



Research Article

Preliminary Pathological Studies on Equine Skin Lesions with Specific Reference to Equine Sarcoid and Detection of Bovine papillomaviruses 1 and 2

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ARTICLE HISTORY

Received: 2013-09-09
Revised: 2013-10-15
Accepted: 2013-10-20

Key Words: Equine, Sarcoid, BPV 1&2, Histopathology, Skin lesions

ABSTRACT

A preliminary pathological study on equine skin lesions with specific reference to equine sarcoid and association of Bovine Papillomaviruses (BPVs) was carried out. A total number of 16 skin biopsy specimens from equines (4 horses and 12 mules) were studied. These animals had proliferative skin lesions which were small, solitary or variable in size and located on eye lids and different parts of the body. Histopathologically, these biopsies were diagnosed as neoplastic (13) and non-neoplastic conditions (3). Amongst neoplastic, 10 cases were benign in nature including fibroid (6), occult sarcoid (2), fibromatosis (1) and fibropapilloma (1) while 3 cases were malignant diagnosed as squamous cell carcinoma of eyelids (2) and vagina (1). Remaining 3 non-neoplastic conditions were diagnosed as necrotizing dermatitis (2) and myositis (1). These skin biopsies were tested for presence of bovine papillomaviruses (BPVs) -1 and -2 DNA by Polymerase Chain Reaction; 5 were positive for BPV-1, 3 for BPV-2 and 6 for their mixed infections. Present study revealed that equine skin biopsies should be analyzed for better formulation of treatment strategy.

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ARTICLE CITATION: Jangir BL, Singh VK, Saikumar G, Pawde AM, Churamani CP and Somvanshi R (2013). Preliminary pathological studies on equine skin lesions with specific reference to Equine Sarcoid and Detection of Bovine papillomaviruses 1 and 2. *Adv. Anim. Vet. Sci.* 1 (X):xxxxx

INTRODUCTION

The equine sarcoid is a locally aggressive, fibroblastic skin tumour that affects horses, mules, donkeys and zebra throughout the world (Chambers et al., 2003; Nel et al., 2006). Sarcoids can occur in horses at any age and there is no gender predisposition (McConaghy et al., 1994; Scott and Miller, 2003). It may occur in any part of the body either as solitary or multiple lesions ranging from small wart-like lesions to large ulcerated fibrous growths. The most common sites are face (muzzle, ears and periocular region), neck, ventral abdomen, distal limbs and at sites that have been previously traumatized (Carr, 2009; Finlay et al., 2009). These are locally invasive, non-metastatic, fibroblastic skin tumours with unpredictable behaviour and are considered to cause substantial economic loss to the equine industry. Sarcoids represent 12 to 67% of all equine tumours and 70% of all skin tumours in horses (Teifke et al., 1994; Lepage et al., 1998) while squamous cell carcinomas (SCCs) account for up to 37% of cutaneous neoplasms (Scott and Miller, 2003).

Although papillomaviruses (PVs) have been described as highly species-specific (Campo, 2006) but some PVs infect a variety of hosts. Cross-species infection occurs in horses and other equids by BPV-1 and -2. Nowadays, it is also known that BPV plays a major role in equine sarcoid pathogenesis (Chambers et al., 2003; Bogaert et al., 2005; Bogaert et al.,

2008). BPV-1 and -2 DNA have been demonstrated in sarcoids all over the world, either one or both types in the same horse. In India, only a few case reports of sarcoid are available (Singh et al., 1986; Sudarsanam and Christopher, 1992; Pratap et al., 2000; Dabas et al., 2004). It is difficult to assess status of sarcoids in India due to lack of systematic studies. Keeping scanty information in the view; a preliminary pathological study on equine skin lesions with specific reference to equine sarcoid was carried out to characterize the suspected skin tumours and to assess presence of BPVs in such lesions.

MATERIALS AND METHODS

Samples

Equine tumour samples were collected from the Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, Bareilly and Central Military Veterinary Laboratory, Meerut Cantt., Uttar Pradesh for diagnosis and analysis. Biopsies were collected after administration of local anaesthesia around the growth and samples were preserved in 10% buffered formalin for histopathological studies. A part of it, from few samples was also stored in sterile vials at -20°C and samples from field cases preserved in 10% buffered formalin were used for molecular studies.

