

Original Research Article

Open Access

Histopathological, and Molecular Characterization as well as Autogenous Immunization Trial of Bovine Papillomatosis (Wart) in Cattle, in Mekelle, Northern Ethiopia

Kidane Workelul^{1*}, Yohannes Tekle Asfaw¹, Guesh Negash¹, Haftay Abraha¹, Nigus Abebe Shumuye², Yisehak Tsegaye Redda¹

ABSTRACT

Bovine papillomatosis (wart) is one of the economically important bovine skin diseases worldwide, caused by a group of viruses named papilloma viruses (PVs). However, no previous studies have described its status in Mekelle, northern Ethiopia, and hence, it has often been misdiagnosed as other skin diseases and remained untreated. In order to determine the status of the disease twenty-two farms were visited, and, out of 235 cattle with skin lesion examined, fourteen (14/235) were found infected with cutaneous papillomatosis. Papilloma biopsies were taken for molecular and histopathological characterization. The therapeutic trial of an autogenous vaccine was evaluated on infected animals. The overall status of bovine papillomatosis in this study was estimated at 5.96% (14/235). The disease was found to be statistically significance in the age groups less than two years ($X^2 = 26.69$, $P = 0.0001$). The more prominent histological characterized lesions in the sampled tissue were identified as squamous papilloma and fibro-papilloma. The Polymerase Chain Reaction (PCR) based identification, revealed that all the clinically and histo-pathologically characterized papillomatosis cases were infected with Bovine Papilloma Virus1 (BPV1), indicating that BPV1 was the most common and sole causative agent of the disease in the study area. The autogenous vaccine therapeutic trial demonstrated promising outcomes. Upon visual inspection, the cutaneous bovine papillomatosis lesions completely regressed, resulting in full recovery and no recurrence of the infection. Hence, it was concluded that bovine papillomatosis is an economically important disease of young age cattle as well as a treatable disease. So, production of marketable autogenous vaccines against bovine papillomatosis should be started and be given at an early stage.

Keywords: *Autogenous vaccine, BPV, PCR, Wart,*

Affiliations:

¹Department of Veterinary Basic and Diagnostics, College of Veterinary Sciences, Mekelle University, Mekelle, Tigray, Ethiopia

²Department of Veterinary Clinical Medicine and Epidemiology, College of Veterinary Sciences, Mekelle University, Mekelle, Tigray, Ethiopia

*Corresponding author: kiduney06@gmail.com

INTRODUCTION

Bovine papillomatosis is a common neoplastic, infectious and contagious disease of cattle that is distributed worldwide among herds. It is caused by a diverse group of viruses called papilloma viruses (PVs) (Antonsson and Hansson, 2002). The disease is characterized by the presence of multiple benign tumors (papillomas) that can regress spontaneously or progress to malignant neoplasms due to the synergistic action of genetic or environmental co-factors and the disease leads to economic depreciation of animals, and the deterioration of their body condition and leather (Campo, 2002; Leal et al., 2003; Turk et al., 2005; Yagui et al., 2006; Borzacchiello and Roperto, 2008; Bocaneti et al., 2014). Macroscopically, the lesion (papilloma) is a small to medium sized (2-3cm) growth on the skin or mucous membranes of cattle. Bovine Papilloma Virus (BPV) infection is endemic in both dairy and beef cattle breeding, although it is more prevalent in dairy cattle (Araldi, 2015). The virus infects epithelial cells of the skin or mucous membranes and produces hyperproliferative lesions (Nasir and Campo, 2008).

Studies based on molecular phylogeny suggest that PVs genomic diversification occurred together with mammals' diversification, being influenced by multiple evolutionary forces (Bravo et al., 2010). Although there is limited epidemiological study that allows the definition of Bovine Papilloma Virus (BPV) distribution, BPV-1 and 2 seem to be the most frequently identified virus types (Pathania et al., 2012; Melo et al., 2014; Santos et al., 2014; Alcântara et al., 2015; Araldi et al., 2015a; Cota et al., 2015). These viruses are associated with both benign and malignant neoplasms (Gopalkrishna et al., 1995; Nasir and Campo, 2008; Cota et al., 2015). However, the global distribution of BPV is not homogenous (Santos et al., 2014). BPV-1 and 2 have closely related serotypes (Shafti-Keramat et al., 2009), associated with urinary bladder malignant neoplasms (Wosiacki et al., 2005; Balcos et al., 2008; Roperto et al., 2008; Maiolino et al., 2013; Cota et al., 2015). BPV-4 infection is an important cause of upper digestive tract cancer development (Tsirimonaki et al., 2003; Nasir and Campo, 2008; Lucena et al., 2011). Studies also show that BPV-13 is associated with urothelial carcinomas (Roperto et

al., 2015).

The lack of epidemiological studies on BPV distribution in countries like Ethiopia could underestimate the true value of infected animals, representing a significant challenge in developing vaccines as the predominant virus types are unknown. (Claus et al., 2009; Araldi, 2015; Araldi et al., 2015a).

