Passive immunity stimulated by vaccination of dry cows with a trivalent vaccine against neonatal calf diarrhea

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Abstract
Passive transfer of colostrum immunoglobulins from cow to newborn is extremely important because calves under 5 weeks of age do not have active immunity and colostrum antibodies are the only source of immunoglobulins to protect calves from infectious diseases immediately after birth. One of the most common causes of calf death is acute neonatal diarrhea caused by pathogens such as rotavirus, coronavirus and Escherichia coli. In the first weeks of life, calves acquire maternal antibodies from colostrum and milk can have a local protective effect against intestinal enteropathogens. Vaccination of pregnant cows reduces the morbidity and mortality rates of the calf. Vaccination of cows even in the early stages of pregnancy (6 months before calving) can provide passive protection in newborn calves against etiological agents such as enterotoxigenic Escherichia coli. Previous studies have shown that continuous administration of colostrum from immunized cows prevents severe diarrhea and mortality in calves. In North America and Europe, various vaccines against neonatal calf diarrhea have been developed to increase antibody titers in colostrum and cow's milk. However, there are not many studies on maternal vaccination to protect against diarrhea in calves in Romania. In this article we have detailed the results of a field study on improving passive immunity in calves by administering a multipurpose maternal vaccine available in Europe.
***Key words**: calf, colostrum, neonatal calf diarrhoea, passive immunity, vaccination.*

Introduction

Neonatal diarrhea mainly affects calves under the age of 4 weeks. It is characterized by diarrhea that leads to dehydration and acidosis, which can have systemic consequences and can lead to death [5]. More than 50% of all cases of neonatal diarrhea occur in the first week of life and only 15% occur after the second week of life [4]. Calf loss, disease management and health consequences, growth rate and reproductive potential of surviving calves can lead to significant economic loss [1, 5].

The main infectious agents causing diarrhea, especially in calves under 12 days of age, are bovine rotavirus (BoRV), bovine coronavirus and enterotoxigenic Escherichia coli (ETEC), but other pathogens may also be responsible for this disease [1 , 4, 5]. The good part is that vaccines against some of the most common pathogens responsible for this disease are available on the market. [2. 3]

The aim of this study was to evaluate the titer of specific BoRV, BoCV and E.coli F5 (K99) antibodies in the serum of pregnant cows, in colostrum and in the serum of newborn calves in order to highlight the immune response in cows and the passive transfer in calves.

Material and methods

In order to carry out the study, 2 experimental groups were established: the control group (M), to which no specific prophylaxis method against bovine rotavirus (BoRV), bovine coronavirus and *Escherichia coli* was applied and the experimental group (E) which was immunized with a trivalent Rotavec ™ Corona vaccine, MSD Animal Health, within 220-230 days of gestation. Each group consisted of 20 pairs of cow-calves from which 104 serum samples and 40 colostrum samples were collected between November 2019 - December 2019. In group E, 2 twin calvings were registered. Blood samples were collected from cows at 21 days post-vaccination, respectively between 241-251 days of gestation, and colostrum samples were collected immediately after calving. Calves received a dose of 4 liters of fresh colostrum harvested from their mothers in the

first hour of life and blood samples were taken before an after colostrum consumption at 24 hours of age.

Specific antibodies were detected using the competition enzyme immunoassay kits ELISA Bovine Rotavirus BIO-X Diagnostics, ELISA Coronavirus BIO-X Diagnostics, and ELISA blocking E. coli F5 (K99) BIO-X Diagnostics. The analyzes were performed in the Immunology laboratory of FMV Iaşi and the descriptive statistical data and intra-group comparisons were performed using the IBM SPSS Statistics Subscription program.

Results and discussions

In the Figure 1 are represented graphically the mean values of bovine anti-rotavirus antibodies (BoRV), expressed as percent inhibition (PI%). It can be seen that the PI% of cow serum antibodies - colostrum antibodies - calf serum antibodies have higher values in group E. The level of anti-rotavirus antibodies in the serum of pregnant cows in group E shows a significant difference compared to group M, in group E registering an average of 77% PI, compared to 41.5% PI in group M (p value <0.001). Increased serum antibody titer in group E cows resulted in a higher synthesis of specific antibodies in colostrum (79.3% PI). In the serum of calves from group E there is an average of 94.4% PI and in the group M there was an average of 59%. The difference between the averages from the 2 groups is statistically significant with a p value <0.001.

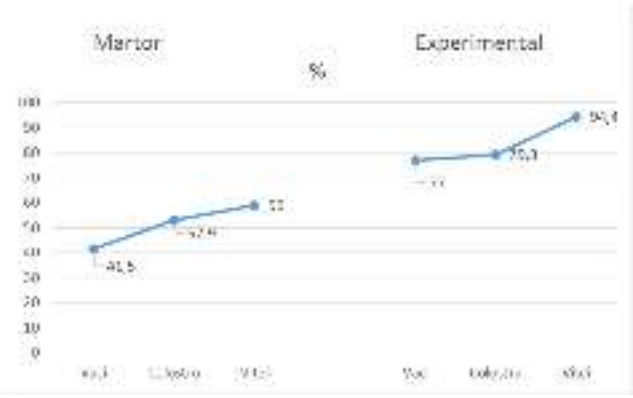

Fig. 1 – Graph of mean values of BoR expressed as percent inhibition measured with competitive ELISA from pregnant cow sera, colostrum and calf sera

