STUDIES ON BOVINE AND BUBALINE PAPILLOMATOSIS WITH SPECIAL REFERENCE TO ITS EPIDEMIOLOGY, CLINICOPATHOLOGY AND THERAPEUTICS

Synopsis of Thesis
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Bovine papillomatosis (BP) is a chronic contagious proliferative disease characterized by warts or papilloma that occur mostly in cutaneous form and less frequently in mucosal form as exophytic papilloma. Cutaneous fibro-papillomatosis in cattle and buffalo is very common and is characterized by the presence of multiple benign exophytic proliferations of the epidermis and of the underlying derma, which is again associated with infection with a BPV. Cutaneous warts in buffalo or bubaline cutaneous papillomatosis (BUCP) although a less studied disease in the country and abroad it has been emerging as an important clinical entity in West Bengal, not reported and studied earlier. Not only cutaneous papillomatosis but also teats and udder warts, defined to be the teat papillomatosis (TP) in both cattle (CTP) and buffalo (BTP) has been documented and recognized in different districts of West Bengal. Bovine papilloma virus (BPV) affects mainly the epidermis but also the dermis of both cattle and buffalo and were found to cause benign proliferative solid outgrowth or the cutaneous neoplasm, categorized to be either papilloma or fibro-papilloma respectively. The present investigation was proposed to undertake etiopathological studies on bovine and bubaline papillomatosis including therapeutic studies. The objective of the present study was to evaluate the clinicopathologic findings and cytomorphologic classic alterations associated with papillomavirus infection through anatomopathologic exam of tumour samples of cattle and buffalo origin and to verify them both by histopathological examination and EM studies. Involution of the papilliform lesions after treatment with different therapeutic agents were also studied.

Clinically, CWTs were observed in organised farms as well as rural areas in both the cattle and buffaloes. Surveys of both BOCP and BUCP including CTP and BTP were conducted for the period from August-2008 to January-2013 by different mode of survey like field survey, farm survey, institutional survey and abattoir survey in different districts of West Bengal. Altogether one thousand seven hundred sixty nine wart cases were recorded of which cattle wart cases including CTP were one thousand six hundred forty and buffalo wart cases including BTP were one hundred twenty nine. Warts recorded in the random field survey were nine hundred thirty nine. In institutional survey, i.e. survey in veterinary clinics altogether five hundred fourteen cases were documented. In organized farm survey warts cases recorded, were two hundred thirty two and in special survey for CTP in organized farm it was forty three and for BTP it was forty one. Year wise recorded clinical incidence of warts through all modes of survey in cattle and buffalo as studied since August 2008 to January 2013 revealed highest incidence in Year-2010 (BOCP-597 & BUCP-48, n = 645), followed by Year-2011(BOCP-413 & BUCP-11, n = 424) , year-2012 (BOCP-391 & BUCP-26, n =
Gender based affection as recorded in cattle revealed higher incidence in females (n = 1280) one thousand two hundred eighty (i.e. 78.06% incidence in female bovines) as compared to warts affliction in males (n = 360) three hundred sixty (i.e. 21.93%). In case of BUCP, gender based affliction as recorded in buffaloes revealed higher incidence of warts was recorded in females (n = 115) one hundred fifteen (i.e. 89.84%) as compared to males (n = 13) thirteen (i.e. 10.15%).

Clinically all three different grades of infection (mild, moderate and severe) were recognized in cattle. Whereas in great majority of cases, mild to moderate grade infection and scarcely the severe grade infection was recorded in buffaloes. Warts appeared elsewhere on the animal body having either solitary, diffuse or scattered lesions as localized infection or extensive distribution of lesions with multi-centric affection all throughout the body, called generalized papillomatosis or whole body affection. Topographic distribution of warts in cattle as observed in the present investigation revealed highest incidence in anterior region (n = 857), followed by posterior region (n = 533) including teat papilloma, antero-posterior (AP) region (n = 197) and whole body affection (n = 63) respectively. CCWT and BCWT as recorded and or observed on external body surface had varying morphological appearance; either stalked (pedunculated) or sessile (broad based) bearing hard, horny or rough and tough papillomatous structures. Grossly the warts occurred around eyes, ears, ear pinnae, head, face, neck, shoulder, limbs, thorax, abdomen, back, axilla, udder, teats and around genitalia or at perineum and occurred predominantly in young animals. The lack of susceptibility of adults to natural cutaneous infection being ascribed to immunity acquired by apparent or in apparent infection when young.

