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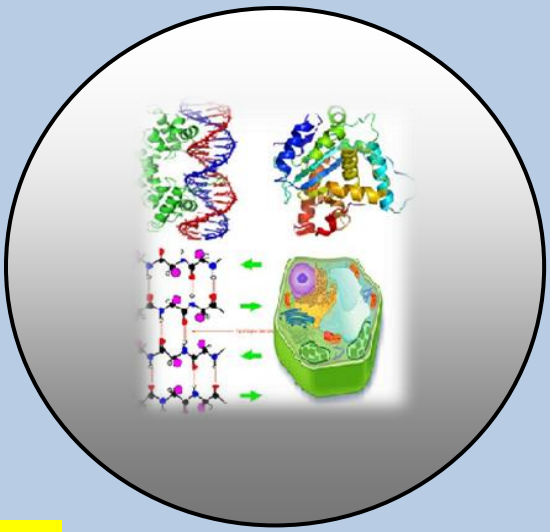

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Antibody Transfer Pattern in Calves from Dams Immunized with *Salmonella* Antigen

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ABSTRACT

The new born of ruminants is completely vulnerable to prevalent infectious diseases as it lacks immunity to those diseases due to the type of placenta possessed by the dams that prevents passage of immunoglobulins to the foetus during pregnancy. The new born is thus born unprotected and should therefore be fed colostrum immediately after birth within the first six hours. This has the ability to confer maternal immunity for a limited number of days after birth. Feeding colostrum should be followed by active vaccination which must be accurately timed if vaccine failure is to be avoided. Active vaccination requires knowing the time passive immunity is sufficiently waned to allow higher efficacy of active vaccination. The objective of this study was to determine the appropriate time vaccination of calves from salmonella –vaccinated dams should be carried out for higher vaccine efficacy in the calves. Using indirect ELISA to monitor antibody depletion pattern in the calves of salmonella-vaccinated dams involving the use of test sera from the calves, positive and negative control sera and re-constituted substrate indicator system (80 mg of PAHBA: 5-amino-2-hydroxy-benzoic acid), the following result was obtained. Day 1 found no antibody in serum of calves. Onday 5 obtained a titre that could confer immunity to enteric diseases (average antibody titre of 1.02 and % value of 221.9%) was attained. It remained above 100% at day 87. After the87thday, antibody titre dropped showing signs of need for active vaccination.The following conclusions were made for this study: based on the result from the calves' sera, Immunoglobulin concentration of 0.624 absorbance antibody titres with a % value of 134.19 marked the end of the less risky period for the calves in terms of immunity and this fell on the 87th day after calving. It was recommended that the calves receive active vaccination after the 87th day of calving.
Keywords: Salmonella Antigen, Maternal Immunity, Calves and Dams.

INTRODUCTION

The route by which maternal antibodies reach the foetus is determined by the structure of the placenta. The placenta of ruminants is syndesmochonial, that is, the chorionic epithelium is in direct contact with uterine tissues. In animals with these types of placenta, the transplacental passage of immunoglobulin molecules is totally prevented, and the new born of these species are entirely dependent on antibodies received through the colostrum (Philips, 2001) When a mammal is born, it emerges from sterile uterus into an environment where it is immediately exposed to a host of microorganisms. If it is to survive, the newborn animals must be able to control microbial invasion within a very short time. In practice, the immune system is unable to get off to a very rapid running start by itself. In animals with a long gestation period (such as the major domestic animals), although the immune system is fully developed at the time of birth, it is unreasonable to expect it to function at full adult levels for at least several weeks. The complete development of immune capability is dependent on antigenic stimulation. The development of antigen-sensitive cells depends on antigen driven cell multiplication. Thus newborn mammals and birds are highly vulnerable to invasion for the first few weeks of life. They need assistance in defending themselves at this time. This temporary help is provided by the mother in the form of antibodies and possible T cells (Amanda et al 2011). The passive transfer of immunity from mother to newborn is essential for survival. Knowledge of transfer of antibodies from the dam to the calves is therefore vital for protection of ruminant stock and prevention of endemic infectious diseases' infection (Weaver, 2000). Objective of the study was to determine the trend of passive antibody transfer to the calves as they feed on colostrum and milk from dams immunized with Salmonella antigen. By this study, it would be possible to know when active vaccination against such diseases would be conducted

