Molecular Detection Of Bovine Papilloma Viruses Associated With Cutaneous Warts In Some Breeds Of Nigerian Cattle

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Abstract

Bovine Papillomaviruses (BPVs) cause cutaneous papillomatosis, a common disease of the skin that manifests as non-threatening, non-invasive cancer(warts), seen mostly in young cattle. Various methods have been used in the identification of bovine papillomatosis associated with skin warts worldwide such as clinical signs, immunohistochemistry, histopathology and electron microscopy. Herein, we describe the molecular detection of papillomatosis in some Nigerian cattle(n=31) through partial amplification of the L1 gene using polymerase chain reaction. Skin biopsies were obtained by both plucking and excision techniques from twenty bulls and eleven cows of White Fulani (17), Red Bororo (4), Sokoto Gudali (9) and a cross between White Fulani and Red Bororobreeds at an International cattle market in Ibadan, Nigeria. White Fulani breed had more positive cases of bovine papillomatosis than other breeds clinically and when polymerase chain reaction technique was performed. Six out of the thirty one skin samples were positive for the presence of the DNA of bovine papillomaviruses across the breeds of cattle. Amplification of the viral DNA from the positive samples produced 450bp bands on agarose gel electrophoresis. Thus, this report provides new information on the molecular diagnosis of papillomaviruses associated with this cutaneous benign tumour in some Nigerian native cattle. Therefore, polymerase chain reaction can be used as a rapid confirmatory diagnostic tool in cases of bovine papillomatosis.

Key words: Bovine Papillomatosis, Cattle, Polymerase chain reaction.
INTRODUCTION

Papillomaviruses (PVs) are small, non-enveloped, double-stranded DNA viruses with circular genomes that replicate intranuclearly in infected cells. (Alfieri et al., 2008; Flores et al., 2010). Papillomaviruses have high affinity for stratified squamous epithelia of warm-blooded animals including cattle. (Egawa, 2005). Papillomatosis occurs in all animal species throughout the world (Radostits et al., 2007), and are highly species-specific (Borzacchiello and Roperto 2008). However, the only reported cases of cross-species infection had occurred in horses and other equids (Campo, 2002). The Papillomaviridae family comprises 16 virus genera which were classified based on genomic DNA homology, using the late L1 structural protein gene (Alfieri et al., 2008; Flores et al., 2010). Papillomatosis affecting cattle is a frequent infectious viral disease of the skin, seen mostly in young cattle, that manifests as self-limiting wart and caused by bovine papillomavirus (BPV) (Zuckerman et al., 2000; Hamad et al., 2011). Thirteen types of the bovine papillomaviruses (BPVs), have been characterized in cattle; namely BPV-1 to BPV-13, which are divided into three genera, as Deltapapillomavirus (BPV-1, -2 and -13), Epsilonpapillomavirus (BPV-5 and -8) and Xipapillomavirus (BPV-3, -4, -6, -9, -10, -11 and -12), and an as yet unclassified PV genus (BPV-7) (Bernard et al., 2010; Hatama et al., 2011; Zhu et al., 2011; Freita et al., 2011, Lunardi et al., 2013). Bovine papillomaviruses exhibit site specificity or site preference (Jarret et al., 1984). BPVs-1, -2, -3, -5, -6, -8, -9 and -10 have been shown to cause skin related papillomatosis in animals (Jarret et al., 1984; Campo et al., 1994; Borzacchiello and Roperto, 2008; Hamad et al., 2011).

Diagnosis of bovine papillomatosis is not always a hard task as the lesions can always be seen on the skin manifesting as firm fibropapillomatous growths usually hairless and greyish in colour (Khan et al., 2010; Terziev et al., 2015). Confirmatory diagnosis of warts can be achieved by histopathological examination of wart lesions, which shows marked parakeratotic hyperkeratosis with long, thick, hair-like, cornified surface projections and papillate, epidermal hyperplasia, with patchy areas of erosion, ulceration, and neutrophil infiltration (Khan et al., 2010; Salib and Farghali, 2011). Identification of the virus by electron microscopy reveals the presence of viral particles (about 40nm in diameter) in the nucleus of cells in the stratum corneum, stratum spinosum, and stratum granulosum of the skin (Misdorp, 2002; Terziev et al., 2015). Bovine papillomatosis can also be confirmed with immunohistochemical examination of the basal layer of the affected epithelial and connective tissue using antibodies against specific antigen of BPV-1, Proliferative Cell Nuclear Antigen (PCNA) and Ki-67 protein (Ozsoy et al., 2011).

