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Cutaneous bovine papillomatosis in the Sudan : detection of the group-specific virus antigen in warts from affected cattle

ABU ELZEIN (E. M. E.), TAGELDIN (M. H.), BAKHIET (H. A.), ABBASS (Z.). Papillomatose contagieuse bovine au Soudan : détection d'antigène au virus spécifique dans les verrues de bovins atteints. *Revue Elev. Méd. vét. Pays trop.*, 1988, **41** (1) : 41-43.

Cette communication rapporte la première identification du virus de la papillomatose bovine au Soudan. L'apparition de la maladie a touché 50 p. 100 des veaux âgés de 3 mois à 2 ans dans deux fermes de la province de Khartoum, Soudan. Les élevages d'animaux de races indigènes ou importées ont été touchés de façon égale. La maladie a été reproduite avec succès chez des veaux et l'antigène au virus spécifique a été détecté dans les verrues des animaux infectés, par la technique de péroxidase anti-péroxidase. *Mots clés* : Bovin - Papillomatose - Technique immunologique - Soudan.

INTRODUCTION

Bovine papillomatosis (BP) is a ubiquitous, host-specific infectious viral disease of cattle. Cutaneous and mucosal forms are known. The causal virus belongs to the family *Papovaviridae genus papillomavirus* (2). Nine virus types are so far identified (OLSON, personal communication). These are readily differentiated by characteristic restriction endonuclease cleavage patterns of their DNA and degree of polynucleotide sequence homology (2).

The disease may impose a threat to animal breeding and production when warts involve the udder or the reproductive organs of affected cattle.

In the Sudan, clinical bovine papillomatosis was described in 1982 (1), but no confirmation for presence of the virus was made. The present study was undertaken to examine whether viral bovine papillomatosis exists in the Sudan.

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MATERIALS AND METHODS

In November 1983, attention was drawn to outbreaks of cutaneous disease characterized by presence of warts involving young cattle aging three months to two years, of both exotic and local breeds in two farms at Khartoum Province, Sudan.

The warts involved the head, neck and shoulders of affected cattle. No spread to other parts of the body was seen. The affected animals showed normal behaviour and looked healthy. Their rectal temperatures were within the normal range. Fifty p. 100 of the calves population in the farms contracted the disease. The disease was not seen in adult cattle nor in any other animal species in the two farms.

Warts from infected animals were collected in 50 p. 100 glycerol buffered saline, pH 7.4, containing penicillin (1000 IU/ml), streptomycin (Img/ml) and mycostatin (50 U/ml). A portion of the sample was fixed in 10 p. 100 formalin, processed, sectioned and peroxidase anti-peroxidase strained for presence of the bovine papillomavirus group-specific antigen. The procedure was as described by SUNDBERG *et al.* (3). The steps in brief were as follows .

— Deparafinization : using three changes of xylene at different times, followed by a change of 2 minutes in absolute ethanol.

— Blocking of endogenous peroxidase : by immersing slides in a solution of 0.3 p. 100 H_2O_2 in methanol for 30 minutes.

— Rehydratation of slides : by immersing sequentially in two changes of absolute ethanol and three respective changes in 95, 90 and 75 p. 100 ethanol.

— Following washing with different concentrations of phosphate buffered saline (PBS), the rabbit anti bovine-papillomatosis virus antiserum was added at a pretitrated dilution. The time of incubation and the right temperature were as described (3).

— Following washing in PBS, containing 1 p. 100 ovalbumin, swine antirabbit serum diluted 1/40 was added and slides were incubated as described (3), and washed.

— Rabbit anti-peroxidase complex was then added at a dilution of 1/80 and slides were incubated at room temperature for 30 minutes. Slides were then soaked in PBS, without ovalbumin, for 6 min. and then washed twice in PB-ovalbumin, for 6 minutes per wash.

— The substrate (3.3 diaminobenzidine tetrahydrochloride 0.03 p. 100 and hydrogen peroxide 0.01 p. 100 in PBS) was added for 10 minutes.