Papilloma viruses (PVs) identification has been implemented using different methods, such as Southern blot (Leto et al., 2011), immunohistochemistry (Araldi et al., 2015b), chromogenic in situ hybridization (Melo et al. 2015), electron microscopy (Araldi et al., 2014b) and polymerase chain reaction (PCR) using specific and/or degenerate primers (Stocco et al., 1998; Araldi et al., 2013, 2014a, 2015b; Melo et al., 2014). Among those techniques, PCR has been the most commonly used method of diagnosis due to its high sensitivity (Leto et al., 2011).

Different treatment methods have been used around the globe of which formalinized suspension inactivate virus prepared as an autogenous vaccine shows effective treatment outcomes and prophylaxis against bovine papillomatosis (Barthold et al., 1976; Hunt, 1984). Moreover, intra-lesional immunotherapy by *Corynebacterium parvum* was

also reported as a possible therapy (Hall et al., 1994).

Thus, the aims of the present study were to detect and characterize the circulating serotypes of BPV and evaluate the treatment effect of an autogenous vaccine.

MATERIALS AND METHODS

Study area, Study design and Sample collection

The study was conducted in Mekelle city and its vicinities, Tigray, Ethiopia (Figure 1). Mekelle is located at a latitude and longitude of 13°29'N 39°28'E and 13°29'N 39°28'E, respectively. It has an elevation of 2084 meters above sea level. Cross-sectional study was conducted to investigate and characterize the circulating serotypes of BPV and evaluate the lesion regression effect of an autogenous vaccine. During the study, dairy farms were selected purposively based on the willingness of farm owners to participate in the study. Accordingly, 22 dairy farms from seven sub cities in Mekelle, where 235 cattle shown skin lesions from these farms were included in the study for further examination and testing for the existence of papilloma lesion. The infected cattle underwent a physical clinical examination, and all visible warts were recorded along with age, sex, anatomical location and the number of warts present on each body part of the animals.

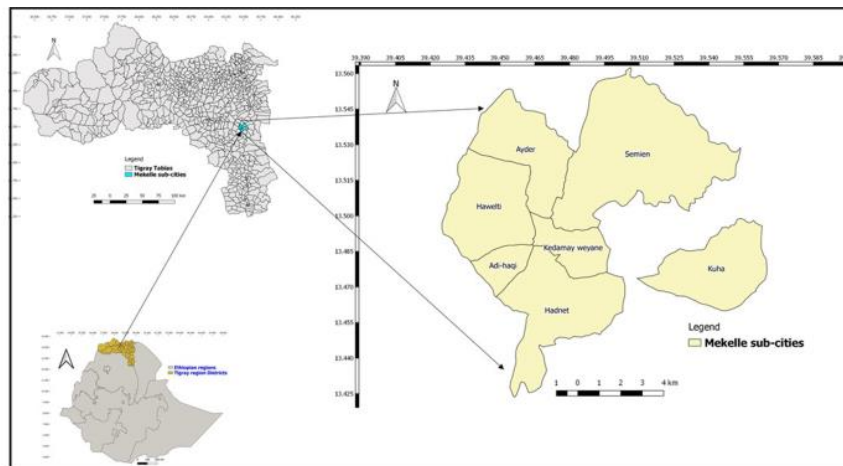


Figure 1: Map of the study area.

Histopathology

Biopsy specimens of papilloma lesions (warts) were collected and preserved in 10% neutral buffered formalin for histopathology examination. The fixed specimens were trimmed, washed, and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin sectioned (4 - 6 microns), and stained with hematoxylin and eosin according to

Bancroft et al. (1996). Every microscopic slide was carefully examined, interpreted, and recorded as described by Ginn et al. (2007).

Polymerase Chain Reaction (PCR) Amplification of Viral DNA

Papilloma-like tissue lesions (warts) were collected and immediately preserved at -20°C until the tissue

was going to be processed for DNA extraction. DNA for PCR amplification was extracted from the frozen samples using HiPurAR Multi Sample DNA Purification Kit (HiPurAR, India), according to the manufacturer's instructions. A polymerase chain reaction (PCR) was performed to determine the serotype of BPV according to Lindsey et al., (2009). Briefly, a PCR mixture with a final volume of 25 μ l containing 1 μ l DNA template, primers (0.5 μ l forward and reverse primer of each specific to BPV1 and BPV2 serotypes (Table 1) and 12.5 μ l of 2x Taq PCR MasterMix (TianGen®, China) and nuclease

free water up to the final volume. The PCR was carried out with the following protocol: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30s, annealing at 55°C for 30s, and extension at 72°C for 2 min, and a final elongation step at 72°C for 10 min. The amplified viral DNA amplicons were run on electrophoresis using a 1% agarose gel with ethidium bromide dye. Visualization of PCR products (bands) was performed using the Gel Documentation system (BioTop, Solo).