There are several skin diseases of cattle in Nigeria. Most common among such skin infections are dermatophilosis, lumpy skin disease and cutaneous papillomatosis known as warts (Radostits et al., 2007).

Cutaneous papillomatosis is widespread among indigenous and sometimes imported beef and dairy cattle in Nigeria. There is shortage of information on the use of polymerase chain reaction as a confirmatory tool in the diagnosis of bovine papillomatosis. The present study reports for the first time, the molecular detection of bovine papillomavirus DNA from cutaneous warts in some indigenous breeds of cattle in Nigeria using polymerase chain reaction technique.
MATERIALS AND METHODS

Bovine papilloma lesions
Thirty-one bovine skinbiopsies were collected from some breeds of cattle showing the classicalpresentation of cutaneous papillomatosis. Such cattle breeds included Sokoto Gudali, Red Bororo, White Fulani(Bunaaji) and their crosses of both sexes. Two main techniques were employed to obtain the skin biopsy samples from each animal. The techniques included both excision and plucking methods which have been described elsewhere (Campo, 2006, Borzacchiello and Roperto, 2008). The sampling took place in an International cattle market in Ibadan, Oyo State, Nigeria, located on Longitude 7° 31’30.01” and Latitude 3° 54’54.82” (Google Earth Software, USA;2007). The samples were obtained over a period of two months from cattle that originated from Niger, Kwara and Oyo States(Nigeria) and their ages, sexes and sources of origin were documented. The ages of all the cattle sampled were estimated using dentition technique as described by Lasisi et al. (2002). The samples were kept in microcentrifuge tubes filled with 500 μL of virus transport medium(Copan Diagnostics Inc, USA) and stored at-20°C until tested.

Preparation of DNA samples
A fragment of approximately 5 mg of each papilloma collected was macerated in virus transport medium using a manual tissue homogenizer and subjected to DNA extraction using DNeasy® blood and tissue kit (Qiagen, Valencia, CA) according to the manufacturer’s instruction. Extracted DNAs were kept in sterile Eppendorf tubes and stored at-20°C.

Polymerase chain reaction
The primers employed for the PCR designated Primer B1 and Primer B2 with the 5’-GCMCAGGGWCATAAYAATGG-3’ and 5’-CGTCCMARRGGAWACTGATC-3’, respectively (Ogawa et al., 2004; Flores et al., 2010). Polymerase chain reactions were performed in a 20-μl reaction mixture, using approximately 2.5 μl of total DNA, 1 μl of 0.1μM of each primer, 12.5 μl of PCR Master Mix (2X)-(DreamTaqbuffer, 0.4 mM of each deoxyribonucleotidetriphosphate, and 4 mM MgCl₂) and 3 μl of nuclease free water. The PCR was done in a 9700 PCR Thermal Cycler (Applied Biosystems, USA) with the following conditions: initial denaturation(94°C/2 min/1 cycle), followed by 35 cycles (denaturation(95°C/20 secs); annealing at 48.5°C/min ;72°C/90 secs; and a final extension of 7 min at 72°C). Five microliters of each PCR products was subjected to electrophoretic fractionation in a 2.0 % agarose gel containing 1 μg ethidium bromide and visualized under ultraviolet light.

Statistical analysis
All data obtained were subject to descriptive statistics.

RESULTS
White Fulani breed and bulls were encountered more frequently than other breeds and cows respectively in the study. Out of the 31 cattle showing the classical
lesions of papillomatosis, White Fulani breed of cattle (54.84%) were encountered more among these cattle breeds during the course of the study (Table 1).

**Table 1: Breed susceptibility of cattle showing clinical papillomatosis**

<table>
<thead>
<tr>
<th>Cattle breeds showing classical papillomatosis lesions</th>
<th>Red Bororo</th>
<th>Sokoto Gudali</th>
<th>White Fulani</th>
<th>White Ful. X Red Bororo</th>
<th>Total number of all cattle breeds sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number (%)</strong></td>
<td>4(12.90%)</td>
<td>9(29.03%)</td>
<td>17(54.84%)</td>
<td>1(3.23%)</td>
<td>31</td>
</tr>
</tbody>
</table>

Bull calves (64.51%) were encountered more in the course of the study than their heifer counterparts (35.48%). Among the White Fulani breed, 11 bull calves were found to be clinically showing cutaneous papillomatosis lesions while only one crossed bull calf of White Fulani and Red Bororo breeds with the clinical skin lesions, was sampled (Table 2).