MATERIAL AND METHODS

Materials for this study were obtained through collaboration with the Federal Institute for Public Health Protection of consumers and Veterinary Medicine, Berlin Germany

Indirect ELISA in measuring passive transfer of AB to calves

Control sera, positive and negative

Peroxidase (PO) conjugated IgGfraction from experimental animals was immunized against IgG from relevant animals. After reconstitution of the freeze dried conjugate, it was further diluted 1:10 and stored in 0.5 ml amounts using glass bottles at -20°C

Test sera

Test sera were obtained from calves whose dams were vaccinated with salmonella antigen

Substrate indicator systems

80 mg of PAHBA (5-amino-2-hydroxy-benzoic acid) was dissolved in 100 ml H_2O at 70°C in a dark bottle. After cooling, 10 ml was withdrawn and replaced by 10 ml of distilled water containing 50 µl of 30% H_2O_2 . The pH was adjusted to 6 by drop wise addition of 1N NaOH

Indirect antiglobulin procedure for measuring antibodies

Antigen at working dilution (1:200) in carbonate buffer was placed into cups of alternate rows 2,4,6, and so on. The remaining cups were charged with carbonate buffer, volume 50 µl. The plate was placed into a moist chamber at 37°C (incubator) for 1 hour.

The antigen was removed by flicking the plate, washing with tap water and subsequently soaking with PBS-T for five minutes. Test and control sera in single dilution (1:50) were two times double diluted in PBS-T i.e. in antigen containing and antigen free rows from A-H, Volume 50 µl, incubated for 30 min at 37°C, and washing with 3 PBS-T soaking periods. Anti-species Ig-PO-conjugate at working dilution in PBS-T (1:250 to 1:1000) was added in 50 µl amounts to all cups , incubated for 30 minutes at 37°C, washed and soaked again. Finally, 50 µl of freshly prepared substrate indicator system (PAHBA) was added to all cups, incubated for 30 min at 37°C. Positive reactions were indicated by a colour change from whitish to brown and the result was read visually butphotometer readings were recorded for analysis with a cut-off point of 0.465 (% antibody value of 100%).

RESULTS

Day 1 found no antibody in serum of calves. Only day 5 obtained a titre that could confer immunity to enteric diseases (average antibody titre of 1.02 and % value of 221.9%). It remained above 100% at day 87. After that day antibody titre dropped showing signs of need for active vaccination. Figures 1 and 2 clearly show the pattern of Antibody depletion in the calves of dams vaccinated with salmonella antigen.

Table 1. Average antibody titres and average percent antibody values days post calving.

Age of calf (day)	1	5	10	13	17	20	24	27	31	42	49
Av. Ab. titre	0.00	1.03	1.02	1.05	1.06	1.01	0.99	0.95	0.86	0.82	0.96
Av. % value	0.00	221.9	218.9	255.6	227.1	217.6	212.0	203.4	183.9	176.6	205.8
Age of calf (day)	56	64	77	87	119	140	Con 1	Con 2			
Av. Ab. titre	0.57	0.64	0.47	0.62	0.42	0.25	0.99	1.14			
Av. % value	122.1	136.8	100.0	134.2	91.2	54.4	212.0	224.3			

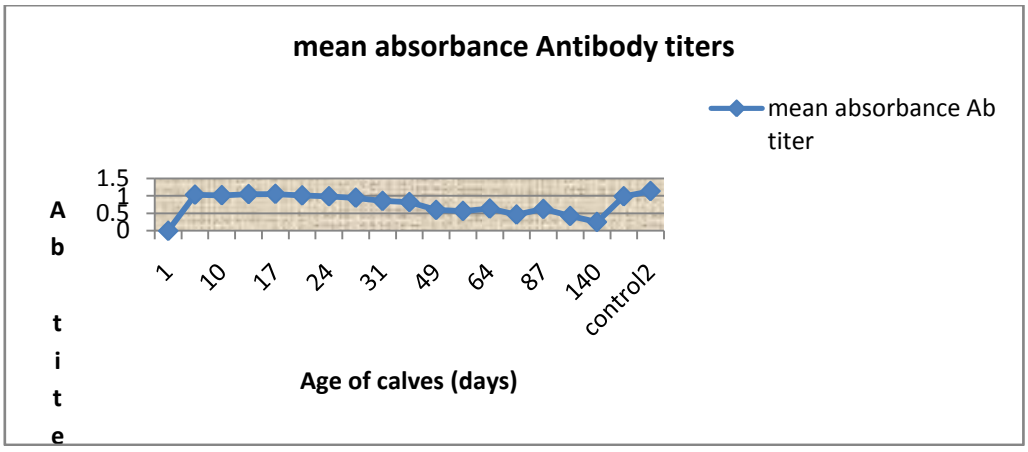

Figure 1. Antibody titre (% value) according to age.