In Figure 2 are represented graphically the mean values of bovine anti-coronavirus antibodies (BoRV), expressed as percent inhibition (PI%). As in the case of the analysis of anti-rotavirus antibodies, it can be observed that in group E the level of anti-coronavirus antibodies in the serum of pregnant cows increased considerably compared to group M, in group E registering an average of 96.2% PI, compared to of 32.6% PI in group M (p value <0.001). Elevated serum antibody levels in group E cows resulted in a high level of synthesis of specific antibodies in colostrum (98.9% PI). An average of 97.3% PI was recorded in the serum of calves from group E, and an average of 32.6% PI was recorded in the group M. The difference between the averages of the 2 groups is statistically significant with a p value <0.001.

Fig. 2 – Graph of mean values of BoCV expressed as inhibition percentages measured by competitive ELISA from pregnant cow sera, colostrum and calf sera

The values of anti-E. coli F5 (K99) antibodies, expressed as percent inhibition measured with ELISA blocking in the sera of pregnant cows and the sera of calves are plotted in Figure 3. In group M, both in the serum of pregnant cows and in the serum of calves, a minimal presence of anti-E.coli F5 (K99) antibodies was detected, with an average of 0.3% PI cows and 0.2% PI calves, respectively. The differences between group E and group M are statistically significant ($p < 0.001$), in group E registering an average of 90.9% PI in the case of pregnant cows serum and 97.4% PI in the case of calves serum.

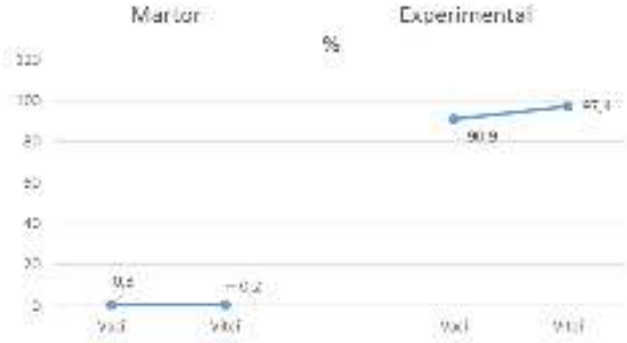

Fig. 3 – Graph of mean values of anti-E.coli F5 antibodies (K99), expressed as percentages of inhibition measured by competitive ELISA in pregnant cows and calves

The calf has an essential feature in terms of immunology: the titer of antibodies at birth is zero, during intrauterine life the transfer of antibodies by transplacental route from mother to fetus is zero. Consequently, it is born without adequate humoral immunity and is totally dependent on passive transfer via colostrum. To test this hypothesis, blood samples were collected from calves in group E before colostrum administration and 24 hours after administration to show the level of anti-rotavirus, anti-coronavirus and anti-E.coli antibodies. F5 (K99). The results are shown in Table 1.

Table 1.

Specific antibodies in calf serum before and after colostrum, expressed as% inhibition measured by competitive ELISA

Statistical parameters	Antibody values expressed in PI%							
	BoRV, strain UK-Compton, serotype G6 P5 (inactivated)			BoCV strain Mebus (inactivated)			E. coli F5 (K99) agglutinate	
	Calves serum before colostrum intake	Colostrum	Calves serum after colostrum intake (24 h life)	Calves serum before colostrum intake	Colostrum	Calves serum after colostrum intake (24 h life)	Calves serum before colostrum intake	Calves serum after colostrum intake (24 h life)
$\bar{X}$	0	79,3	94,4	0	96,4	97,3	0	97,4
SE	0	6,4	1,1	0	1,8	1,0	0	0,9
Median	0	89,2	95,1	0	98,9	99,1	0	98,7
SD	0	20,3	3,7	0	5,8	3,5	0	3,2
Min.	0	35,0	88,3	0	80,2	89,7	0	88,5
Max.	0	97,9	98,5	0	99,1	99,2	0	99,1
CI 95%	0	14,5	2,3	0	4,2	2,2	0	2

The presence of bovine anti-rotavirus, anti-bovine coronavirus or anti-E.coli F5 (K99) antibodies was not detected in the 22 serum samples collected before colostrum, which highlights the importance of colostrum administration in newborn calves for passive immune transfer of anti-infective specific immunoglobulins.

Conclusion

Because rotavirus and coronavirus infections are endemic in cattle, most adult cows have been infected naturally. The sustained response of antibodies found in the serum and colostrum of cows in group M is most likely due to such infectious exposure. Also, the immune response of group E cows may be due to a primary infectious exposure prior to systemic vaccination, but vaccination causes a marked increase in specific serum antibody levels. If in the case of rotavirus and bovine coronavirus were detected after analysis titers of maternal antibodies synthesized following active infections (group M), in the case of anti-E.coli F5 antibodies (K99) in the study group they were almost nonexistent. The obtained results showed that by the active immunization of cows in the last gestation period the specific antibody titers increased significantly in group E compared to group M, in all 3 infectious strains. This proves the effectiveness of vaccination in the herd.

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