**Table 2: Sex Susceptibility of cattle breeds ampeld to Papillomatosis**

<table>
<thead>
<tr>
<th>Cattle breeds showing classical papillomatosis lesions</th>
<th>Red Bororo</th>
<th>Sokoto Gudali</th>
<th>White Fulani</th>
<th>White Ful. X Red Bororo</th>
<th>Sex (%) distribution among cattle breeds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (%)</strong></td>
<td>M=3</td>
<td>M=5</td>
<td>M=11</td>
<td>M=1</td>
<td>M=20 (64.51)</td>
</tr>
<tr>
<td></td>
<td>F=1</td>
<td>F=4</td>
<td>F=6</td>
<td>F=0</td>
<td>F=11 (35.48)</td>
</tr>
</tbody>
</table>

In the field, the diagnosis of cutaneous papillomatosis (also known as warts) in cattle is by the observable firm fibropapillomatous growth on the skin of affected animals. The confirmation of the tentative diagnosis is often by histochemical reactions. To develop a better and faster method of confirmatory diagnosis for the skin infection in some breeds of Nigerian cattle, we attempted for the first time, to test the ability of PCR to detect the presence of papillomavirus in the skin lesions of several breeds of Nigerian cattle showing the clinical skin disease. Six samples (19.35%) were confirmed positive for bovine papillomatosis while twenty five samples (80.65%) were confirmed negative for bovine papillomatosis after amplification with the set of primers B1 and B2. Figure 1 shows the results obtained.
Molecular Detection Of Bovine Papilloma Viruses Associated

DISCUSSION

Despite the fact that, bovine papillomavirus which induces exophytic papillomas of cutaneous epithelia in cattle is perhaps the most extensively studied animal papillomavirus, there is a sheer lack of information on the susceptibility of various Nigerian breeds of cattle to the virus. That White Fulani cattle were the most susceptible to the classical papillomatosis is worth noting. This finding is in agreement with the work of Dim, et. al., (2012) which reported that the Bunaaji/White Fulani cattle were the most populous and most widely distributed breed in Nigeria. This is because this breed is more heat tolerant (Hansen, 2004) and has lower mortality rate than other indigenous breeds in Nigeria. Although the White Fulani cattle have been said to be more resistant to diseases than other breeds especially the Red Bororo breed (Boutrais, 2007), our finding here that these cattle were found to be most susceptible...
to cutaneous papillomatosis seems to be an exception. This may be due to the thin skin possessed by this breed of cattle (Hansen, 2004). Also sex susceptibility to cutaneous papillomatosis among the Nigerian cattle breeds in the present study is very exciting. More males were found with the clinical skin condition among the cattle sampled. This finding is contrary to the work of Salib and Farghali, (2011) which showed that bovine papillomatosis has a higher prevalence in females than in males. This variation could be explained by the fact that more farmers are willing to sell off most of the bull calves with clinical papillomatosis due to their poor aesthetic appearance and the projected lower economic value in terms of production compared to the heifers when they reach adulthood. Working with various breeds of Nigerian cattle has been done elsewhere (Lasisi and Isehunwa, 2011) in which the blood glucose, erythrocyte indices and serum proteins of three breeds of Nigerian cattle were studied across various ages. Therefore, this present study is in agreement with such earlier work. That only a very small fraction of the samples (obtained from Nigerian cattle breeds showing clinical cutaneous lesions) from the total samples were confirmed positive for the papilloma virus using the selected set of primers is very noteworthy. This finding is in line with the work of Flores et al., (2010) in which 12 out of 49 Brazilian cattle samples (24%) collected were amplified with MY09/MY11 (Primer B). This shows that Primer B is very useful in the detection of bovine papillomaviruses, associated with cutaneous warts, in Nigeria. The high number of negative samples using Primer B, despite being obtained from clinically affected cattle, is worth noting. This is in line with the work of Freitas et al., (2013) in which some samples showed the presence of virus, but with no virus expression. This difference might be related to genetic reassortment as it relates to viral infections. Hence, further scientific work needs to be focused on primer design that will be efficient in the detection of bovine papillomatosis in Nigerian cattle.

CONCLUSION
Conventional polymerase chain reaction is a reliable diagnostic method that can be used to confirm cutaneous-associated papillomatosis in clinically affected cattle across all breeds in Nigeria. This technique has been used in diagnosing bovine trypanosomiasis in Burkina Faso (Solano et al, 1999), lumpy skin disease in Egypt, (El-Kholy et al., 2008) and also, bovine babesiosis in South Africa (Mtshali and Mtshali, 2013).

REFERENCES