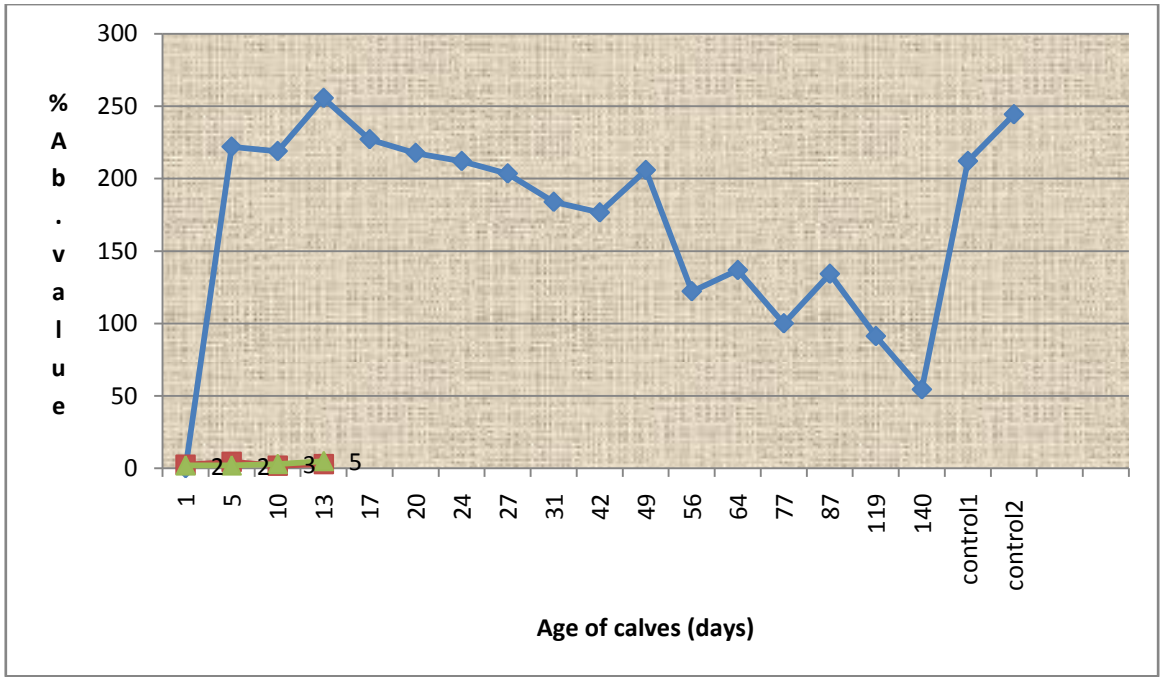

Figure 2. Percent Antibody value calculated from absorbance data.

Antibody titre was below the control until day 5 after calving when it equalized with the control that is an indication of immunity. It also shows how the special qualities of colostrum decline from the moment lactation starts.

DISCUSSION

The Development of the immune system

The results clearly show that the calf is born unprotected and thus points to the importance of feeding colostrum to calves immediately they are dropped. It agrees with the development of the immune system in the calf. The thymus is the primary lymphoid organ and the spleen and the lymph nodes are secondary. Development of these organs as immunoglobulin containing cells produce antibodies (later in foetal life), popularly described as cell mediated immune responses.

Secretion and composition of colostrum

Colostrum represents the accumulated secretions of the mammary gland over the last few weeks of pregnancy together with proteins actively transferred from the blood stream under the influence of estrogens and progesterone. This colostrum, the yellow and rather creamy liquid that takes the place of true milk for the first three or four days after calving has special qualities and is vitally important to the calf for these reasons: (i) laxative – with a high fat content and encourages the expulsion of the first faecal material (meconium) from the calf; (ii) protective-owing to the presence of readymade antibodies against certain ailments such as scour. According to Weaver et al (2000), during the first 24 hours of life, these are absorbed through the intestinal wall; (iii) nutritive – because it contains certain protein substances (phosphor-casein)readily available to the calf; and (iv) lastly it is fortifying, through its high vitamin A content (there is little or no vitamin A in a calf's liver when it is born (Norman, 2010; Campbell et al 2007).