Pathological Studies

The detailed clinical gross observations with specific locations of tumorous growths were recorded. After proper fixation in the 10% neutral buffered formalin, tissues were cut into small sections with thickness of 2–3 mm and embedded in the paraffin by standard procedures. The paraffin embedded tissues were cut into 6 micron thick sections and stained with hematoxylin and eosin as per conventional procedures (Culling, 1995). Masson's trichrome staining was done on selected duplicate tissue sections to differentiate between collagen and smooth muscles as per standard procedures (Luna, 1968).

Molecular Studies

DNA extraction was performed from tissues using DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following manufacturer's protocol. BPVs were detected by PCR, targeting the L1 gene of BPV-1 and -2 with specific primers (Operon Biotechnologies, Genetix Biotech Asia Pvt. Ltd., Huntsville, AL, USA) and these were similar to the previous studies (Yagui et al., 2008; Pangty et al., 2010). Primers of BPV-1 (forward- 5'-gga gcg cct gct aac tat agg a-3'; reverse-5'-atc tgt tgt ttg ggt ggt gac-3') and BPV-2 (forward- 5'-gtt ata cca ccc aaa gaa gac cct-3'; reverse-5'-ctg gtt gca aca gct ctc ttt ctc-3') were expected to

amplify the specific viral DNA template of 301 and 164 bp sizes, respectively. Amplified DNA fragments were run in 1.5% agarose gel containing ethidium bromide (0.5 g/ml) and visualized by transillumination under UV light (Geldoc, USA). Selective PCR products were directly sequenced commercially on ABI-PRISM dye terminator at DNA Sequencing Facility at Department of Biochemistry, Delhi University, South Campus, New Delhi using specific primers supplied from our laboratory. The generated sequences were blasted online on National Center for Biotechnology Information (NCBI) and analyzed by MegAlign (DNASTAR Inc.). The generated sequences were submitted to NCBI and EMBL data base.

RESULTS

A total of 16 sarcoid-like cases were observed on different body parts of mules and horses. Grossly, the tumours were located on eyelids and various parts of the body with varied appearance (Figure 1A). The detailed observations are presented in Table-1. Histopathologically, these cases were categorized into three different types; neoplasms including benign and malignant tumours and non-neoplastic inflammatory conditions.

Table 1: Etiopathological Findings of Equine Skin Lesions

Case No	Class of animal	Clinical Diagnosis	Histopathological Diagnosis	PCR results	
				BPV-1	BPV-2
E1	Horse (Adult)	Wounded sarcoid-like lesion in thigh region	Fibroid	+ve	-ve
E2	Mare (Adult)	A big tumorous lump hanging from vagina	Squamous cell carcinoma	-ve	-ve
E3	Mule (General Service)	Tumorous growth in nasal cavity	Fibromatosis	+ve	+ve
E4	Mule (General Service)	Tumour on lower eye lid (Left side)	Fibroid	+ve	-ve
E5	Mule (General Service)	Tumour on lower eye lid (Right side)	Squamous cell carcinoma	+ve	-ve
E6	Mule (MA)	Cutaneous neoplasm on lower belly	Necrotizing myositis	+ve	+ve
E7	Mule (Young Stock)	Tumour orbital fossa	Necrotizing dermatitis	+ve	-ve
E8	Mule (General Service)	Tumour on medial aspect of thigh of right hind limb	Fibroid	+ve	+ve
E9	Mule (General Service)	Cutaneous neoplasm on preputial fold	Occult sarcoid	-ve	+ve
E10	Horse (Ride A)	Hard growth on elbow of left for limb	Fibroid	-ve	+ve
E11	Mule (General Service)	Tumour on mid ventral region of belly	Fibroid	+ve	+ve
E12	Mule (General Service)	Melanoma (On left eyelid)	Occult sarcoid / Fibroid (?)	+ve	+ve
E13	Mule (General Service)	Tumour nearby right teat	Fibroid	-ve	-ve
E14	Mule (Young Stock)	Tumorous growth on tail	Fibropapilloma	+ve	+ve
E15	Horse (Young Stock)	Bursati/ Ulcer (Left eye)	Necrotizing dermatitis	+ve	-ve
E16	Mule (General Service)	Tumour growth on third eyelid (Right side)	Squamous cell carcinoma	-ve	+ve