Table 1: Primers used to amplify target serotype of Bovine Papilloma Virus

Gene	Primer set	Sequence (5' to 3')	Annealed Bases	GC%	Tm (°c)	Product size (bp)
BPV1	Forward	GGAGCGCCTGCTAACTATAGGA	22	55	59	301
	Reverse	ATCTGTTGTTTGGGTGGTGAC	21	48	57	
BPV2	Forward	GTTATACCACCCAAAGAAGACCCT	24	46	58	164
	Reverse	CTGGTTGCAACAGCTCTCTTTCTC	24	50	60	

Experimental design and treatment trial

Four apparently healthy cattle with a bovine papillomatosis (Wart) lesion were selected for the therapeutic trial after the animals were grossly diagnosed clinically based on the clinical sign, size and clinical appearance of the lesion on the animal's body skin (Figure 2). The animals were identified using a unique tag number, and were kept according to appropriate management practices with good handling. The autogenous vaccine was prepared as per Pearson et al., (1958). Briefly, 5 grams of newly formed, active wart tissue were surgically excised and preserved in saline solution. The tissue was then divided into tiny pieces, mixed with 30 ml/g of a 50% glycerol-saline solution, and filtered through a muslin cloth (sieve). To stop bacterial growth, antibiotics (Strepto-Pencillin, 2

mg/ml) were added. The virus was inactivated by adding 0.4 ml of formalin per 100 ml of filtrate, which was then stored in the refrigerator for 24 hours before use. The autogenous vaccine was administered subcutaneously in doses of 10 ml and revaccinated at 10-day intervals for three consecutive time as per Pearson et al. (1958). Three of them were considered as the experimental group and administered the prepared autogenous vaccine while the negative control group (one animal) was given the same dose of saline solution (without autogenous vaccine) subcutaneously for the same time interval as the experimental animals. The effect of the autogenous vaccine was recorded through papilloma lesion regression visualization by comparing before and after.

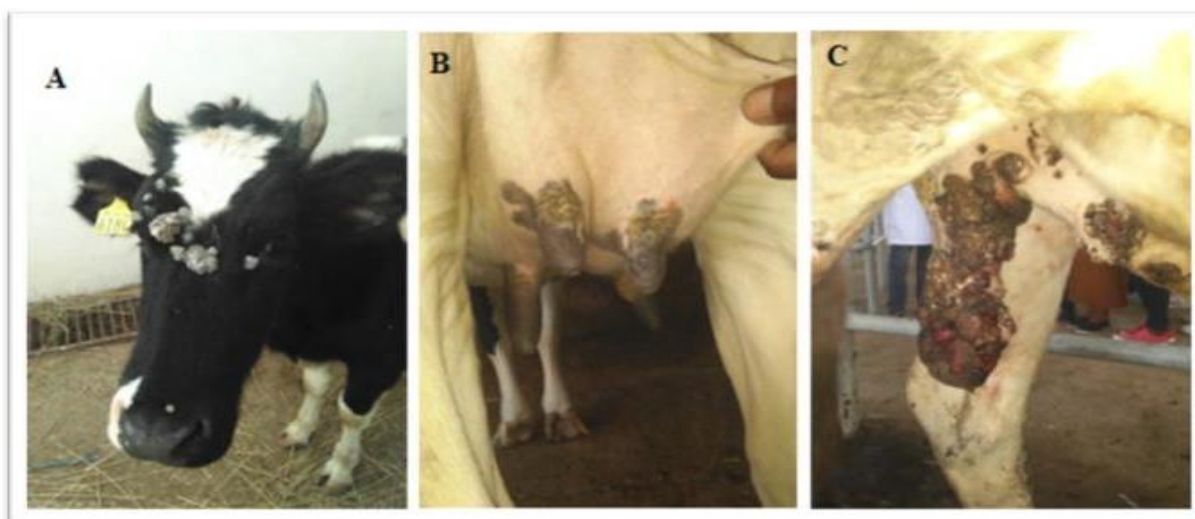


Figure 2: Anatomical location of infected part of the animal with *Bovine Papillomatosis* (Wart) A-Face area; B- Female Animal Udder part; C- Male animal Testicle

Data analysis

Data obtained from the collected samples was

properly coded and entered into Microsoft Excel-2010 spread sheet. The data was filtered for any invalid entry and then transferred to SPSS version 20 software for statistical analysis. Descriptive statistics was used to summarize the data generated from the study. Chi square (X^2) and p-value also used to assess the degree of association between the associated risk factors and bovine papillomatosis.

RESULTS

Disease status

From the total 235 selected cattle with skin lesions only 5.95% (14/235) were found to be positive for

papilloma lesions. The higher percentages of the diseases were reported in female animals (5.10%) and animals aged less than 2 years (4.68%). However, only the occurrence of the disease in the different age groups was found statistically significant ($X^2 = 26.69$, $P = 0.0001$), as indicated in Table 2. With regard to the anatomical location, a high number and percentage of papillomatosis lesions were recorded around the face 5(35.71%) compared to other locations i.e. neck 2(14.28%), legs 2(14.28%), udder 1(7.14%), testis 1(7.14%) ear 2(14.28%) and generalized 1(7.14%).

