectorul bovinelor. Scopul acestui studiu a fost de a evalua eficacitatea acestui produs hiperimun, administrat în tulburările infecțioase digestive ale vițeilor neonatali, în același timp această terapie fiind comparată cu cea convențională, care constă în administrarea de antibiotice. În acest sens, prezentul studiu a inclus 30 de indivizi cu tulburări digestive, viței din rasa Holstein, care au fost divizați în două grupuri: primul este grupul experimental (n=20), incluzând viței cărora li s-a administrat plasmă hiperimună, iar cel de-al doilea este grupul de control (n=10), vițeii din acest lot fiind tratați cu antibiotice. Pentru a evalua eficacitatea terapiei s-a urmărit evoluția titrului de IgG seric, astfel cuantificarea anticorpilor s-a realizat înainte de administrarea oricărei forme de terapie, la o oră și 24 de ore de la administrarea plasmei hiperimune și la 72 de ore și 96 de ore de la instituirea terapiei cu antibiotice. În ambele cazuri, s-au notat creșteri ale titrului de IgG seric, însă în cazul grupului experimental, titrul de anticorpi a crescut de la o medie de 12,69 ± 2,39 mg/mL, măsurat înainte de administrarea plasmei, la o medie de 123,30 ± 5,52 mg/mL la doar o oră de la începerea terapiei. În cazul lotului martor, titrul anticorpilor serici a crescut de la o medie de 13,53 ± 2,46 mg/mL la 74,46 ± 14,74 mg/mL, medie înregistrată la 96h după instituirea terapiei cu antibiotice. Rezultatele încurajează utilizarea plasmei hiperimune, nu doar cu scopul de a îmbunătăți titrul anticorpilor serici al vițeilor nou-născuți, cât și ca alternativă la terapia diareii neonatale bazată pe antibiotice.

Cuvinte cheie: diaree neonatală, viței, IgG seric, plasmă hiperimună, reducerea consumului de antibiotice

Neonatal diarrhoea is the most frequent pathology that affects calves in the first period of their lives, and the usual pathogens implicated in the disease are *Escherichia coli* (K99), *Cryptosporidium* spp., rotavirus, and coronavirus (22). These microorganisms are often isolated from samples taken from calves with diarrhoea, which suggests that these pathogens are responsible for the initiation of digestive diseases in the first days of life (14). Neonatal diarrhoea that develops in the first 2-4 days post-parturition is caused only by *Escherichia coli*, enterotoxigenic strain (K99), and after 5-21 days pathology occurs under the action of *Cryptosporidium* spp., rotavirus, or coronavirus (10, 16). The development of neonatal diarrhoea is closely related to the failure of passive transfer of immunity (5, 6), which occurs

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when the serum concentration of IgG in calves is lower than 10 mg/mL after consumption of colostrum (11). Failure of passive transfer is not a pathology in itself but rather an immunodeficiency that predisposes the calf to various diseases (11). The dependence on colostrum consumption in the first hours postpartum derives from the particular structure of the ruminant placenta that does not allow the passage of antibodies from the mother to the foetus (23). In this way, the consumption of colostrum is mandatory in the first 24 hours after calving, because after this period the absorption of these molecules in the intestine wall of the newborn is stopped (9). Antimicrobials are often used systemically to prevent and treat neonatal diarrhoea in calves, although an etiologic diagnosis is often not made. This leads to excessive and unnecessary use of antibiotics in species intended for human consumption and the potential development of antibiotic resistance (1) and severe dysbiosis, which will alter the gut microbiota. The World Health Organisation has recognized antibiotic resistance as one of the three major public health targets (24). The consumption of antibiotics in animals is currently double that of humans, and the average antibiotic intake per kilogramme of beef is 45 mg, while in pork it is 172 mg and in broiler chicken 148 mg (24). It is projected that by the year 2030, antibiotic consumption in animals will increase by 67% from the level in 2010. Globally, China is the largest producer and consumer of antibiotics, using 97,000 t per year for animal agriculture (24). At the 2016 level, Raboisson et al. estimated a total cost per dairy calf between € 52 and € 285 due to failure of passive transfer of antibodies from colostrum, and in veal calves this cost is double (19).