Neoplastic Conditions

Benign tumours

Benign tumours were diagnosed histopathologically into different types including fibroid (6), occult sarcoid (2), fibromatosis (1) and fibropapilloma (1). The tumours diagnosed as fibroid revealed focal epidermal hyperplasia with a few rete pegs extending into the dermis and proliferation of fibrous connective tissue that was confluent with the epidermis. Proliferating fibroblasts were packed densely in spindle form and haphazardly arranged fascicles and interlacing bundles in whorls pattern with presence of small amount of collagen (Figure 1B). Occasionally, severe haemorrhages with engorgement of blood vessels, focal areas of necrosis with infiltration of neutrophils, mononuclear cells and marked eosinophils were also seen. Fibromatosis showed moderate proliferation of fibrous connective tissue with hyalinization of tissues and areas of infrequent engorgement of blood vessels with RBCs, haemorrhages and focal areas of lymphocytic

infiltration. Duplicate sections stained with Masson's Trichrome revealed proliferating fibrous connective tissues with abundant collagenous fibres that stained blue, while the nuclei stained black and other fibres, muscles stained red in variable shades, which was very less as compared to collagenous fibres (Figure 1C). Occult sarcoid showed mild thickening of epidermis with elongated basal cells. Lamina propria showed diffuse infiltration of mononuclear cells, mainly lymphocytes, as well as perivascular mononuclear cell infiltration (Figure 1D). Sebaceous glands were degenerated. Immature fibrous connective tissue was arranged in whirling pattern. In one case, areas of ulceration were seen while the non-ulcerated areas showed epidermal hyperplasia with prominent rete pegs extending into the dermal proliferation of fibroblasts. Melanosis was also evident with presence of scanty melanin pigment. Fibropapilloma revealed moderate degree of cornification with parakeratosis along with basket-weave appearance of stratum corneum. Hyperplasia of stratum

spinousum with presence of koilocytes and islands of proliferating dermal connective tissue surrounded by hyperplastic epidermal cell layers with well developed rete pegs

was seen. Basal cell layer was hyperplastic with hyperchromatic nuclei.

