PERSISTENCE OF COLOSTRAL ANTIBODIES AGAINST VIRUS-INFECTION-ASSOCIATED (VIA) ANTIGEN AND VIRUS NEUTRALIZING ANTIBODIES INDUCED BY NATURAL INFECTION WITH FOOT-AND-MOUTH DISEASE VIRUS IN INDIAN BUFFALO (*BUBALUS BUBALIS*) CALVES

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SAMARA, S.I.; PINTO, A.A.; ABUHAD, T.G.; KOSEKI, I.; GONÇALVES, E.I.; MANO FILHO, A.C. Persistence of colostral antibodies against Virus-Infection-Associated (VIA) antigen and virus neutralizing antibodies induced by natural infection with foot-and-mouth disease virus in indian buffalo (Bubalus bubalis) calves. Rev.Fac.Med.Vet.Zootec.Univ.S.Paulo, 24(1):35-40, 1987.

SUMMARY: Antibody against virus-infection-associated (VIA) antigen and neutralizing serum antibody to foot-and-mouth disease virus were studied in 32 non vaccinated Indian buffalo calves. Antibody levels against VIA antigen were detected by double immunodifusion in 76.5% of the animals at 3 ± 1/2, 30% at 4 ± 1/2 and 6.2% at 5± 1/2 months of age. Antibody against VIA antigen was no longer detectable at 7 ± 1/2, 8 ± 1/2 and 9 ± 1/2 months of age. Neutralizing serum antibody levels were studied in 12 of 32 animals. At 3 ± 1/2 months of age neutralizing serum antibody for O_1, A_{24}, and C_3 virus were found respectively in 83.3%, 83.3% and 66.6% of samples. At 5 ± 1/2 months of age neutralizing serum antibody was present against the three types only in 20% of the animals and was not detected at 7 ± 1/2 months of age. Twenty one days after the outbreak in cattle when the buffalo calves were 12 ± 1/2 months old antibody against VIA antigen was again detected in 20 of 32 (62.6%) of the animals. Neutralizing serum antibody was found in up to 63.3% of animals against type A and 16.6% against type O_1 and C_3.

UNITERMOS: FMD, Indian Buffaloes; Antibodies against VIA, neutralizing antibodies

INTRODUCTION

In 1966, COWAN & GRAVES, 5 described virus-infection-associated (VIA) antigen in foot-and-mouth disease (FMD) showing that this antigen is virus specific, but not type specific. Early investigations using double immunodiffusion test for VIA antigen provided a rapid and accurate method of differentiating animals immunised by inactivated vaccine from those which had experienced infection (McVICAR & SUTMOLLER, 12, 1970; FERNANDEZ et alii, 6, 1975; ROSENBERG et alii, 15, 1977; PINTO & HEDGER, 14, 1978; PINTO & GARLAND, 13, 1979)

Foot and mouth disease virus (FMDV) has been maintained in free-living African buffalo (Syncerus caffer) and in water buffalo (Bubalus bubalis) in the absence of observable clinical signs (CONDY et alii,4, 1969; HEDGER et alii, 8, 1969; HEDGER et alii, 9, 1972; JEREZ et alii, 10, 1979; SAMARA & PINTO, 17, 1983; HEDGER & CONDY, 7, 1985)

In Brazil, antibody studies have shown that FMDV is widespread not only in cattle and pigs but also in water buffalo (JEREZ et alii, 10, 1979; SAMARA et alii, 18, 1981; SAMARA & PINTO, 17, 1983)

Serum antibodies to VIA antigen induced by infection with FMDV can be detected in cattle up to 490 days after inoculation with the virus (FERNANDEZ et alii, 6, 1975), similar persistence also occurrs in the African buffalo (PINTO & HEDGER, 14 , 1978)

Colostral antibodies to VIA antigen persist up to 180 days of age in African buffalo calves born from mothers naturally infected with FMDV, while serum neutralizing antibodies are detectable up to 300 days (PINTO & HEDGER, 14, 1978)

The present study was undertaken to provide information on those topics for the Indian buffalo (Bubalus bubalis) and specifically to determine the persistence of antibodies to VIA antigen and serum neutralizing antibodies.

