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Persistence of Maternal Antibodies In Calves Born to Dams Naturally Infected With *Bovine Ephemeral Fever Virus*

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Bovine ephemeral fever virus (BEFV) is an arthropod born *rhabdovirus*, which causes disabling febrile infection among cattle and water buffalo. The disease is characterized by inflammation of mesodermal tissue as achieved by muscular shivering, stiffness, lameness and enlargement of the peripheral lymph nodes. Recently, the disease caused several outbreaks during 2000, 2001 and lastly 2004. The persistence of immunity after natural infection is varied from 6 months to several years. Assessment of maternal antibodies in calves born to naturally infected dams is a matter of interest in order to determine the proper time of vaccination. Twenty-five pregnant Frisian cows in the last stage of pregnancy were naturally infected with BEFV during the Egyptian outbreak in summer 2004. Four of them were aborted and excluded from experimental. The disease was diagnosed clinically and confirmed by the detection of BEFV antigen by immunoperoxidase staining. The aim of the present study was to determine the efficiency of transfer of BEFV antibodies from naturally infected cows to newborn calves through colostrums. According to the level of serum neutralizing antibodies (SNA), the cows were allotted into three groups. In the first group (n = 9), the mean SNA in naturally infected cows was 46.2, which gave a mean titer neutralizing antibodies (NA) of 28.4 in their colostrums. Calves fed on these colostrums had a mean SNA of 12.2, 6.9 and 2 after one week, one month and 2 months, respectively. In the second group (n = 7), the mean SNA in naturally infected cows was 12.6, which gave a mean neutralizing antibodies (NA) titer of 7.7 in their colostrums. Calves fed on these colostrums had a mean SNA of 3.7, 0.9 and 0.0 after one week, one month and 2 months, respectively. In the third group (n = 5), the mean SNA in naturally infected cows was 2.4, which gave a mean titer neutralizing antibodies (NA) of 0.8 in their colostrums. Calves fed on these colostrums had a mean SNA of 0.4, 0.0 and 0.0 after one week, one month and 2 months, respectively. It could be concluded that: 1) The level of SNA in the pregnant cows determines the level of NA in their colostrums. 2) The level of SNA in calves dependent on the level of NA in the colostrums taken beside the general rules of transfer of maternal immunity through colostrums as for example the quantity and proper time of colostrums intake. 3) The assessment of SNA in calves is very essential to determine the proper time of vaccination especially after natural outbreak. This point needs further investigation.

INTRODUCTION

Bovine ephemeral fever virus (BEFV) is an arthropod born rhabdovirus, which causes disabling febrile infection among cattle and water buffalo. The disease is characterized by inflammation of mesodermal tissue as achieved by muscular shivering, stiffness, lameness and enlargement of the peripheral lymph nodes (Radostitis *et al.* 2000). During summer 2000, a severe outbreak of BEF was recorded in Egypt. Clinically the disease occurred suddenly in both foreign and native breeds of cattle with severe economic losses (Zaghawa *et al.*, 2000). The virus is mainly transmitted by insect especially Culicoides (Yeruham *et al.*,

2003). Recently the bovine ephemeral fever virus (BEFV)-induced apoptosis and involved viral molecules was demonstrated in several cell lines (Chang *et al.*, 2004)

The route by which maternal antibodies reach the fetuses is determined by the type of the placenta. The placenta of ruminants is syndesmochorials in which the transplacental passage of immunoglobulin molecules is totally prevented and their newborn are entirely dependent on antibodies received through the colostrums (Tizard, 2000). Many reasons for the failure of adequate colostral transfer are possible. One occasion colostrums may be abnormally low in IgG, inadequate colostral intake by the newborn animals, poor mothering, improper time of colostral intake (to avoid proteolytic activity of newborn intestine and intestinal permeability) (Tizard, 2000).

The variation of maternal antibodies in calves is a reflection antibodies of dams colostrums at time of parturition and this is correlated with time of infection of dams prior to calving and their immune response as well as the level of colostral uptake (El-Naggar, 2003).

Calves less than 6 months old are not affected by the natural BEFV infection. Calves as young as three months are as susceptible as adults to experimental infection. Newborn colostrums deprived calves are susceptible to infection (Radostitis *et al.* 2000).

In endemic areas colostrally acquired antibodies may play a role in the Epidemiology of the disease (Kahrs, 1981).

The aim of the present study was to determine the efficiency of transfer of BEFV antibodies from naturally infected cows to newborn calves through colostrums.

MATERIALS AND METHODS

1) Experimental design.

Twenty five pregnant Fresian cows naturally infected with BEFV, during summer 2004 outbreak in Egypt, were used. Four animals were aborted and therefore they excluded from the experiment. Serum samples were collected from pregnant cows one week before parturition and their colostrums directly after parturition as well as sera of their suckling calves.