— Slides were counter stained with fresh Light Green stain for 2 minutes. Slides were then immersed in the following solutions :

75 p. 100 ethanol - 10 dips.

90 p. 100 ethanol - 10 dips.

95 p. 100 ethanol - 10 dips.

Absolute ethanol - 10 dips.

Xylene - 10 dips.

— Slides were then mounted and read as described (3).

Inoculation of the calves with the suspected warts material

A portion of the wart sample, in glycerol buffered saline, was chopped aspetically with sterile scissors and homogenized using Silverson homogenizer (Silversons Ltd. U.K.). The homogenate was then centrifuged at 1,000 g for 15 min. in the cold. To the supernatant fluid, antibiotics were added as above, and the supernatant was used to inoculate three indigenous one-year-old calves as follows :

— each calf received 0.1 ml doses at various sites of the neck and shoulders. One calf was injected intradermally, the second intramuscularly and the third subcutaneously. A control calf was inoculated with normal bovine skin suspension.

RESULTS

Animal inoculation

The first appearance of warts was seen nine days post inoculation on the calf which received the intradermal injections. On day 34 post inoculation, warts appeared on the calf which received the subcutaneous inoculation. Fifty-six days post inoculation, warts were seen on the calf which was inoculated intramuscularly. No lesions were seen on the control calf.

Warts from the experimentally-infected calves were sectioned and PAP-stained, for presence of the papillomavirus group-specific antigen, as described earlier.

The PAP staining results

BP virus group specific antigen was successfully, detected in sections of warts from infected bovines, using the PAP test.

DISCUSSION

The purpose behind the present study was to examine whether the skin diseases which involved the calves in the described outbreaks, was due to infectious viral bovine papillomatosis.

Results, reported here, established and for the first time in the Sudan presence of cutaneous viral bovine papillomatosis. The disease was successfully reproduced in experimental animals and the group-specific virus antigen was detected in warts from infected animals.

Since research in the field of papillomatosis is rather expensive and as there is no facility for that in the Sudan, it is hoped that sometime the Sudanese isolates of BP virus are to be typed abroad using the current molecular biological techniques. This is expected to add some knowledge to the international epidemiology of the disease and will further clarify the peculiarities of the disease in the Sudan.

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ABU ELZEIN (B. E. M.), TAGELDIN (M. H.), BAKHIET (H. A.), ABBASS (Z.). Cutaneous bovine papillomatosis in the Sudan : detection of the group-specific virus antigen in warts from affected cattle. *Revue Elev. Méd. vét. Pays trop.*, 1988, **41** (1) : 41-43.

This paper records the first identification of bovine papillomatosis virus in the Sudan. Outbreaks of the disease involved 50 p. 100 of the calves aging between three months and two years in two farms at Khartoum Province, Sudan. Both indigenous and exotic breeds were involved. The disease was successfully reproduced in calves and the virus group-specific antigen was detected in warts from affected animals, using the peroxidase anti-peroxidase (PAP) technique. *Key words* : Bovine - Papillomatosis - Immunological technique - Sudan.

ABU ELZEIN (E. M. E.), TAGELDIN (M. H.), BAKHIET (H. A.), ABBASS (Z.). Papilomatosis contagiosa bovina en el Sudán : detección de antigeno al virus especifico en las verrugas de bovinos enfermos. *Revue Elev. Méd. vét. Pays trop.*, 1988, **41** (1) : 41-43.

Los autores relatan la primera identificación del virus de la papilomatosis bovina en el Sudán. La enfermedad ocurró en 50. p. 100 de los terneros de 3 meses a 2 años de edad en dos granjas de la provincia de Khartoum, Sudán. Atacó igualmente las ganaderias de razas locales o importadas. Se la reproduzcó con exito en los terneros y se evidenció el antigeno al virus especifico en las verrugas de los animales infectados por medio de la técnica de peroxidasa anti-peroxidasa. *Palabras claves* : Bovino - Papilomatosis - Técnica inmunologica - Sudán.

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