The warts varied in size from 0.5 cm upwards and they were dry, horny, cauli-flower like in appearance in great majority of CCWT and BCWT cases. Warts on teats were flat, round in shape; dome shaped frond type, filliform and rice grain type. Some warts in complicated cases were oozing owing to myiasis and secondary bacterial invasion could be seen. The CWTs were found to be transmitted from cattle to buffaloes and vice versa and the incidence of TP in both the species also confirmed the condition. In some organized farms warts were confined mostly to ear pinna which could be due to the fact that these were transmitted from the infected to healthy one by tattooing machine. Grossly it was observed that few CWT cases of cattle spread extensively to the adjacent areas forming large cauliflower like masses but in buffaloes, almost all cases showed solitary growths.

Warts were more concentrated at anterior region and its incidence was more in tender aged animals (young, adolescent or sub adult group). The wart like cutaneous outgrowths showed cauliflower, club-shaped, nodular, dome shaped, filiform or rice grain-like
appearance on the surface of the skin. The higher incidence of the disease was recorded in young animals aged between 1-3 years and the incidence rates of the disease decreased by age. The lesions frequently occurred on the surface of the head, neck, around the eyes, limbs, thorax, barrel, back, perineum, udder, teats and external genitalia. Papillomas appearing on the body surface were benign and none died. In some buffaloes warts appeared around the vulva and at anal periphery. Those were typically hard, rough and tough with white colouration all throughout the tumour surface and were tightly adhered to the skin with broad base.

Since cutaneous virus is shed from the epidermis in dehydrated squamous it is not surprising that transmission can occur via fomites. Besides direct transmission from infected to susceptible healthy individuals certain vectors like ticks and flies were also suspected for the spread or transmission of the disease. Since in the investigation it was found that most of the wart bearing animals had suffered from ecto-parasitism (like ticks infestation) like other authors our suspect also goes to the ticks as the vector helping in transmission of the disease.

Haematological studies of CWT affected cattle and buffaloes showed significant increase in the TLC and lymphocytes and decrease in PCV and Hb. Serum biochemical studies in infected cattle and buffaloes revealed significant increase in albumin and A/G ratio. Cholesterol was decreased in BUCP and increased in BOCP cases. Total serum protein was decreased in both the infected cattle and buffaloes.

Warts were diagnosed by physical examination and were defined by morphology, location and host immune response. Papilloma virus induced lesions in the study were usually multiple and were sufficiently characteristic to confirm the diagnosis macroscopically. However there may be simulants of warts, which needs a definitive diagnosis requiring identification of the virus or its cytopathic effects (CPE) on the individual cell i.e. a change known as koilocytic atypia or koilocytosis. Diagnosis was based on clinical signs and confirmed by histopathology of bovine and bubaline cutaneous and teat warts, followed by ultra-structural studies by both SEM and TEM studies. Further confirmation was made by DNA identification by polymerase chain reaction (PCR) technique in biopsy or tissue scrappings.

