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Bovine Papillomatosis and its Management with an Autogenous Virus Vaccine in Kiambu District, Kenya

A G Thaiya[1] *, P Gitau[1] , G K Gitau[1] and P N Nyaga[2]

[1]Department of Clinical Studies and [2]Department of Veterinary Pathology, Microbiology and Parasitology
Faculty of Veterinary Medicine, University of Nairobi. P O BOX 29053-00625 Kangemi, Nairobi, Kenya

*Corresponding author email: agthaiyah@yahoo.com

Abstract

Six cases of bovine papillomatosis were reported to the University of Nairobi veterinary clinic. Diagnosis was based on presented clinical signs and histopathology of affected skin lesions. The histological samples of the warts confirmed the diagnosis of papillomatosis. An autogenous formalin killed bovine specific wart vaccine was prepared from the wart samples and injected into four calves on day 0, 10 and 30, while two calves were left as undosed controls. The warts started regressing three weeks post vaccination and completely disappeared by the seventh week. This case represents a successfully management of a case of papillomatosis with a bovine specific autogenous vaccine. Keywords: bovine papilloma virus, papillomatosis, autogenous vaccine

Introduction

The papilloma viruses belong to a large family of human and animal viruses that normally infect epithelial cells causing benign hyperproliferative lesions (warts, papillomas, fibropapillomas) which can progress to cancer (Campo, 2006). There are 6 virus types that cause bovine papillomatosis and these have been thoroughly characterized: Bovine papilloma virus (BVP)-1, BVP-2 and BVP-5 cause fibropapilloma while BVP-3, BVP-4 and BVP-6 cause true epithelial papillomas (Campo, 1999, 1997). BPV-1/2 infection in cattle results in benign coetaneous fibropapillomas, productive for infectious progeny virus, which regress in response to a cell-mediated immune response (Okabayashi et al., 1991; Coleman et al., 1994; Frazer, 1996; Knowles et al., 1996). These neoplasms most often regress spontaneously, occasionally persist, and, in the presence of additional critical genetic or environmental factors, can progress to cancer (Campo, 1987). It is thought to be a multistep affair (Koller and Olson, 1972; Lancaster and Olson, 1982). Furthermore, papillomavirus infection in cattle could be connected with serious disorders of the metabolism (mainly mineral, energetic and nitrous) probably caused by damage to the liver and kidney with mutagenic, carcinogenic and immunosuppressive cadmium, arsenic and lead, observed in the serum of tested animals (Lesnik et al., 1999). Infection by BPV occurs as a result of the virus exposure to single or multiple lesions of the epithelium. Papilloma viral infection, transformation and multiplication of basal cells, lead to wart formation, but most warts are benign and do not proliferate indefinitely (Shah and Howley, 1996). A formalinized suspension of bovine warts with inactivated virus provides a vaccine for effective treatment and prophylaxis of bovine papillomatosis (Barthold et al., 1976; Hunt, 1984; Lesnik et al., 1999; Süveges and Schmidt, 2003). Intra-lesional immunotherapy by Corynebacterium parvum has also been reported (Hall et al., 1994).

Clinically, the disease presents as warts of various sizes that are dry, horny, cauliflower-like on any part of the body but especially on the head (around eyes), neck and shoulder. Warts persist for 5-6 months and in some cases for up to 18 months but in most animals they regress spontaneously. However, animal owners reporting to the clinic have consistently complained that the animals appear unsightly due to the lesions. In addition farmers are very concerned that their animals are experiencing severe pain and would prefer a quick alleviation of this pain and sometimes prefer to slaughter valuable animals resulting in economic losses. There is, therefore, a very urgent need to obtain a cure for the disease. This case represents a successful management of a case of papillomatosis with an autogenous vaccine.

Materials and Methods

Animals

Six calves were reported sick to the University of Nairobi veterinary clinic from a medium sized mixed farm located in Kabete, Central Kenya, with nodular swellings around the head. On examination the calves varied in age from 8 to 14 months and all were female. Examination of the head revealed wart like lesions around the head (Figure 1 and 2) especially around the eyes, ears and progressing to the nose and the neck. The lesion characteristic varied from flat to pedunculated lesions measuring 0.5-10mm in diameter. The herd consisted of 34 heifers but only 6 had the clinical disease. A tentative diagnosis of papillomatosis was arrived at from the clinical presentation. For confirmation of diagnosis, a sample of the warts was taken for histopathology.