MATERIAL AND METHODS

1. Animals

The experiment was carried out on a farm with cattle* and buffaloes* located in the township of Gusolândia, State of São Paulo, Brazil. Thirty two young buffaloes born to mothers several times vaccinated and in which infection has not been confirmed either clinically or by virus isolation, were bled systematically at 3 ± 1/2 months of age (1st bleeding), 4 ± 1/2 months (2 nd), 5 ± 1/2 months (3rd), 7 ± 1/2 months (4th), 8 ± 1/2 months (5th), 9 ± 1/2 months (6th) and 12 ± 1/2 months of age (7th bleeding)

The 7th and final bleeding was carried out 21 days after the discovery of the first clinical case of foot-and-mouth disease due type A virus in cattle on this farm. The clinical status, the aspects of the detection of foot-and-mouth disease carriers and virus infection associated (VIA) antibody among adult and young water buffalo after an outbreak of the disease in cattle have been described (SAMARA & PINTO, 17, 1983)

Viral Assay

Foot-and-mouth disease virus (type A) were isolated from cattle epithelial tissue and from oesophageal-pharyngeal fluid sample collected from 2 out of 10 randomly selected female buffaloes during an outbreak of the disease previously described (SAMARA & PINTO, 17, 1983). This virus was used for experimental studies in serum neutralisation test.

Serological identification of the FMDV sample isolated was done by the complement fixation reaction according to the technique of CAMARGO et alii, 1 (1950).

Via Antibody test

The tests were carried out by double immunodiffusion on agar gel as previously described by McVICAR & SUTMOLLER, 12 (1970) and as modified by PINTO & HEDGER, 14 (1978).

The VIA antigen used for all tests was prepared from FMDV type O_1 Campos grown in BHK 21 cells and the procedure used has been described by LOBO et alii, 11 (1974) with some modification (PINTO & HEDGER, 14, 1978)

Serum Neutralizing Antibody

The sera of 12 of the 32 buffaloes selected at random were screened for serum neutralizing antibodies in order to determine the immune status of these animals. The samples collected at 3 ± 1/2, 5 ± 1/2, 7 ± 1/2 and 12 ± 1/2 months of age were tested against the viral strains O_1 Campos, A_{24} Cruzeiro and C_3 Indaial, as well as the strains A buffalo (Virus isolated from buffalo oesophageal pharyngeal fluid) and A Cattle (Virus isolated from cattle lingual epithelium during the course of this outbreak), all adapted to grow in monolayers of the IB-RS-2 Clone 26-3 cell line (CASTRO, 3, 1972). Sera were diluted 1:100 in Hanks physiological saline and mixed with an equal volume of an appropriate viral dilution containing approximately 100 plaque forming units of virus. The serum-virus mixtures were mixed and incubated at 37º C for 30 minutes and then inoculated into flaskes containing cell cultures held in a incubator at 37ºC for one hour. The preparations were then washed twice Hanks physiological saline and the cell monolayers covered with tris agar medium. The procedure continued as previously described by CASTRO, 2 (1970)

The degree of serum neutralization was determined by counting the number of plaque forming units which developed. Sera that induced the neutralization of 50 to 100 per cent and 0 to 49 per cent of plaque forming units produced by the virus were considered positive (+) and negative (-) respectively.

RESULTS

Data concerning the anti-VIA antigen antibodies detected in the sera of 32 young buffaloes from 3 ± 1/2 to 12 ± 1/2 months of age are presented in Tab. 1. The VIA test was positive in 23 out of 30 animals (76.6 per cent) at 3 ± 1/2 months of age, in 9 out of 30 animals (30.0 per cent) at 4

* Animals vaccinated against foot-and-mouth disease with commercial vaccines prepared with strains 0_1 Campos, A_{24} Cruzeiro, A Venceslau and C_3 Indaial, according to the scheme provided by the Ministry of Agriculture of Brazil.