1) Serum samples:

Serum samples were collected from 21 pregnant cows one week before parturition and from their suckling calves one week, one month and 2 months after birth. Sera were used for detection of BEFV antibodies.

2) Colostrums:

Colostrums samples were collected directly after parturition from twenty one naturally infected dams with BEF virus. The colostrums samples were centrifuged after removal of the cream layer and the supernatant was used for detection of BEFV antibodies (Zaghawa, 1997).

3) Detection of BEFV antigen:

Heparinized blood samples were collected from febrile animals during BEF outbreak. The buffy coat samples were separated and washed three times with phosphate buffered saline (Nagano *et al.*, 1990). Films were prepared from buffy coat and used for direct detection of BEF virus antigen by immunoperoxidase technique.

4) Titration of antibodies to BEFV:

Serum neutralizing antibody titers were determined by a constant virus-varying serum, microtitre plate technique using 100 TCID50 of Vero-cell-culture-adapted BEFV and two fold serum dilutions (Young and Spradbrow, 1990).

5) Statistical analysis

Data were transformed as log x + 1.5 to treat the non homogenous variances. Log geometric mean, one way ANOVA and multiple regressions were analyzed according to Glover and Mitchell (2002).

RESULTS AND DISCUSSION

Twenty-five pregnant Frisian cows in the last stage of pregnancy were naturally infected with BEFV during the Egyptian outbreak in summer 2004. Four of them were aborted and they were excluded from the experiment. The disease was diagnosed clinically by presence of fever, stiffness, lameness, muscular shivering and some of them showed subcutaneous emphysema. A sever outbreak of BEF was observed in Egypt in summer 2000.The disease is characterized clinically by fever, stiffness, lameness, dyspnea, abortion, subcutaneous emphysema and recumbency.

The diagnosis was confirmed by the detection of BEFV antigen by immunoperoxidase staining in buffy coat films from feverish animals. The immunoperoxidase staining is a rapid, simple and specific technique for detection and identification of BEF virus antigen (Khaliel *et al* .2002).

The preliminary titration of BEF virus neutralizing antibodies in naturally infected dams allow us to divide the animals into three groups according to the level of the neutralizing antibodies as shown in tables (1, 2 &3). Table (1) showed the individual neutralizing antibody titers (NA) of the 1st group in serum and colostrums of dams after natural infection with BEF virus and serum of their suckling calves In this group (n = 9), the mean SNA in naturally infected cows was 46.2, which gave a mean neutralizing antibodies titer (NA) of 28.4 in their colostrums. Calves on fed these colostrums had a mean SNA of 12.2, 6.9 and 2 after one week, one month and 2 months, respectively.

Table (2) showed the individual neutralizing antibody titers (NA) of the 2nd group in serum and colostrums of dams after natural infection with BEF virus and serum of their suckling calves In this group (n = 7), the mean SNA in naturally infected cows was 12.6, which gave a mean neutralizing antibodies titer (NA) of 7.7 in their colostrums. Calves fed on these colostrums had a mean SNA of 3.7, 0.9 and 0.0 after one week, one month and 2 months, respectively.

The results presented in table (3) revealed the individual neutralizing antibody titers (NA) of the 3rd group in serum and colostrums of dams after natural infection with BEF virus and serum of their suckling calves In this group (n = 5), the mean SNA in naturally infected cows was 2.4, which gave a mean neutralizing antibodies titer (NA) of 0.8 in their colostrums. Calves fed on these colostrums had a mean SNA of 0.4, 0.0 and 0.0 after one week, one month and 2 months, respectively.

Before statistical analysis the data were transformed as log x + 1.5 to treat the non homogenous variances. Log geometric mean, one way ANOVA and multiple regressions were analyzed according to Glover and Mitchell (2002).

The results presented in table (4) showed the geometric means of SNA titers of dams and newborns. Table (5) showed the regression coefficients of the dependent variable (titer in

colostrums, serum of newborns at one week, one month and two months) on the dependent variable (titer in the dam's serum) as well as intercepts. Table (6) showed the regression coefficients of the dependent variable (titer in serum of new born at one week, one month and two months) on the dependent variable (titer in the colostrums) as well as intercepts.

The presented data (Tables 1, 2&3) as well as the statistical analysis (Tables 4, 5&6) indicated a strong relation between the level of antibodies in the dam sera, their colostrums and the sera of their newborn calves. The route by which maternal antibodies reach the fetuses is determined by the type of the placenta. The placenta of ruminants is syndesmochorials in which the transplacental passage of immunoglobulin molecules is totally prevented and their newborn are entirely dependent on antibodies received through the colostrums (Tizard, 2000).