Histopathology

Tissue samples of the warts were aseptically taken and preserved in 10% neutral buffered formalin for 48 hours. Fixed specimens were dehydrated through graded alcohols at 80% for 4hrs and 96% for 4hrs and were then dehydrated in isopropanol for 4hrs. Clearing of the tissues was done in amylacetate and xylene for one and 4.5 hrs, respectively, after which the tissues were infiltrated with molten wax at 60^{o} C for 6hrs. The tissues were then embedded in paraffin wax; 5-6 μm thick serial sections were cut, stained with haematoxylin and eosin (H&E), and examined with a light microscope.

Autogenous vaccine preparation and administration

The autogenous vaccine was prepared according to a laboratory protocol reported previously by Hunt (1984). Approximately 5g of actively growing wart sample was excised with a portion of the skin from each animal and placed in a bottle with phosphate buffered saline (PBS) in ice until processing. All wart samples were mixed together and cut up into thin sections using a scalpel blade and placed in a mortar. The sample was then pounded with a pestle to macerate the cells and release the virus particles. PBS was then added to the tissues and the maceration continued. The resultant fluid was then placed in 10ml bottles and centrifuged at 3,000 rpm for 5 minutes. The supernatant was placed into 10ml bottles and 0.5ml of 40% formaldehyde added into each bottle and mixed. The solution was then incubated at 37^{o}C in a water bath for 2hrs after which it was stored at 4^{o}C for 7days before use. An equal volume of Aluminum hydroxide was then added as an adjuvant and the vaccine administered to the calves.

Four heifers were each injected with 1ml of vaccine on days 0, 10 and 30 subcutaneously while two untreated heifers were left as undosed controls. The animals were then observed weekly once the vaccination was completed for the progression of the warts for the next 7 weeks and the results classified as either complete or incomplete regression.

Results

Diagnosis was based on the clinical presentation of wart like lesions on the skin (Figure 1 and 2) and histopathological findings. The results of histopathology indicated that the animals had fibropapillomas characterized by dermal fibroblastic proliferation with overlying, often ulcerated hyperplastic epidermis (Figure 3b). Figure 3a shows a normal skin tissue. Clinically, the papillomas in vaccinated heifers started regressing three weeks post vaccination and had totally disappeared by the 7th week (Figure 4a and b). However, in the untreated heifers the papillomas continued to persist.

Discussion

The results of this experiment indicate the successful treatment of bovine papillomatosis in Kenya using a species specific autogenous vaccine. Reports of bovine papillomatosis treatment with vaccine produced from formalinized suspension of wart tissue indicate variable results. Lesnik et al. (1999) reported that treatment with vaccine showed 93.5% efficiency with no difference in the used vaccine after 105 days of vaccination. Süveges and Schmidt (2003) showed autogenous vaccination made from sterile homogenized tumour tissue and,

performed twice, prevented new cases and with sick animals recovering after vaccination. On the contrary, treatment with autogenous wart vaccine sometimes failed (Smith, 1990, Ndarathi and Mbuthia, 1994). However, individual bovine-specific autogenous vaccine has been shown to work (Ndarathi and Mbuthia, 1994). Commercial vaccines for cattle rarely seem to effectively promote regression of existing warts or to prevent malignant progression, although they may be capable of preventing the development of new lesions if the same strain is involved (Smith, 1990; Campo, 1991; Scott and Anderson, 1992).

In this present study, vaccination resulted in complete regression of the tumor masses in all treated animals. In an earlier reported case (Ndarathi and Mbuthia, 1994), a single wart sample from one calf was used to prepare the vaccine and vaccinate all affected animals but this failed to cure the animals. In this present case, wart samples were collected from all the affected calves and combined to prepare the vaccine. Bovine papilloma viruses 1, 2 and 5 cause fibropapillomas (Campo, 1999, 1997) and in an outbreak it is possible that one or all three could be involved. By using the current method, all virus types can be collected resulting in a more successful response during vaccination. The present case involved young animals up to 14 months of age. This is in agreement with the literature where older animals have been reported to be resistant to this infection and this could be due to development of immunity acquired from apparent or inapparent infections when young (Radostits et. al., 1994). Only 6 heifers were infected and this could have been through contact as they were grazing together. This case of bovine papillomatosis in Kenya was successfully treated with an autogenous bovine specific vaccine and veterinarians encountering such cases that have persisted for sometime should go for vaccination. It is also important to collect as many wart samples as possible in an outbreak so as to prepare a vaccine with many virus types and this will enhance the response as demonstrated in the present case.