The use of hyperimmune products to support the animal's body in the absence of maternally transferred antibodies is becoming an increasingly effective method for treating newborn ailments (13). More than that, this innovative therapy attempts to overcome all the drawbacks of conventional therapies, including those related to animal welfare (8) and biosecurity (1). In this study, calves with digestive pathologies were selected and subsequently administered hyperimmune plasma as an alternative to conventional therapy.

MATERIALS AND METHODS

The present work was conducted for a period of 4 months (January-April) in a dairy farm located in the South of Romania. The experimental batch consisted of 30 Holstein calves that had diarrhoea of infection origin confirmed by the rapid test kits (Fassisi BoDia, Germany) that detected the antigens tracked in the faeces. Based on the future therapy, the animalswere randomly separated into two groups, the control group (n=10) which received antibiotics (Exabiopen, Syva, Spain) and fluid therapy during the study (Ringer Lactate, Braun, Romania), and the experimental group (n=20), the calves within it being treated with hyperimmune plasma acquired by a previously outlined procedure by Constantin et al. (2023). After a clinical examination of each affected calf, the administration of hyperimmune plasma (one unit) was done intravenously through a catheter placed in the jugular vein, and after a 37°C water bath, slow thawing of it. For a sterile administration, the jugular groove was trimmed, cleaned, and disinfected with betadine before the venous catheter placement. After placing the venous catheter, the procedure for administering the hyperimmune product was started. Initially, for 20 minutes, the transfusion rate was slow, at 10 mL/kg/h, to prevent the occurrence of allergic reactions. During this time, the calves were monitored, constantly evaluating their pulse, respiratory rate, colour of apparent mucous membranes, and any abnormal behaviours. Allergic reactions could have been caused by the proteins in the plasma and consisted of the appearance of tachycardia, tachypnoea, tremors, sweating, hives, dyspnoea, and collapse (21). Even though the doses of epinephrine (0.001-0.002 mg/kg) were already prepared in order to combat possible adverse reactions, none of the calves suffered allergic reactions during this procedure. In the absence of any adverse reactions, the transfusion rate was increased, and the calves received the entire amount of plasma within 30-40 minutes.

The determination of IgG concentration in the serum of calves that received hyperimmune plasma was carried out using the Bovine IgG ELISA Kit (Abcam, UK), which allows the quantification of immunoglobulins in the serum under investigation by coupling them to anti-IgG antibodies included in the kit. To obtain the test samples, blood was collected three times from the jugular vein: before the start of therapy, one hour after administration, and 24 hours after administration. Blood samples were collected using 18 G needles and BD Vacutainer K2 EDTA (Plymouth, UK) collection tubes from both the control group calves and the calves treated with hyperimmune plasma. Further, the specimens were centrifuged at 1500× g for 15 min at 4°C, and plasma was harvested and frozen at −80°C until antibody evaluation. The quantification of antibody titres was first performed before the administration of any form of therapy, both antimicrobial and hyperimmune plasma, one hour after plasma administration, and 24 hours after the completion of the transfusion. For the calves in the control group, blood samples were collected before administering antibiotics, at 72 hours, and 96 hours after the first administration. This change in sampling times was necessary because the half-life of antibiotics is greater than 1 hour, the interval at which the first blood sample was collected to quantify the antibody titre post-administration of hyperimmune plasma, and the immune system's response, with antibody formation, to the action of pathogens takes longer.

The authors of this study ensured that they followed all regulations on the welfare of animals, as outlined by the European Union and national legislation (Directive 2010/63/UE;Law 34/2014).None of the animals involved in the study experienced any suffering during the procedures. The data was analysed and interpreted using SPSS Statistics 26 (IBM Inc. Chicago, IL, USA). Various statistical measures, such as mean and standard deviation, were calculated for the collected blood samples.

RESULTS AND DISCUSSION

Hyperimmune plasma therapy has already entered routine therapeutic protocols in equine neonatology by producing plasma at commercial rates. Recently, following encouraging results in other species, this innovative therapy has also started to be applied to cattle, and the premises are favourable. According to the results obtained (Table 1), the serum IgG level of the calves increased after just one hour post administration and maintained its upward trend one day after the administration of the hyperimmune plasma.