± 1/2 months of age, and in 2 of 32 animals (6.2 per cent) at 5 ± 1/2 months of age. Only one animal (N. 116) which was positive to the VIA test at 3 ± 1/2 months and which became negative at 4 ± 1/2 months, showed antibody against VIA antigen again at 5 ± 1/2 months, and become negative thereafter. Animal N. 112 become negative at 3 ± 1/2 months, and turned positive at 4 ± 1/2 months as well as at 5 ± 1/2 months. No antibodies to VIA antigen were detected in any of the animals studied at 7 ± 1/2, 8 ± 1/2 and 9 ± 1/2 months of age. It should be pointed out that the 32 buffaloes had been vaccinated against foot-and-mouth disease 7 days before the bleeding performed at 7 ± 1/2 months of age. No antibodies to VIA antigen were detected in these animals at that time or even at 8 ± 1/2 months of age, 40 days after vaccination. Antibodies were again detected in 20 out of 32 animals (62.5 per cent) at 12 ± 1/2 months of age, or more precisely at 21 days after an outbreak of foot-and-mouth disease caused by type A virus. Clinical manifestations were observed during this outbreak only among the cattle raised on this ranch, as previously described by SAMARA & PINTO, 17 (1983)

Tab. 2 sumarizes the qualitative data for the presence of serum neutralizing antibodies in 12 samples collected from the 32 buffalo calves studied. Of the sera from of 12 animals examined at 3 ± 1/2 months of age, 10 (83.3 per cent) exhibited antibodies against type O_1 Campos virus, 10 (83.3 per cent) against type A_{24} Cruzeiro virus, 10 (83.3 per cent) against the type A buffalo viral strain, 8 (66.6 per cent) against the cattle type A viral strain, and 8 (66.6 per cent) against type C_3 Indaial virus. When 5 buffalo calves were examined at 5 ± 1/2 months of age, only 1 (20 per cent) exhibited antibodies against all the viral strains utilized in the experiment. When 4 buffaloes were examined at 7 ± 1/2 months of age, none was found to have significant levels of antibodies against the viral strains used. Twelve sera of buffalo calves were examined at 12 ± 1/2 months of age, when 21 days had elapsed after the beginning of an outbreak of FMD among the cattle raised on the same ranch. Two of these 12 animals (16.6 per cent) showed antibodies against type O_1 virus, 7 of 12 (58.3 per cent) against type A(24), 7 of 11 (63.3 per cent) against the type A buffalo viral strain, 7 of 11 (63.3 per cent) against the cattle type A viral strain, and 2 of 12 (16.6 per cent) against type C_3

DISCUSSION

In these experiment the presence of antibody against virus infection associated (VIA) antigen were detected in 76.6% of the animals at 3 ± 1/2 months of age, decreasing to 30.0% at 4 ± 1/2 months and to 6.2% at 5 ± 1/2 months (Tab. 1). From 7 ± 1/2 months to 9 ± 1/2 months, antibody against VIA antigen was not detected in any animals when tested by double immunodiffusion on agar gel. As soon as these animals contracted inapparent infection at 12 ± 1/2 months old, VIA antibody was again detected in 62.5% of them. The presence of VIA antibody 21 days after the FMDV outbreak among the cattle confirms the occurence of viral replication (COWAN & GRAVES, 5, 1966; ROWLANDS et alii, 16, 1969) and consequently inapparent infection among the buffalo calves apparently healtly, which then become carriers, as reported by, McVICAR & SUTMOLLER, 12 (1970); PINTO & HEDGER, 14 (1978); PINTO & GARLAND, 13 (1979) and SAMARA & PINTO, 17 (1983). In a similar study of waning maternal immunity in only six uninfected African buffalo calves (Syncerus caffer) from an infected herd, antibody to VIA antigens was detected for periods of up to 6 months after birth (PINTO & HEDGER, 14, 1978). Indeed, there was a good agreement among the results of the VIA test in boths species, Bubalus bubalis and Syncerus caffer.