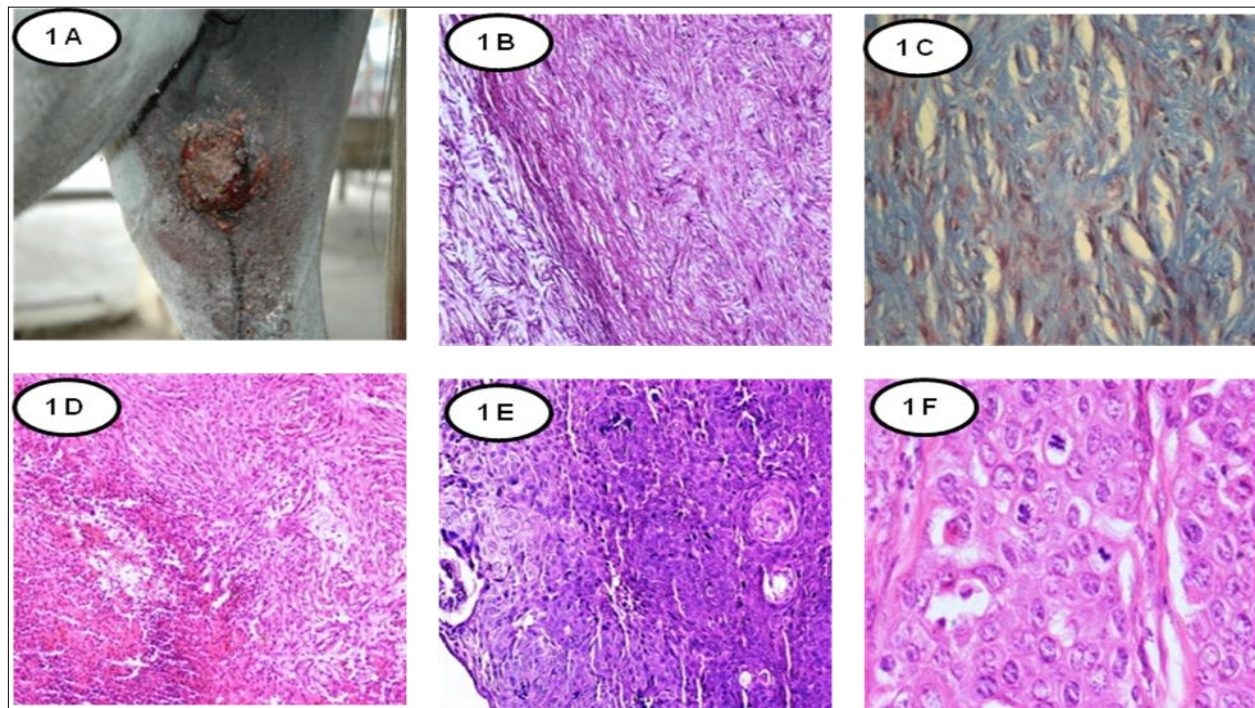


Figure 1A: Round, raised growth on the inner aspect of thigh region of right hind limb in a horse; 1B: Proliferating fibrous connective tissue with densely packed spindle cells with fibres showing whirling pattern; Fibroid, H & E $\times 100$. 1C: Proliferating fibrous connective tissues with abundant collagen fibres stained blue, nuclei stained black and other fibres and muscles stained red. Fibroid, Masson's Trichrome $\times 400$. 1D: Proliferating fibrous tissues, haemorrhages and focal area of necrosis along with mononuclear cell infiltration; Fibroid, H & E $\times 100$; 1E: Neoplastic squamous cells with hyperchromatic nuclei forming thick cellular cords with occasionally mitotic figures. Squamous cell carcinoma, H & E $\times 100$; 1F: Neoplastic squamous cells showing marked mitotic figures in different stages of cell-cycle; Squamous cell carcinoma, H & E $\times 400$.

