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Detection of Antibodies Against Camel Contagious Ecthyma in Sudan Using Passive Hemagglutination Test (PHT)

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Abstract: Antibodies against camel contagious ecthyma virus (CCEV) in camel sera were detected by passive hemagglutination test (PHT) with a mean antibody prevalence of 35%. The test revealed that the infection is widespread in all parts of the Sudan where camels are raised with variable prevalence rate. The antibody prevalence was 42% in Butana, 41 % in Darfor and 19 % in Blue Nile areas. The antibody prevalence was higher post rainy season (87.5%) compared to post rainy season (2.8%) confirming seasonality associated with the rainy season (June-October). The prevalence in age group 1-4 years was relatively higher (41%) in comparison with calves less than one year (32%) and adults (35%).

Key words: Camel contagious ecthyma-serology- passive hemagglutination test-Sudan

Introduction

Camel contagious ecthyma (CCE) is an infectious disease of camel, characterized by development of pustules and scabs around the mouth, lips, nostrils, eyelids, gingiva and sometimes all over the body. The causal virus is a DNA virus belonging to the parapoxvirus (PPV) genus of the family poxviridae. Camel contagious ecthyma is a sparsely studied disease. The disease, which is often misdiagnosed as camel pox, has been reported in Kazakhstan (Tulepbaev, 1971); Mongolia (Dashtseren *et al.*, 1984), Kenya (Munz *et al.*, 1986); Somalia (Moallin and Zessin, 1988); Libya (Azwai *et al.*, 1995) and Sudan (Ali *et al.*, 1991; Khalafalla *et al.*, 1994 and Khalafalla and Mohamed, 1997). The genus PPV genus is currently grouped into four members, Orf virus or PPV ovis, bovine popular stomatitis virus (BPSV) or PPV bovis 1, pseudocopox virus (PCPV) or PPV bovis 2 and PPV of red deer in New Zealand (PVNZ) (Inoshima *et al.*, 2001 and Büttner and Rziha, 2002).

In a previous publication (Khalafalla and Mohamed, 1997) we reported on the epizootiology of CCE in eastern Sudan. The majority of CCE infections occurs in young camels up to 3 years of age and 98% of them were in camel calves less than one year old. The mean morbidity and mortality rates in this age group were 60% and 9%, respectively. The disease in Sudan and probably else where had a marked seasonality associated with rainy season and camel skin abrasion caused by eating thorny acacia trees.

Reports on CCE antibody detection are scarce. Azwai *et al.* (1995) used parapoxvirus ovis (ORFV) in a sandwich ELISA for the detection of total and specific IgG and IgM antibodies in camel sera. The causative agent (CCEV) was first isolated in cell culture by our group (Khalafalla *et al.*, 1998). This report describes the development of a passive hemagglutination test (PHT) and the results of a serological survey for antibodies against the disease utilizing CCEV as antigen.

Materials and Methods

Collection of Blood Samples: Blood samples were collected from free-ranging camels of different age groups in Butana area of eastern Sudan (n = 69), Blue Nile area of central Sudan (n = 36) and Darfor area of western Sudan (n = 22). Sampling was done during February - May (pre- rainy season) and during Novemper - December (post-rainy season) between 1996 and 1997. All serum samples were warmed at 56°C for 30 minutes to inactivate complement just before being examined by PHT.

Antigen Preparation: The antigen for PHT was a cell culture lysate prepared from a local isolate of CCEV. The isolate (CE/SUD 3) was originally isolated in chick embryo kidney cell culture (CEK) as described previously (Khalafalla *et al.*, 1998). The virus undergone 3 passages in CEK cells and its titer was found to be 105.5 TCID /0.1 ml.

Control Positive and Negative Sera: Control positive serum was prepared in rabbits using an orf virus vaccine (Scabivax, Cooper Animal Health, UK) and the negative control serum was collected from un-inoculated rabbits (Khalafalla *et al.*, 1998).

Passive Hemagglutination Test Procedure: The test was performed as described by Scott and Maeda (1985), with some modifications. Briefly, blood was collected from healthy sheep into tubes containing EDTA, stored overnight

and then washes 3 times with phosphate buffered saline (PBS).
Five ml of 2.5 % RBC suspension in PBS were mixed with 0.6ml of 2.5 % gluteraldehide solution and 2ml of CCE antigen (2X104.5 TCID). Sensitization was allowed to proceed slowly at room temperature with gentle magnetic stirring at 100 rpm for 60 minutes. Sensitized cells were then washed thrice in PBS and finally suspended in PBS containing 0.3 % bovine serum albumin and 0.02 % sodium azide to give suspension of 0.6 % sensitized cells. Control cells were prepared in the same manner except antigen was omitted. Two-fold serial dilutions in duplicate of test serum in PBS were prepared in U- shaped microtiter plates. Two rows containing positive and negative rabbit sera were used included in each plate. Then 0.1 ml of the 0.6% sensitized RBCs were added to each well. The cells allowed settling at 4°C for 24 hours. The end point (titer) was determined as the highest serum dilution with complete or up to 50% agglutination.