Serum samples from 12 animals selected at random from the 32 buffaloes were analyzed in order to quantify the presence of serum neutralization antibodies against the viral types O_1 Campos, A_{24} Cruzeiro, C_3 Indaial and against the type A virus isolated from 2 of 10 samples of oesophageal-pharyngeal fluid samples recovered from adult buffaloes taken 21 days after an outbreak of FMD in cattle and from the lingual epithelium of cattle during the outbreak of the disease in the latter species. The results revealed the presence of high titres of antibodies, probably originating from colostrum, against all viral strains used in 66.6% to 83.3% of all animals at 3 ± 1/2 months of age, with a gradual decrease over the subsequent months (Tab. 2). Thus at 5 ± 1/2 months, only one of the calves showed serum neutralization antibodies, and at 7 ± 1/2 months the serum neutralization test was negative for all animals.

We should not rule out the possibility that the neutralizing antibodies induced following multiple vaccination of the dams were transfered to the calves via colostrum.

Twenty-one days after the outbreak of FMD among the cattle on this farm, when the buffalo calves were 12 ± 1/2 months old, a marked increase in serum neutralization antibodies against the type A_{24} Cruzeiro virus (58.3% of all animals) was observed, as well as against the type A virus isolated from the oesophageal-pharyngeal

fluid of buffaloes (63.3%) and the lingual epithelium of cattle (63.3%), a fact that strongly supports the initial assumption that these animals had been infected in a non-apparent form during the outbreak, thus becoming carriers of type A FMDV as showed by SAMARA & PINTO, 17 (1983). Only two of these buffaloes had antibodies against type O and type C viruses, possibly owing to the single vaccination they had received 7 days before sampling at 7 ± 1/2 months of age.

Finally, these observations demonstrate that most buffalo calves are completely susceptible to the different types of FMDV during the next months when non-apparent infection may occur and the animals may become asymptomatic carriers.

SAMARA, S.I.; PINTO, A.A.; ABUHAB, T.G.; KOSEKI, I.; GONÇALVES, E.I.; MANO FILHO, A.C. Persistência de anticorpos colostrais anti-antigeno via ("Virus-Infection - Associated") e anticorpos soroneutralizantes induzidos por infecção natural pelo virus da febre aftosa em bezerros búfalos Indianos (Bubalus bubalis). Rev. Fac. Med. Vet. Zootec.Univ.S.Paulo, 24(1):35-40, 1987.

RESUMO: Anticorpos anti-antigeno VIA e anticorpos soroneutralizantes foram estudados em 32 bezerros búfalos indianos não vacinados, a partir de 3 ± 1/2 meses de idade até 1 ano. Esses anticorpos foram detectados até aos 5 ± 1/2 meses de idade dos animais, e não mais o eram nas maiores idades, até sofrerem infecção natural do virus da febre aftosa, o que ocorreu aos 12 ± 1/2 meses de idade. O significado destes achados em relação à susceptibilidade dos bubalinos a essa doença é discutido.

UNITERMOS: Febre aftosa, búfalos indianos; Anticorpos anti-antigeno VIA; Anticorpos suroneutralizantes

TABLE 1 – Antibody response to VIA antigen in sequential samples of serum taken from 32 buffalo calves, Guzelândia, SP, 1980-1981.