Table 1

Serum IgG levels obtained from the serum samples of calves transfused with hyperimmune plasma

Sample number	Before plasma administration (mg/mL)	1 hour from plasma administration (mg/mL)	24 hours from plasma administration (mg/mL)
1	11.8	122.7	110.9
2	10.5	120.5	130.8
3	13.4	135.5	140.5
4	12.5	125.5	130.3
5	10.8	120.1	125.5
6	15.5	122.8	130.0
7	10.8	130.5	140.2
8	11.5	120.7	125.5
9	9.5	121.5	124.3
10	10.7	123.8	140.8
11	12.3	118.7	129.5
12	14.8	120.2	130.3
13	13.5	122	135
14	12.8	121.1	134.5
15	16.7	130.8	140.5
16	18.2	120.5	125.8
17	10.8	109.8	120.2
18	13.4	120.2	135.8
19	12.3	128.5	140.5
20	17.5	130.7	148.2
Mean	12.96	123.3	131.95
Standard deviation	2.39	5.52	8.47

Correlated with the results obtained, the administration of hyperimmune plasma causes a 9.5-fold increase in the serum antibody titre of the calves just one hour after its administration. The increased concentration of antibodies is maintained, according to the results obtained, even 24 hours after administration. Studies conducted by other researchers support the idea that the calves' production of IgG antibodies begins 36 hours postpartum and continues until 3 weeks, at a rate of only 1g/day (2). After the intestinal barrier no longer allows the passage of colostral antibodies into the calf's bloodstream, the administration of hyperimmune plasma constitutes an effective method to increase antibody titres (15). This idea is supported by the current study, which demonstrates an increase in the mean IgG titre from 12.96 ± 2.39 mg/mL, measured before plasma administration, to a mean IgG of 123.30 ± 5.52 mg/mL just 1 hour after completion of the transfusion process.

This increase in antibody titre, achieved through the administration of hyperimmune plasma, demonstrates its positive impact on combating infectious diseases, especially in cases of diarrhoeal origin. Maintaining the upward trend of antibody titres even at 24 hours after administration of hyperimmune plasma, with average values recorded at 131.95 ± 8.47 mg/mL, encourages the use of this product in infectious gastrointestinal pathologies syndromes that frequently evolve, and more critically, in calves with weakened passive immunity.

The antibody titres of the calves that were administered antibiotics were measured before administration, at 72 hours post-administration, and again at 96 hours after the first administration. The difference in antibody titre measurements across the days of blood sampling between the group administered hyperimmune plasma and the control group stems from antibiotic administration supporting antibody formation by the calves' immune system, but these antibodies are produced much later. In other words, while antibiotics assist in stimulating the calves' immune systems to produce antibodies, there is a delayed response compared to the immediate boost observed with hyperimmune plasma administration. According to the obtained results (Table 2), the antibody titres increase to a lesser extent after administering antimicrobial substances compared to the increase observed after administering hyperimmune plasma.

Table 2

Serum IgG levels obtained from the serum samples of control calves

Sample number	Before antibiotic administration	72 hours after antibiotic administration	96 hours after antibiotic administration
1	10.5	60.7	75.2
2	11.0	73.5	95.5
3	12.3	81.5	87.9
4	10.8	50.5	65.7
5	13.1	85.7	89.5
6	14.7	96.7	98.9
7	15.8	50.7	65.5
8	17.3	40.2	50.5
9	12.5	65.7	75.8
10	17.3	86.5	90.1
Mean	13.53	69.17	79.46
Standard deviation	2.46	17.64	14.74

In the control group (n = 10), the calves treated with antibiotics for diarrhoea, the mean IgG titre was only 5.11 times higher than before the start of treatment. At the 72-hour mark after starting the antimicrobial therapy, the serum antibody concentrations ranged between 40.2 mg/mL and 96.7 mg/mL, which is lower compared to the calves administered hyperimmune plasma. The latter group exhibited an average serum IgG of 123.3 ± 5.52 mg/mL just one hour after receiving the hyperimmune product (Fig. 1.). These results underscore the prompt immune response when the body receives antibodies, bypassing the time-consuming process of antibody production. In addition to the low rate of antibody

titre growth, administering antibiotics to combat neonatal diarrhoea has several negative effects on the gastrointestinal tract. These include alterations in the structure and function of the intestinal wall, delays in growth, and prolonged duration of diarrhoea (3).