Results and Discussion

The PHT used in the present study performed well with a titer of 1/256 in control positive rabbit serum and negative result in the negative rabbit serum. Besides, camels with known history of CCE gave positive results. Forty five samples (35.4%) out of 127 serum samples collected from Butana, Darfor and Blue Nile regions of the Sudan were found positive when examined by PHT. This indicated that the disease has a widespread nature and occur in most areas of the country where camels are raised but with variable prevalence rate and proving the existence of past or recent exposure to the virus. The prevalence was 42% in Butana, 41% in Darfor and 19 % in Blue Nile as shown in Table 1.
The antibody prevalence was higher in serum samples collected after the rainy season (87.5%) than in samples collected before the rainy season (2.7%) as shown in Table 2. This confirmed the seasonality of CCE infection and supports the findings of Khalafalla *et al.* (1997), who reported that the disease had a marked seasonality with 97% incidence rate in the rainy season.
Table 3 demonstrates that the prevalence of antibodies in young camels of one to four years old (41%) was highest when compared with adult (35%) and those camels less than one year old (32%). This is in accordance with the previous observations of Buchnev *et al.* (1987) and Khalafalla *et al.* (1994). According to Khalafalla and Mohamed (1997) the majority of CCE occurs in young camels up to 3 years and 98% of them were in camel calves less than one year old. The mean morbidity and mortality rates in this age group were 60% and 9%, respectively.
The prevalence of antibodies was higher in female (41%) than in male (25%).
Overall the result of the PHT revealed low antibody titer in the range 1 and 6 (log 2). Among the sera examined 13.3 %, 40.9%, 29.5%, 13.3% and 4.4% of the total positive serum samples showed antibody titers of 1, 2, 3, 4 and 5 (log 2), respectively. Also among the serum samples collected from camels in Butana region before rainy season 6.8%, 34%, 37.9%, 13.8% and 6.8% of the total sera examined showed antibody titer of 1, 2, 3, 4 and 5 (log 2), respectively. In Blue Nile area 71%, 14.3% and 14.3% of the total sera examined showed antibody titers of 1, 2 and 3(log 2), respectively. In Darfor region 44.4%, 33.3%, 11.1% and 11.1% of total sera examined showed antibody titer of 1, 2, 3 and 4 (log 2), respectively.
Table 4 demonstrated that in serum samples collected from young camels less than one year 40%, 40%, 10% and 10% showed antibody titer of 2, 3, 4 and 5 (log 2), respectively. While 27%, 27%, 36.4% and 9% of the total sera collected from young camels of the age group 1-4 years showed CCE antibody titers of 2, 3, 4 and 5 (log 2), respectively. Antibody titer of 1, 2, 3, and 4 were detected in 25 %, 45.8%, 25% and 4.1% of adult camel's sera of 5-20 years old, respectively.

Table 1: detection of antibody response to CCE virus in camel sera collected from different areas in the Sudan

Region	No. of tested sera	No.of positive sera	*Percent positivity
Butana	69	29	42%
Blue Nile	36	7	19%
Darfor	22	9	41%
Total	127	45	35%

*Sera having antibody titers of 1 (log 2) or more

Table 2: Detection of antibodies against CCE as measured by PHT in camel sera collected in the Sudan pre- and post rainy season

Region	No of tested sera	No. of negative sample	No. of positive sample	Positive percentage
Pre- rainy season	37	36 (97%)	1	2.7%
Post- rainy season	32	4 (12.5%)	28	87.5%
Total	69	40 (57%)	29	42%

*Sera having antibody titers of 1 (log 2) or more

Table 3: Detection of antibody response to CCE virus in sera collected from different age group

Age group	Total number of tested sera	No. of positive sample	*Positive percentage
0- 1 year old	31	10	32%
1-4 years old	27	11	41%
5-and older	69	24	35%
Total	127	45	35%

*Sera having antibody titers of 1 (log 2) or above

Table 4: Antibody titer against CCE as measured by PHT in camel sera collected in the Sudan

Tter age group	1 (log_2)	2 (log_2)	3 (log_2)	3 (log_2)	4 (log_2)
0-1 year	-	40%	40%	10%	10%
1-4 year	-	27%	27%	36%	9%
5-and older	25%	46%	25%	4 %	-

No significant correlation was detected between sero-positivity and region and sex. However, the study indicated indirect relationship between age and titer (Table 4). The decrease of antibody titer as the animal became older could be explained by the short-lived nature of antibodies against PPV (Haig *et al.*, 1997 and Czerny *et al.*, 1997). Various serological tests have been used for detection of antibodies against Orf in sheep. These included neutralization test (Nagigton and Whittle, 1961 and Poulain *et al.*, 1972), hemagglutination test (Sawheney, 1966) and passive hemagglutination test (Scott and Maeda (1985). It was evident from these reports that the production of antibodies against virus antigens is variable and probably depends on the degree of response of individual animals. In camels, only one report described the use of ELISA (Azawi *et al.*, 1995) for detection of anti-PPV antibody. The overall prevalence of ORFV reactive antibodies detected by that ELISA in 520 Libyan camels was 11.2%. Although ELISA is more sensitive than PHT but it requires expertise and special equipment and is much expensive if compared with PHT. Since the PHT developed and used in this communication is simple, rapid and giving clear distinction between positive and negative sera that match clinical observations it is therefore recommended for routine serological surveys and immune response of PPV infection in camels.

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