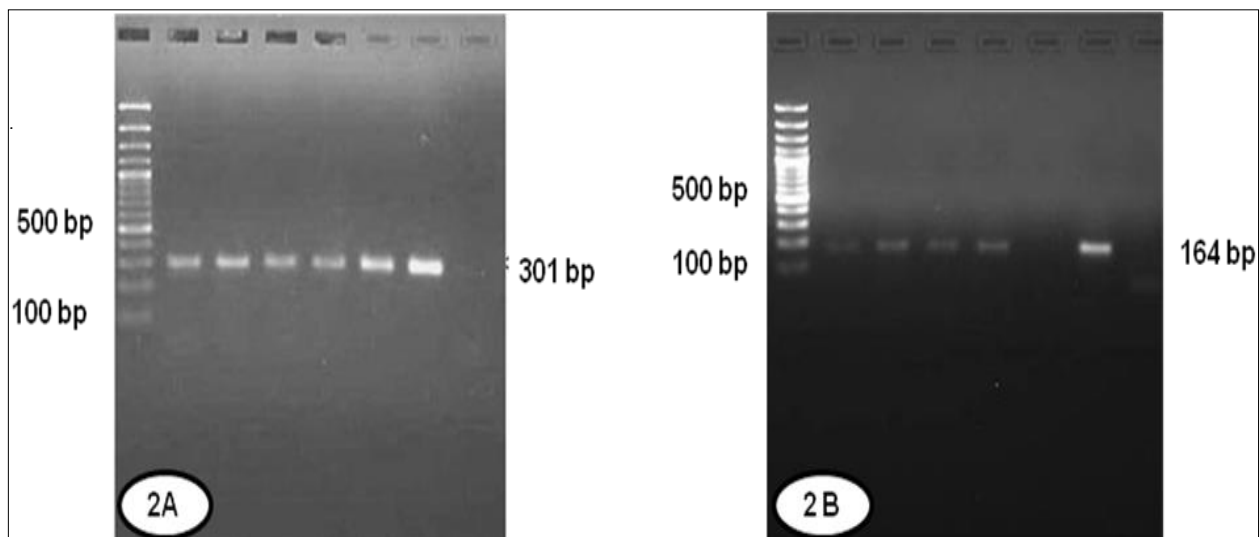


Figure 2A: Equine sarcoids: PCR products in ethidium bromide stained 1.5% agarose TBE gel electrophoresis. M-100bp marker; L1-L6: BPV-1, 301 bp; L7: NTC; 2B: Equine sarcoids: PCR products in ethidium bromide stained 1.5% agarose TBE gel electrophoresis. M-100bp marker; L1-L4 & L6: BPV-2, 164 bp; L7: NTC.

Malignant Tumours

Three tumor cases involving a growth in vagina and two on eyelids were histopathologically diagnosed as squamous cell carcinoma. The neoplastic squamous epithelial cells invaded deep down the dermis in the form of thick cellular cords, irregular sheets and independent cellular islands. In between the cellular islands, proliferation of fibrous connective tissue was seen. Aggressively proliferating neoplastic cells in large irregular sheets frequently exhibited nuclear indentations, mitotic figures and increased nuclear-cytoplasmic ratio (Figure 1 E&F). Necrotic areas with infiltration of inflammatory cells including polymorphonuclear and mononuclear cell and engorged blood vessels were also seen elsewhere.

Non-Neoplastic Conditions

Of three non-neoplastic conditions, one was diagnosed as necrotizing myositis and two were necrotizing dermatitis (Table 1). Microscopically the former was characterized by severe necrosis of muscle and connective tissue with infiltration of neutrophils and lymphocytes and presence of few isolated intact myocytes amidst necrotic debris. Necrotizing dermatitis was characterized by severe necrosis with infiltration inflammatory cell comprising of lymphocytes, neutrophils and eosinophils in addition to engorged blood vessels

PCR Results

All 16 DNA samples from sarcoid-like lesions from mules and horses were subjected to PCR. Results are presented in Table-1. Out of these, 5 samples were positive for BPV-1, 3 for BPV-2 and 6 showed mixed infection of both BPV-1 and -2. One each case of squamous cell carcinoma and fibroid were found negative for both BPV-1 and -2. The case of necrotizing myositis was positive for both BPVs while cases of necrotizing dermatitis were positive for BPV-1 only. Overall, 68.75% (11/16) and 56.25% (9/16) positivity was seen for BPV-1 and -2, respectively. Gel electrophoresis showed the specific PCR product size of 301 and 164 bp for BPV-1 and -2, respectively (Figure 2 A&B). Selective PCR products were sequenced and the generated sequences of BPV-1 and -2 were submitted to NCBI and EMBL database, respectively and accession numbers were assigned (KF114852, KF114854, KF114855 and KF148688). Partial BPV-1 sequences generated in the present study exhibited close homology among themselves. Similarly, generated BPV-2 sequences were also identical. On comparison, BPV-1 & -2 partial sequences generated in present study revealed close homology as well as with the earlier published BPV-1 & -2 sequences on NCBI.

DISCUSSION

Equine sarcoids and other tumors like squamous cell carcinomas seriously compromise the welfare of affected horses, donkeys and other equids and attributed to considerable financial losses to the owners. There are several different clinical manifestations of equine sarcoids, which have variable macroscopic appearances, growth patterns and behavior ranging from slow growing occult lesions or even, less frequently, spontaneous regressing lesions to locally aggressive fast growing and ulcerative fibroblastic lesions (McConaghy et al., 1994; Scott and Miller, 2003; Knottenbelt, 2005). In the present study, 16 skin lesions/growths of equines (Table 1) were studied. Macroscopically, these lesions/growths were small, solitary, variable in size and located on eye lids and different parts of the body. These findings are in accordance to earlier reports (Knottenbelt, 2005; Wobeser et al., 2010; Kainzbauer et al., 2012). Histopathologically, these cases were diagnosed as neoplastic (81.25 %) and non-neoplastic (18.75%) conditions. Among neoplastic conditions, benign tumours (62.50%) included fibroid (37.5%), occult sarcoid (12.5%), fibromatosis (6.25%) and fibropapilloma (6.25%) whereas, malignant