Furthermore, increasing antibody titres to a greater extent following the administration of hyperimmune plasma shows that the body's reaction time to pathogens is shorter. Achieving nearly a 10-fold higher titre of serum immunoglobulins within just one hour after administering a hyperimmune product enhances the body's defence capability. The immune response to disease-causing agents is more prompt compared to antibiotic administration.

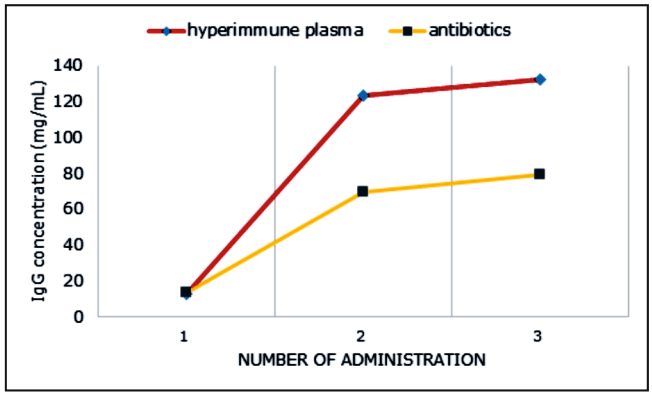

Fig. 1. Graphical representation of serum IgG titres after depending on the administered product

The maternal IgG half-life is known to be around 20 days (17), but the duration of immunoglobulins in hyperimmune serum is not currently understood (18). There is a pressing need for studies focused on the potential effects of stimulating the immune system of calves transfused with hyperimmune plasma. Besides its rich immunoglobulin content, this product also contains other components such as coagulation factors, complement, cytokines, and interleukins (4), which could potentially have immunostimulant effects on the newborn's immune system (Bresciani et al. 2016). The results of the study presented in this paper are supported by another research. Selim et al. (1995) administered hyperimmune plasma containing antibodies against the J5 membrane antigen of *Escherichia coli* to a group of calves with diarrhoea. They observed significant improvements in the antibody titres in the serum samples collected from the treated animals.

In addition to assessing the increase in antibody titres of calves treated with hyperimmune plasma or antibiotics, they were also evaluated clinically. Just two days after administering the hyperimmune product, the diarrheal discharges of individuals in this group significantly decreased. Moreover, the overall condition of the calves improved, with restored appetite, normalisation of body temperature, and their presentation in a quadrupedal stance at the time of examination. Out of the total calves in the group treated with hyperimmune plasma (n = 20), only two calves died due to infectious neonatal diarrhoea, resulting in a survival rate of 98%. In contrast, calves treated with antibiotics had a longer clinical course of the disease, showing general deterioration and diarrhoea lasting over 5 days. Out of the total number of calves in the control group (n=10), only 5 calves survived the illness, resulting in a treatment success rate of only 50%. The prolonged and lingering course of the disease in calves with low post-colostral antibody titres treated with antibiotics led to their demise after a prolonged illness progression.

The administration of hyperimmune plasma remains an effective therapy for neonatal diarrhoea, especially in calves who's post-colostral antibody titres do not reach an optimal level of defence against infectious agents. This increases vulnerability to the pathogenic action of the most common pathogens, namely *Escherichia coli*, bovine rotavirus, bovine coronavirus, and *Cryptosporidium* spp.

CONCLUSIONS

Just one hour after the transfusion of hyperimmune plasma, the serum level of immunoglobulins recorded values approximately 9.5 times higher than before administration, with the average IgG value increasing from 12.96 mg/mL to an average of 123.30 mg /mL. The upward trend in serum antibody titres of the calves persisted at 24 hours post-administration, reaching average values of 131.95 mg/mL. In the case of the control group where antibiotics were administered as a therapeutic measure for neonatal diarrhoea, the serum antibody titre measured at 72 hours post-administration showed an increase of only 5.11 times compared to levels measured before administration. The average values before administration were 13.53 mg/mL, which reached average values of 69.17 mg/mL at 72 hours and 79.46 mg/mL at 96 hours post-administration of antibiotics.

In cases of reduced passive transfer of immunity, transfusion with hyperimmune plasma represents a suitable alternative to enhance the antibody titres in newborn calves of the bovine species. Apart from the rapidity with which the animal's body becomes capable of responding to environmental pathogens, hyperimmune plasma also replaces traditional antibiotic-based therapies. This aspect is crucial in the livestock industry, particularly concerning the alarming rate of antibiotic resistance spread.

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