Table 1: Overall and factor-specific status of Bovine Papillomatosis

Risk Factors	Total animals	No. of infected cattle	Status of the Diseases (%)	Chi square (X^2)	P-value	
Sex	Male	19	2	0.85	0.770	0.0612
	Female	216	12	5.1		
	Total	235	14	5.95		
Age	< 2 years	45	11	4.68	26.69	0.0001
	2-5 years old	51	1	0.42		
	> 5 years old	139	2	0.85		
	Total	235	14	5.95		

Histopathological findings

Microscopic findings showed that, histopathological changes of the affected tissue with papilloma were identified hyperplasia of fibrotic tissue with excessive deposition of collagen materials in the dermal layers of the skin and severe hemorrhage. Marked acanthosis and hyperkeratosis on the outer most layer of the epidermal layer. Following with hyperplasia of epidermal epithelial cells with excessive down growth of fingerlike inward growth

(rete ridges), with high infiltration of the inflammatory cells in response to the virus antigen. Some cells degenerated, while others were stimulated to excessive growth and the formation of papilloma (wart) (Figure 3). The overall microscopic characteristics of the affected skin were a heavily keratinized layer with hyperplasia of epidermal epithelial cells and fibrotic tissue of the dermal layer accompanied by deformity or absence of skin structures (like sweat glands and hair follicles)

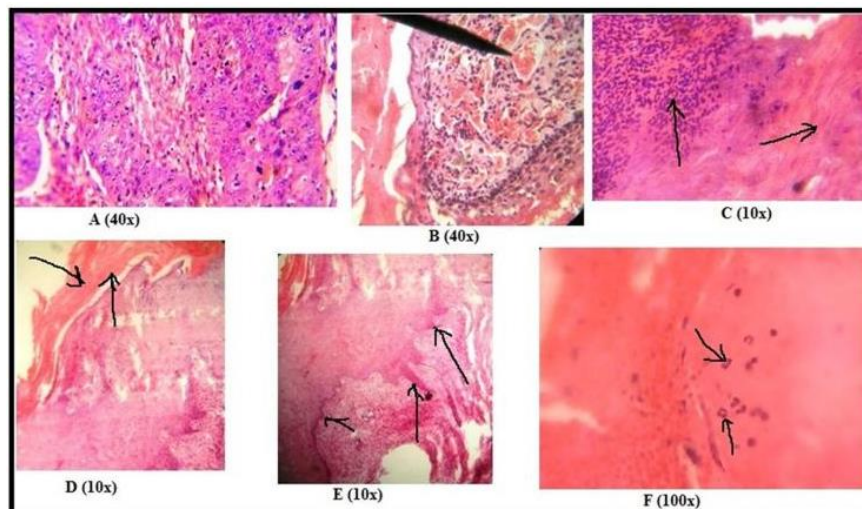


Figure 3: Microscopic lesions of the infected cattle with Bovine Papilloma Virus. A. Hyperplasia of epidermal epithelial cells; B. Marked hemorrhage; C. Deposition of collagen materials in the dermal layers of the skin with moderate infiltration of inflammatory cells; D. Marked acanthosis and hyperkeratosis on the out most layer of the epidermal layer; E. Hyperkeratosis of the epidermal layer with excessive down growth of rete ridges like finger projection of the epithelial cells (black arrow); F. Inflammatory cells with arrow (Neutrophil).

Polymerase Chain Reaction Findings

Bovine papillomavirus 1 (BPV1) was detected in all 14 of the papilloma tissues sample (Figure 4). These BPV1 positive samples exhibited a 301bp fragment of the L1 gene region, which was amplified using BPV1 serotype specific primers. However, none of the samples showed amplification for BPV2 using

BPV2 serotype specific primers. The PCR product showed that all 14 (100%) of the analyzed bovine papilloma at the L1 gene were induced by the BPV1 serotype (Figure 4) in the assessed cattle populations. The result showed that BPV1 serotype is circulating in the study area.