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Figure 1. Head region of heifer no.101 with warts (→) around the eyes and around the ears

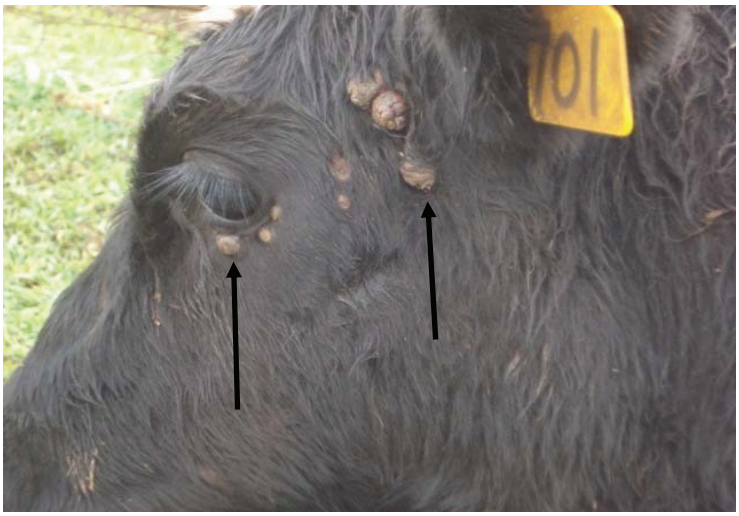

Figure 2. Head region of calf no. 693 with warts (→) around the nose, eyes, ear and neck region.

Figure 3. Histological section of (a) normal skin tissue with a normal epidermis (N) and dermis (D) (Haematoxylin and Eosin Stain X 200).

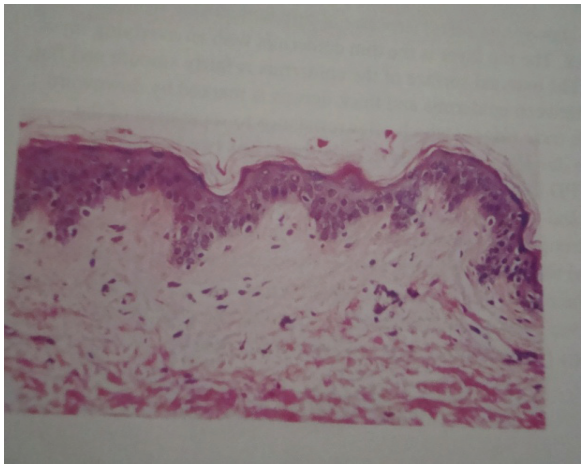

Figure 3 b. Histological section of a cutaneuos fibropapilloma showing an ulcerated epidermis (A) and fibroblastic proliferation of the dermis (B) (Haematoxylin and Eosin Stain x 200).

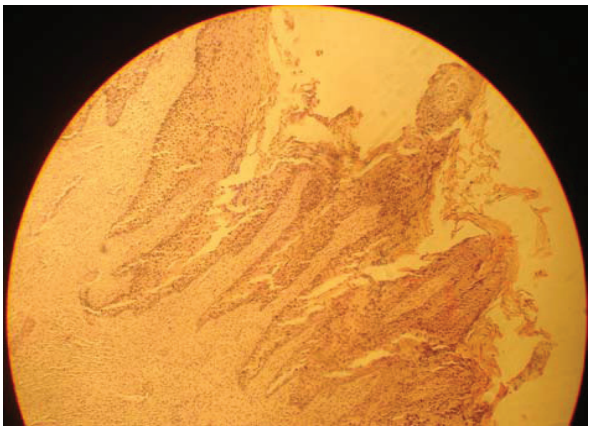

Figure 4. Head regions of calves (a) No. 101 and (b) No. 693 after treatment, with warts completely disappeared.

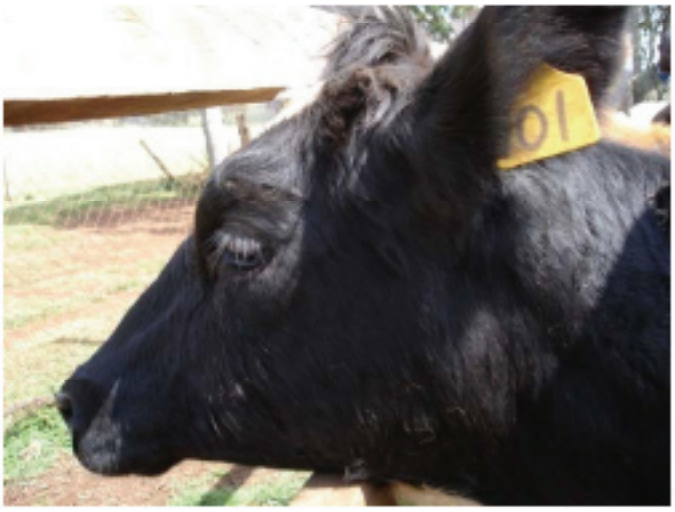

Figure 4 b