tumours (18.75%) were all diagnosed as squamous cell carcinomas. Benign tumours in equines occurring in various body locations have been reported from different parts of the world (Martens et al., 2000; Chambers et al., 2003; Hallamaa et al., 2005). Similarly, occurrence of squamous cell carcinoma in the head and neck region, particularly in the periorbital, parotid areas and in the jugular groove of a Connemara mare (Kainzbauer et al., 2012) has been described. In present study, remaining 18.75% were non-neoplastic conditions and diagnosed as necrotizing dermatitis. Cases of dermatitis associated with BPV infection have been described in horses (Yuan et al., 2007; Wobeser et al., 2012).

The skin biopsies tested for presence of BPV-1 and -2 DNA by PCR revealed that 31.25% were positive for BPV-1, 18.75% for BPV-2 and 37.5% for both types. One fibroid and one squamous cell carcinoma were found negative for BPVs while both cases of necrotizing dermatitis were positive for BPV-1. Earlier Campo (2006) reported that the bovine warts are a rich reservoir of BPV-1 virions and an equally high percentage of sarcoid affected horses show latent BPV infection in the normal skin. The relatively high occurrence of BPV infection in apparently healthy horses living in close contact with papilloma-affected cattle is indicative for a BPV cross-species transmission from bovines to equines. In horses living in direct contact with sarcoid affected horses, a 50% rate of latent infection of BPV has been observed (Bogaert et al., 2008). Our findings were more or less in corroboration with that of Martens et al., (2001) who also detected BPV DNA in 88% of swabs and 91% of scrapings from sarcoid-affected lesions and no BPV DNA could be detected from non-sarcoid lesions. BPV-1 and -2 DNA have been detected in sarcoids all over the world, either alone or as mixed infection of both types in the same horse (Teifke et al., 1994; Nasir and Reid, 1999; Bogaert et al., 2007). Our findings are in agreement with previous observations. In Europe, BPV-1 accounts for a vast majority of DNA detected with only a small number of sarcoids containing BPV-2 (Otten et al., 1993; Teifke et al., 1994). Studies in the United States have shown BPV-1 and BPV-2 in roughly equal proportions in the eastern United States (Teifke et al., 1994) while BPV-2 predominates in the western United States representing 63% of the amplified DNA (Carr et al., 2001). Equine sarcoids are most commonly associated with BPV-2 in western Canada (Carr et al., 2001; Wobeser et al., 2010). In horses with multiple tumours or same sarcoid sample, both types of BPV were demonstrated in the same animal (Martens et al., 2001; Bogaert et al., 2010; Wobeser et al., 2010). Despite the consistent detection of papillomavirus DNA in the sarcoid lesions, papillomavirus particles have not been demonstrated till date. BPV-1 and -2 infection is not uncommon in military dairy farms and cattle in the rural areas around Bareilly, Uttar Pradesh (Pangty et al., 2010; Kumar et al., 2013). Since horses and cattle are housed in the same premises and at times share pastures, the potential scope for cross-species transmission is always there. Systemic studies on equine skin lesions, particularly sarcoids were not conducted earlier in India and our findings suggest that skin biopsies should be analyzed for better formulation of treatment strategy in valuable equids.

CONCLUSION

Sarcoids are common lesion of the skin of equines and are associated with the BPVs. In India only limited field case studies and reports on this aspect is available. BPV-1 & -2 and their mixed infections were detected in histopathological confirmed cases of sarcoids/ squamous cell carcinomas. Further systemic research is needed to determine the importance of BPVs and/or other equine papillomaviruses as a cause of sarcoids and different clinical conditions.

ACKNOWLEDGEMENTS

We are thankful to Head, Division of Pathology and Director, IVRI, Izatnagar, UP, India for all necessary facilities for carrying out this research work.

AUTHORS DECLARATION OF INTERESTS

No conflicts of interest have been declared.

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