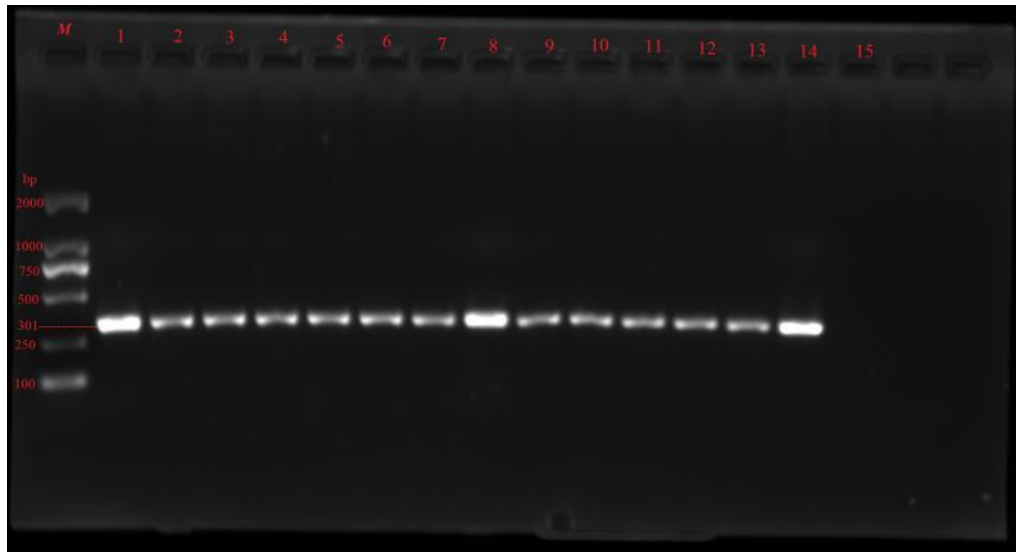


Figure 4: Agarose gel electrophoresis showing PCR amplification. Lane M: Molecular marker 100-2000 bp; Lane (1 to 14): test samples; Lane (15): negative control.

Treatment Response Findings

The warts in the affected locations shed after the start of the treatment trial, and the papilloma lesion began to regress in about 3 weeks. Within 6 weeks, all of the papillomas spontaneously disappeared, and the animal displayed full healing. During the research period's follow-up, no papilloma recurrence was seen

in treated cows even after two months (Figure 5). The control animal shows no change; instead, it continues to persist with the infection for a long time without showing any clinical symptoms of healing. However, once the research period was over, all of the controlled and other positive animals received treatment.

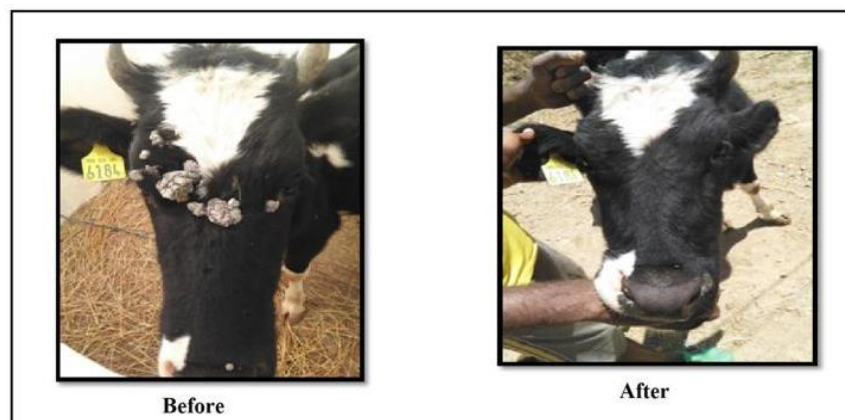


Figure 5: A young calf heifer suffering with Bovine papillomatosis (wart) recovered after administration with autogenous vaccine (before) and (After)

DISCUSSION

Bovine papillomatosis (wart) was found with an overall occurrence of 5.95% in the assessed cattle. Where young cattle less than two years (4.68%) with less immune status are more susceptible than older ones. The reported variation in age susceptibility to bovine papillomatosis could be due to variations in the immune status of animals at different age groups. This is probably because their immune systems are transitioning from the maternal immunity, they received from colostrum for the development of their own immunity to pathogens in their environment (Borku *et al.*, 2007). Though it was not statistically significant, the reported higher number of the disease in females could be due to fewer male animals were kept in dairy farms and physiological stresses (lactation, pregnancy, heat) that compromise the immunity of female animals as compared to male animals (Arelidi *et al.*, 2015a). The findings of the present study were also in agreement with the reports of Salib and Farghali (2011), who reported, that the prevalence of bovine papillomatosis in the Northern Oases (Egypt) was higher in females than males, but this finding was contrary to the work of Jeremiah *et al.*, (2016), who reported that the prevalence of bovine papillomatosis in Nigeria was higher in males than females.

The overall microscopic histopathological characteristics of the affected skin were heavily keratinized layer with hyperplasia of the epidermal epithelial cells and fibrotic tissue of the dermal layer accompanied by deformity or absence of skin structures (like sweat glands and hair follicles). Particularly, the marked acanthosis and hyperkeratosis in most of the outer layer of epidermis, excessive down growth of rete ridges/hyperplasia like finger projection of the epithelial cells in the epidermal layer of the skin, presence of fibro-vascular core and marked hemorrhage with moderate infiltration of inflammatory cells were typical histopathologic features of bovine papillomatosis (Ginn *et al.*, 2007). The findings support the clinical-gross pathological observation. The histopathologic findings of the present study agree with those of previous studies by Jelinek and Tachzy (2005) and Claus *et al.* (2009).

The purpose of PCR characterization was to identify the serotype of the papilloma virus circulating in the region. Accordingly, all of the clinically and pathologically characterized papillomatosis cases were found to be infected with BPV1, indicating that BPV1 was the most common and sole causative agent of the disease in the study area. Similarly, many other researchers have indicated that BPV1 and 2 seem to be the most frequently identified virus types (Pathania *et al.*, 2012; Melo *et al.*, 2014).