The presented data (Tables 1, 2&3) as well as the statistical analysis (Tables 4, 5&6) showed that the level of the neutralizing antibodies in the newly born calves was significantly decreased after 2 months from colostrums intake. Such finding is supported by Radostitis *et al.* (2000), as they stated that calves as young as three months are as susceptible as adults to experimental infection and newborn colostrums deprived calves are susceptible to infection. Furthermore, Kahrs (1981) recorded that in endemic areas colostrally acquired antibodies may play a role in the epidemiology of the disease. The variation of maternal antibodies in calves is a reflection of the level of antibodies of dams colostrums at time of parturition and this is correlated with time of infection of dams prior to calving and their immune response as well as the level of colostral uptake (El-Naggar, 2003).

Two out of 9 newborn calves showed a low serum neutralizing antibodies although their dams and colostrums had high neutralizing antibodies. These results indicated a failure of antibody transfer from cows to newborns through colostrums (table 1). Many reasons for the failure of adequate colostral transfer are possible such as inadequate colostral intake by the

Table (1): Individual neutralizing antibody (NA) titers of the 1st group in sera and colostrums of dams after natural infection with BEF virus and sera of their suckling calves

Serial number	NA in dam serum	NA in Colostrums	NA in calf 1 week old	NA in calf 1 month old	NA in calf 2 month old
1	64	48	4	2	0
2	32	16	8	6	0
3	64	32	16	12	4
4	64	48	24	16	8
5	32	24	16	8	2
6	32	16	8	4	0
7	64	32	24	12	4
8	32	16	2	0	0
9	32	24	8	2	0
Mean	46.2	28.4	12.2	6.9	2

Table (2): Individual neutralizing antibody titers (NA) of the 2nd group in sera and colostrums of dams after natural infection with BEF virus and sera of their suckling calves

Serial number	NA in dam serum	NA in Colostrums	NA in calf 1 week old	NA in calf 1 month old	NA in calf 2 month old
1	16	12	2	0	0
2	8	4	2	0	0
3	16	8	2	0	0
4	16	8	6	2	0
5	8	4	2	0	0
6	8	6	4	2	0
7	16	12	8	2	0
Mean	12.6	7.7	3.7	0.9	0

Table (3): Individual neutralizing antibody titers (NA) of the 3rd group in sera and colostrums of dams after natural infection with BEF virus and sera of their suckling calves

Serial number	NA in dam serum	NA in Colostrums	NA in calf 1 week old	NA in calf 1 month old	NA in calf 2 month old
1	2	0	0	0	0
2	2	2	2	0	0
3	4	2	0	0	0
4	2	0	0	0	0
5	2	0	0	0	0
Mean	2.4	0.8	0.4	0	0

Table (4): Geometric means of neutralizing antibody titers to BEF virus in sera and colostrums of dams and sera of their newborns.

Titer in	Log Geometric Mean ± SD	Geometric Mean
Dam's serum	1.224±0.45	16.75 a
Colostrums	0.995±0.47	9.89 ab
One week old calves	0.757±0.38	5.71 b
One month old calves	0.504±0.38	3.19 c
Two-month old calves	0.285±0.241	1.92 c

Means having different super scripts are significantly different ($P < 0.05$)

Table (5) Regression coefficients of the dependent variable (titer in colostrums, serum of newborns at one week, one month and two months) on the dependent variable (titer in the dam's serum) as well as intercepts.

	Dependent variable			
Independent variable	Colostrums	Serum One week	Serum One month	Serum Two month
	b ± SE	b ± SE	b ± SE	b ± SE
Dam's serum	1.00±0.06*	0.696±0.10*	0.639±0.121*	0.304±0.099*
R^2	.92	.70	.59	.33
Intercept (a)	-0.23	-0.10	-0.23	-0.13

* significant at $P < 0.05$

Table (6) Regression coefficients of the dependent variable (titer in serum of new born at one week, one month and two months) on the dependent variable (titer in the colostrums) as well as intercepts.

	Dependent variable		
Independent variable	Serum One week	Serum One month	Serum Two month
	b ± SE	b ± SE	b ± SE
Colostrums	0.662±0.10*	0.57±0.13*	0.260±0.10
R^2	0.68	0.52	0.26
Intercept (a)	0.09	-0.07	0.03
* significant at P < 0.05			

newborn animals, poor mothering and improper time of colostral intake. The late colostrums intake adversely affect the transfer of immunoglobulins through the action of the proteolytic activity of newborn intestine and intestinal impermeability (Tizard, 2000).

It could be concluded that:

1) The level of SNA in the pregnant cows determines the level of NA in their colostrums.

2) The level of SNA in calves dependent on the level of NA in the colostrums taken beside the general rules of transfer of maternal immunity through colostrums as for example the quantity and proper time of colostrums intake.

3) The assessment of SNA in calves is very essential to determine the proper time of vaccination especially after natural outbreak. This point needs further investigation.

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