Animal nº / Age in months	3 ± 1/2 months	4 ± 1/2 months	5 ± 1/2 months	7 ± 1/2 months[a]	8 ± 1/2 months	9 ± 1/2 months	12 ± 1/2 months[b]
101	+	–	–	–	–	–	–
102	+	–	–	–	–	–	+
103	–	+	–	–	–	–	+
104	+	+	–	–	–	–	+
105	+	–	–	–	–	–	+
106	+	–	–	–	–	–	+
107	–	+	–	–	–	–	+
108	–	–	–	–	–	–	+
109	+	–	–	–	–	–	+
110	–	+	–	–	–	–	–
111	+	+	–	–	–	–	+
112	–	+	+	–	–	–	+
113	+	–	–	–	–	–	+
114	+	–	–	–	–	–	+
115	+	–	–	–	–	–	–
116	+	–	+	–	–	–	–
117	+	–	–	–	–	–	+
118	+	–	–	–	–	–	+
119	+	–	–	–	–	–	–
120	+	–	–	–	–	–	+
121	+	+	–	–	–	–	+
122	+	–	–	–	–	–	–
123	–	–	–	–	–	–	–
124	–	+	–	–	–	–	–
125	+	–	–	–	–	–	+
126	+	–	–	–	–	–	+
127	+	–	–	–	–	–	+
128	+	–	–	–	–	–	–
129	+	–	–	–	–	–	–
130	+	+	–	–	–	–	–
131	NT	NT	–	–	–	–	–
132	NT	NT	–	–	–	–	+
Total	23/30[c]	9/30	2/32	0/32	0/32	0/32	20/32
Percentage (%)	76.6	30.0	6.2	–	–	–	62.5

a – 7 days after vaccination with trivalent FMDV vaccine.
b – 21 days after an outbreak of FMD among cattle.
c – Numbre of serum samples positive for VIA antibody/number of serum samples examined.
+ – reagent.
– – no reagent.
N.T. – Not tested.

TABLE 2 – Summarized results of serum neutralizing antibodies against FMDV in buffalo calves at different ages, Guzelândia, SP, 1980–1981

Age in months / Animal Nº	FMDV type: O_1 Campos				A_{24} Cruzeiro				A Buffalo[a]				A Cattle[b]				C_3 Indaial			
	3±1/2	5±1/2	7±1/2	12±1/2	3±1/2	5±1/2	7±1/2	12±1/2	3±1/2	5±1/2	7±1/2	12±1/2	3±1/2	5±1/2	7±1/2	12±1/2	3±1/2	5±1/2	7±1/2	12±1/2
101	+	NT	NT	–	+	NT	NT	–	–	NT	NT	–	–	NT	NT	–	+	NT	NT	–
102	+	NT	NT	+	+	NT	NT	–	+	NT	NT	–	+	NT	NT	–	+	NT	NT	–
103	–	NT	NT	–	+	NT	NT	+	+	NT	NT	+	+	NT	NT	+	–	NT	NT	–
104	+	–	–	–	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+
105	+	–	–	–	+	–	–	+	+	–	–	+	+	–	–	+	–	–	–	–
106	+	NT	NT	–	–	NT	NT	–	–	NT	NT	+	–	NT	NT	+	–	NT	NT	–
107	+	NT	NT	–	+	NT	NT	+	+	NT	NT	+	–	NT	NT	+	+	NT	NT	–
108	+	–	NT	–	+	–	NT	+	+	–	NT	+	+	–	NT	+	+	–	NT	–
109	+	NT	NT	–	+	NT	NT	–	+	NT	NT	NT	+	NT	NT	NT	+	NT	NT	–
115	+	+	–	–	+	+	–	+	+	+	–	+	+	+	–	+	+	+	–	–
116	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
120	+	NT	NT	+	+	NT	NT	+	+	NT	NT	–	+	NT	NT	–	+	NT	NT	+
Total positive	10	1	0	2	10	1	0	7	10	1	0	7	8	1	0	7	8	1	0	2
Total examined	12	5	4	12	12	5	4	12	12	5	4	11	12	5	4	11	12	5	4	12
%	83.3	20.0	–	16.6	83.3	20.0	–	58.3	83.3	20.0	–	63.3	66.6	20.0	–	63.3	66.6	20.0	–	16.6

a – FMDV sample isolated from buffaloes during the outbreak in cattle on the same farm.
b – FMDV sample isolated from cattle during the outbreak.
\+ – 50% to 100% neutralization.
– – 0% to 49% neutralization.
NT – Not tested.

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Recebido para publicação em 27/08/86
Aprovado para publicação em 26/11/86