Another study by Campo (2002) reported that different BPV genotypes, including BPV1, are associated with papillomas of the teats and udders in cows. However, other similar cases were reported in the incidence of BPV in cattle in Europe, America, Asia, and Oceania, and BPV1, -6, -8, and -10 were found in bovine warts from a German cowshed. It was also reported by other previous researchers (Antonsson and Hansson, 2002) that PCR assays using degenerate primers that amplify partial fragments of the L1 gene, followed by sequencing, suggested the existence of 12 putative new BPV types detected in wart-scarred and healthy teat skins of cattle from Japan and Sweden.

The present study suggests that BPV1 is very well distributed in the study area, control and prevention strategies for bovine papillomatosis, such as future vaccine development, should target specific BPV1 viruses. This is because an autogenous vaccine providing immunity to one type of papilloma virus does not confer immunity to another (absence of cross protection) due to the fact that some papillomas are topographically specific and caused by distinct viruses having different antigenic reactions and DNA compositions (Arelidi *et al.*, 2015b; Munday *et al.*, 2015).

The prepared autogenous vaccine was effective in treating the active bovine papillomatosis with excellent lesion regression outcomes. There was full recovery, and no recurrence of the disease was reported during the follow-up period of two months. These findings were consistent with previous reports by Turk *et al.* (2005). It was observed that successful treatment against papillomatosis has been a great challenge for field veterinary practitioners as no effective treatment for the disease is available. Other therapeutic methods, such as surgical intervention, might not be possible if a large area is involved, which sometimes aggravates the condition. Hence, this study has proven an excellent choice of regression lesion for bovine papillomatosis.

CONCLUSION

Bovine papillomatosis (wart) is a common cattle disease with an economical importance worldwide. Young cattle (under 2 years) are more susceptible than adults. Pathologically identified lesions caused by BPV were characterized as squamous papilloma and fibro papilloma. The PCR based detection of the identified serotype reveals that all 14 of the clinically and pathologically characterized papillomatosis cases were found to be infected with BPV1. An autogenous vaccine had excellent treatment outcomes in treating active bovine papillomatosis, with 100% full recovery and no recurrence of the disease. Further

studies, are required to deepen knowledge of BPVs circulating in different parts of the region in particular and the country in general.

ACKNOWLEDGEMENT

The authors would like to extend their acknowledgement to the different farm owners and farm workers of the study site for their keen interest and cooperation during the collection of the samples and to the College of Veterinary Sciences staff members who directly or indirectly helped us during the research period.

ETHICAL APPROVAL

Ethical approval was obtained from Mekelle University College of Veterinary Sciences, Animal Use and Care Committee CVM/CRC-01/010/19.

Available data and material: all needed data and materials can be supplied as per your request

Competing Interests: Authors declare that they do not have any conflict of interest.

Funding: Mekelle University, College of veterinary sciences

REFERENCES

- Alcântara, B., Alfieri, A., Headley, S., Rodrigues, W., Otonel, R., Lunardi, M., Alfieri, A. (2015). Molecular characterization of bovine Deltapapillomavirus (BPV-1, 2, and 13) DNA in equine sarcoids. *Pesqui Veterinária Bras*, 35:431-436.
- Antonsson, A., Hansson, B.G. (2002). Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. *J. Virol.*, 76: 12537-12542.
- Araldi, R. (2015). Bovine papillomavirus: What We Know and What We Should Know. Lambert Academic Publishing, Germany, 124 p.
- Araldi, R., Carvalho, R., Melo, T., Diniz, N., Sant'Ana, T., Mazzuchelli-de-Souza, J., Spadacci-Morena, D., Beçak, W., Stocco, R. (2014a). Bovine papillomavirus in beef cattle: First description of BPV-12 and putative type BAPV8 in Brazil. *Genet Mol Res* 13:5644-5653.
- Araldi, R., Giovanni, D., Melo, T., Diniz, N., Mazzuchelli-de-Souza, J., Sant'Ana, T., Carvalho, R., Beçak, W., Stocco, R. (2014b). Bovine papillomavirus isolation by ultracentrifugation. *J Virol Methods* 208:119-124.
- Araldi, R., Mazzuchelli-de-Souza, J., Modolo, D., Souza, E., Melo, T., Spadacci-Morena, D., Magnelli, R., Rocha, M., De-Sá-Júnior, P., Carvalho, R., de Sá Júnior, P.L., Carvalho, R.F.D. and Beçak, W. (2015a). Mutagenic potential of *Bos Taurus* papillomavirus type 1 E6 recombinant protein. First description. *Biomed Res Int* 2015:1-15.
- Araldi, R., Melo, T., Diniz, N., Carvalho, R., Beçak, W., Stocco R. (2013). Bovine papillomavirus clastogenic effect analyzed in comet assay. *Biomed Res Int* 2013:1-7.
- Araldi, R., Melo, T., Neves, A., Spadacci-Morena, D., Magnelli, R., Módulo, D., De-Sá-Júnio, P., Mazzuchelli-de-Souza, J., Carvalho, R., Beçak, W., Stocco, R. (2015b). Hyperproliferative action of bovine papillomavirus: Genetics and histopathological aspects. *Genet Mol Res* 14:12942-12954.
- Balcos, L., Borzacchiello, G., Russo, V., Popescu, O., Roperto, S., Roperto, F. (2008). Association of bovine papillomavirus type-2 and urinary bladder tumours in cattle from Romania. *Res Vet Sci* 85:145-148.
- Bancroft, J.D., Stevens, A. and Turner, D.R. (1996). *Theory and Practice of Histological Techniques*. 4th Edition, Churchill Livingstone, New York.
- Barthold, S.W., Olson, L., Larson, B. (1976). Precipitin response of cattle to commercial wart vaccine. *Am. J. Vet. Res.* 37: 449-451.
- Bocaneti, F., Altamura, G., Corteggio, A., Velescu, E., Roperto, F., Borzacchiello, G. (2014). Bovine papillomavirus: New insights into an old disease. *Transbound Emerg Dis* 63:1-10.
- Börkü, M., Atalay, O., Kibar, M., Cam, Y., Atasever, A. (2007). Ivermectin is an effective treatment for bovine cutaneous papillomatosis. *Res Vet Sci* 83:360-363.
- Borzacchiello, G., Roperto, F. (2008). Bovine papillomaviruses, papillomas and cancer in cattle. *Vet Res* 39:45.
- Bravo, I., Sanjosé, S., Gottschling, M. (2010). The clinical importance of understanding the evolution of papillomaviruses. *Trends Microbiol* 18:432-438.
- Campo, MS. (2002). Animal models of papilloma virus pathogenesis. *Virus Res* 89:249-261.
- Claus, M., Lunardi, M., Alfieri, A., Sartori, D., Fungaro, H., Alfieri, A. (2009). Identification of the recently described new type of bovine papillomavirus (BPV-8) in a Brazilian beef cattle