As described by (Weaver et al 2000), the development of the immune system in the foetal calf is in this wise:

Day(s)	**Tissue/organ development**
40	Foetal thymus recognizable
41	Thymus
45	Peripheral Blood lymphocytes
56	Bone marrow, spleen
59	IgM-positive cells
60	Lymph nodes
90	Complement
110	Granulocytes
130	IgG
135	IgG- positive cells
145	Serum IgM
155	Tonsil
175	Peyers patches
Using highly sensitive VN tests	
73	Responded to rota virus
93	Responded to parvovirus
120	Responded to parainfluenza virus
75-80	Phytohemagglutinin, concanavalin A(conA) and pokeweed mitogen (PWM), temporarily lost at birth due to high serum steroid levels

Composition of colostrum and milk (Barron, 1990)

	Colostrum	Milk
IgA	100-700 (mg/dl)	5-11
IgM	300-1300	4-15
IgG	3400-8000	33-120

Absorption of IgG

Young animals that suckle soon after birth take colostrum into their intestinal tract. Thus, naturally suckled calves ingest an average of 2 litres of colostrum although individual calves can ingest as much as 6 litres. In these young animals, the level of proteolytic activity in the digestive tract is low and is further reduced by trypsin inhibitors in colostrum.

Therefore, Colostral proteins are not degraded and used as food source but instead reach the small intestine intact. Colostral immunoglobulin binds to a specialized Fc receptor on the intestinal epithelial cells of newborns (FcRn). This receptor is a MHC class Ib heterodimer containing a large α chain and B_2 – microglobulin. FcRn is probably found in all mammals and is very similar to the Fc receptor found on the yolk sac of chicken. Once bound on the FcRn ,immunoglobulins are actively taken up by epithelia cells through pinocytosin and are passed through these cells into the lacteals and possibly the intestinal capillaries.

Eventually, the absorbed immunoglobulin reaches the systemic circulation, and newborn animals thus obtain a massive transfusion of maternal immunoglobulin. The secretions of the mammary gland gradually change from colostrum to milk. Milk is rich in both Ig G and Ig A in ruminants. For the first few weeks of life, while proteolytic digestion is poor, the immunoglobulins can be found throughout the length of the intestine and the faeces of young animals. As the digestive ability of the intestine increases, only secretory IgA (Sig A) molecules are left intact eventually. Sig A is therefore the most important factor that protects them against enteric infection. The amount of Ig A in the intestine can be relatively large. For instance, a 3- week-old piglet may receive 1.6 g daily from the sow's milk (Shea et al, 2009)

Failure of passive transfer (FPT)

The initial absorption of IgG from colostrum is required for the protection of a young animal against septicaemia disease. The continuous intake of IgA or IgG1 from milk is required for protection against enteric disease. Failure of either of these processes predisposes a young animal to infection. In addition, for reasons unknown, colostrum deprived lambs are neutropenic and their few neutrophils are relatively inefficient at phygocytosis compared with neutrophils from colostrum fed milk. The inflammatory responses of these animals are depressed (Amanda et al, 2011).

There are three major reasons for the failure of adequate colostrum transfer

1. Production failure - the mother may produce inefficient or poor quality colostrum
2. Ingestion failure- there may be sufficient colostrum produced, but inadequate intake by the new born animal
3. Absorption failure – there may be failure of absorption from the intestine despite an adequate intake of colostrum

In this study day 1 found no antibody in serum of calves. Only day 5 obtained a titre that could confer immunity to enteric diseases (average antibody titre of 1.02 with a % value of 221.9%). It remained above 100% at day 87. After that day antibody titre dropped showing signs of need for active vaccination. Figures 1 and 2 clearly show the pattern of Antibody depletion in the calves of dams vaccinated with salmonella antigen.

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions were made for this study: based on the results from the calves' sera, an Ig concentration of 0.624 absorbance antibody titre with a % value of 134.19 marked the end of the less risky period for the calves in terms of immunity and this fell on the 87th day after calving. It was recommended that the calves receive active vaccination after the 87th day of calving.

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