- herd. *Pesqui Veterinária Bras* 29:25-28.
18. Cota, J., Peleteiro, M., Petti, L., Tavares, L., Duarte, A. (2015). Detection and quantification of bovine papillomavirus type 2 in urinary bladders and lymph nodes in cases of bovine enzootic hematuria from the endemic region of Azores. *Vet Microbiol* 178:138-143.
19. Ginn, P.E., Mansell, J.E.K.L., Rakich, P.M. (2007). Skin and appendages. In: Jubb, K.V.F., Kennedy, P.C., Palmer, N.C. (Eds.), *Pathology of Domestic Animals*. Elsevier, Saunders, pp. 748–750.
20. Gopalkrishna, V., Srivastava, A., Hedau, S., Sharma, J., Das, B. (1995). Detection of human papillomavirus DNA sequences in cancer of the urinary bladder by in situ hybridisation and polymerase chain reaction. *Sex Transm Infect* 71:231-233.
21. Hall, H.C., Teuscher, P., Urie, B., Boden, R., Robison. (1994). Induced regression of bovine papillomas by intralesional immunotherapy. *Therapeutic immunol.* 1: 319-324.
22. Hunt, E. (1984). Fibropapillomatosis and papillomatosis. *Vet. Clin. North Am. Large Anim. Pract* 6: 163-167.
23. Jelinek, F., Tachezy, R. (2005). Cutaneous papillomatosis in cattle. *J.Comp. Pathol* 132: 70-81,
24. Jeremiah, O.T., Fagbohun, O.A., Babalola O. J. (2016). Molecular Detection of Bovine Papilloma Viruses Associated with Cutaneous Warts in Some Breeds of Nigerian Cattle.
25. Leal, AM., Ferraz, OP., Carvalho, C., Freitas, AC., Beniston, RG., Beçak, W., Campo, MS., Stocco dos Santos, RC. (2003). Quercetin induces structural chromosome aberrations and uncommon rearrangements in bovine cells transformed by the E7 protein of bovine papillomavirus type 4. *Vet. Comp. Pathol* 1: 15-21.
26. Leto, M., Santos-Júnior, G., Porro, A., Tomimori, J. (2011). Human papillomavirus infection?: Etiopathogenesis, molecular biology and clinical manifestations. *An Bras Dermatol* 86:306-317.
27. Lindsey, CJ., Almeida, ME., Vicari, CF., Carvalho, C., Yagui, A., Freitas, AC., Beçak, W., Stocco dos Santos, RC. (2009). Bovine papillomavirus DNA in milk, blood, urine, semen, and spermatozoa of bovine papillomavirus-infected animals. *Genet Mol Res*, 8: 310-318.
28. Lucena, R., Rissi, D., Kommers, G., Pierezan, F., Oliveira-Filho, J., Macêdo, J., Flores, M., Barros, C. (2011). A retrospective study of 586 tumours in Brazilian cattle. *J Comp Pathol* 145:20-24.
29. Maiolino, P., Ozkul, A., Sepici-Dincel, A., Roperto, F., Yücel, G., Russo, V., Urraro, C., Lucà, R., Riccardi, M., Martano, M. (2013). Bovine papillomavirus type 2 infection and microscopic patterns of urothelial tumors of the urinary bladder in water buffaloes. *Biomed Res Int* 2013:937918.
30. Melo, T., Araldi, R., Pessoa, N., De-Sá-Júnior, P., Carvalho, R., Beçak, W., Stocco. (2015). *Bos taurus* papillomavirus activity in peripheral blood mononuclear cells? Demonstrating a productive infection. *Genet Mol Res* 14:1612-1627.
31. Melo, T., Carvalho, R., Mazzucchelli-de-Souza, J., Diniz, N., Vasconcelos, S., Assaf, S., Araldi, R., Ruiz, R., Kerkis, I., Beçak, W. (2014). Phylogenetic classification and clinical aspects of a new putative Deltapapillomavirus associated with skin lesions in cattle. *Genet Mol Res* 13:2458-2469.
32. Munday, J., Thomson, N., Dunowska, M., Knight, C., Laurie, R., Hills, S. (2015). Genomic characterisation of the feline sarcoid-associated papillomavirus and proposed classification as *Bos taurus* papillomavirus type 14. *Vet Microbiol* 177:289-295.
33. Nasir, L. and Campo, MS. (2008). Bovine papillomaviruses: their role in the aetiology of cutaneous tumours of bovids and equids. *Vet. Dermatol.*, 19(5): 243-254.
34. Pathania, S., Dhama, K., Saikumar, G., Shahi, S., Somvanshi, R. (2012). Detection and quantification of bovine papilloma virus type 2 (BPV-2) by real-time PCR in urine and urinary bladder lesions in enzootic bovine haematuria (EBH)-affected cows. *Transbound Emerg Dis* 59(1): 79-84.
35. Pearson, JKL., Kerr, WR., McCartney, WDJ. (1958). Tissue vaccines in the treatment of bovine papillomas. *Vet.Rec.* 10:971-973.
36. Roperto, S., Brun, R., Paolini, F., Urraro, C., Russo, V., Borzacchiello, G., Pagnini, U., Raso, C., Rizzo, C., Roperto, F., Venuti, A. (2008). Detection of Bovine Papillomavirus Type 2 (BPV2) in the Peripheral Blood of Cattle with Urinary Bladder Tumors: Possible Biological Role. *Journal of General Virology* 89: 3027-

- 3033.
37. Roperto, S., Russo, V., Leonardi, L., Martano, M., Corrado, F., Riccardi, M., Roperto, F. (2015). Bovine papillomavirus type 13 expression in the urothelial bladder tumours of cattle. *Transbound Emerg Dis* 63(6): 628-634.
 38. Salib, FA, Farghali, HA. (2011). Clinical, epidemiological and therapeutic studies on Bovine Papillomatosis in Northern Oases, Egypt. *Veterinary World*, 4(2): 53.
 39. Santos, E., Silva, M., Pontes, N., Coutinho, L., Paiva, S., Castro, R., Freitas, A. (2014). Detection of different bovine papillomavirus types and co-infection in bloodstream of cattle. *Transbound Emerg Dis* 63:e103-e108.
 40. Shafti-Keramat, S., Schellenbacher, C., Handisurya, A., Christensen, N., Reininger, B., Brandt, S., Kirnbauer, R. (2009). Bovine papillomavirus type 1 (BPV1) and BPV2 are closely related serotypes. *Virology* 393:1-6.
 41. Stocco, RC., Lindsey, CJ., Ferraz, OT., Pinto, JR., Mirandola, RS., Benesi, FJ., Birgel, EH., Bragança, CA., Beçak, W. (1998). Bovine papillomavirus transmission and chromosomal aberrations: an experimental model. *J. Gen. Virol.*, 79: 2127-2135.
 42. Tsirimonaki, E., Neil, B., Williams, R., Campo, M. (2003). Extensive papillomatosis of the bovine upper gastrointestinal tract. *J Comp Pathol* 129:93-99.
 43. Turk, N., Zupancic, Z., Staresina, V., Kovac, S., Babic, T., Kreszinger, M., Curic, S., Barbic, L., Milas, Z. (2005). Severe bovine papillomatosis?: Detection of bovine papillomavirus in tumour tissue and efficacy of treatment using autogenous vaccine and parammunity inducer. *Vet Arh* 75:391-397.
 44. Wosiacki, S., Barreiro, M., Alfieri, A., Alfieri, A. (2005). Seminested PCR for detection and typing of bovine Papillomavirus type 2 in urinary bladder and whole blood from cattle with enzootic haematuria. *J Virol Methods* 126:215-219
 45. Yagui, A., Carvalho, C., Freitas, A., Gustavo, L., Góes, B., Dagli, L., Birgel-Júnior, E., Beçak, W., Stocco dos Santos, R. (2006). Papillomatosis in cattle: In situ detection of bovine papillomavirus DNA sequences in