Epidemiological status of BOCP including CTP in Bengal was determined. Besides CCWTs and CTPs, epidemiological aspect of bubaline cutaneous papillomatosis (BUCP) including BTP was also determined. Prevalence of the disease in cattle based on field survey was 3.03% and in buffaloes it was 0.90%, reflecting higher prevalence of the disease in cattle as compared to buffalo. Females were more prone to infection as compared to males, reflecting gender affinity based on the survey carried out in WB both in case of buffalo and cattle. Among one thousand six hundred forty BOCP cases, three hundred sixty were male
(21.95%) and one thousand two hundred eighty were female cattle (78.04%). Seasonal affection as noted in CCWT cases in field survey revealed higher incidence in summer (n = 449), followed by winter (n = 329) and rainy (n = 99) respectively revealing percentile seasonal incidence as 51.19%, 31.51% and 11.28% respectively. In regards to gross regional distribution of warts in cattle, the highest affection was observed in anterior region (n = 857). Second highest was the posterior or caudal regional distribution (32.5%). Posterior or caudal affection including CTP was observed in five hundred thirty three (n = 533) and antero-posterior (cranio-caudal) regional affection was noted in one hundred ninety seven (n = 197) animals. Whole body affection was observed in sixty three cases. The percentile affection in regards to gross regional distribution pertaining to anterior, posterior, antero-posterior and whole body affection was 52.25%, 32.50%, 12.01% and 3.84% respectively. Breed predisposition in regards to clinical affection as studied based on field survey for BOCP cases, revealed four hundred sixty six cases in ND and indigenous cattle (male-140 and female-326) and four hundred eleven in cross bred cattle (CBJ male-42, CBJ female-345, HLX male-1 and HLX female-23) and on institutional survey for BOCP cases, showed two hundred eighty five in ND and indigenous cattle (male-118 and female-167) and two hundred twenty nine in cross bred cattle (CBJ male-19, CBJ female-196, HLX male-0 and HLX female-14). Economic importance of the disease was also documented in both cattle and buffalo. Generalized and or whole body papillomatosis was of great concern leading to the depreciation of animal value. The incidence of papillomatosis was found to be much higher in imported and cross bred cattle than in indigenous cattle. Although the disease is not of much importance, its high infectivity, chronic illness leading to un-thriftiness and the damage of the skin due to generalized warts were matter of concern and economic losses. Extensive warts lead to depreciation in both aesthetic and economic value of the animal due to loss of body condition, hide’s value, increased risk of conditions like mechanical trauma, wounds, haemorrhages, fly blown condition (myiasis), necrotic dermatitis, mastitis, interference in suckling, milking and coitus. In spite of the benign nature of the disease, the benign progression demands attention, for hyperplastic lesions could depreciate the pelt in affected animals; when located in the udder, it led to secondary infections and lactation problems. Several economic consequences were determined, as cows with teat papillomas could not be milked, young calves could not suckle, and often the pedunculated papillomas could snap off, the sites became infected and mastitis would ensue with distortion of the milk canals.

It was noted that both the cattle and buffalo developed papillomas and fibropapillomas (a benign fibroblastic proliferation with overlying acanthosis, hyperkeratosis, and a down growth of rete pegs) depending on the ratio of fibrous connective tissue to hyperplastic epithelium. Histologically, CCWTs showed finger-like projections covered by
hyper-keratinized layers along with hyperplasia of the epidermis where stratum spinosum showed degeneration and prominent vacuolations. Stratum granulosum containing scattered koilocytes and virus infected cells with clear cytoplasm. Fibroplasia was seen in its dermis in most of the cases. Histopathology of CTP revealed multiple papillary projections of fibrovascular connective tissue covered by hyperplastic stratified squamous epithelium. Histologically, acanthosis and hyperplasia of granule cells in the stratum granulosum were the common findings in the teat lesions. Besides exophytic warts or papilloma one endophytic fibro-papilloma case was diagnosed through histo-pathological studies on CCWT but no such case was seen in BCWT.

In BCWT cases besides, hyperplasia of epidermis, there were presence of numerous vacuolated cells and a few mitotic figures. Fibromatosis was pronounced in these animals. All these cases were diagnosed as fibropapilloma. The tumours were seen to consist of homogeneous, smooth connective tissue covered by hyperplastic epidermis with marked acanthosis and hyperkeratosis. Many koilocytes were present in stratum spinosum, characterised by large, mildly eosinophilic and vacuolated cytoplasm.

Ultra-structural studies by SEM revealed top surface architecture of tumour stroma and cyto-morphological alterations of the cells both in CCWT and CTP as well as in BCWT and BTP. Marked proliferative changes in epidermal cells and engorgement of blood vessels in the dermal layer were the characteristic findings. On SEM study lateral view of the wart revealed strong uneven longitudinal folds on its surface, epical view showed multiple pores of varying diameters and multiple grooves inside the pores as seen on higher magnification. Numerous pores all throughout the surface of the wart were self-explanatory for presenting sponge like appearance.

TEM studies revealed both nuclear and cytoplasmic changes induced by the BPV infection both in cattle and buffalo warts. BPV induced both lytic and proliferative changes in cells, most notably in keratinocytes. TEM studies of BCWT and CCWT showed cornified and more or less structure less tumour stroma and tumour cells found to have bizarre forms of nucleus. Few basal cells and scarce cytoplasmic organelles like mitochondria and RER were observed. Secretory vesicles and keratinocytes were also prominent. Bizarre shaped nucleus with electron dense heterochromatin, focal nuclear atypia and cytoplasmic vacuolization and damage of desmosomes or its detachment were most characteristic findings. In nuclear part of the keratinocytes there was productive phase of virus replication and there were presence of plenty of electron dense intranuclear icosahedral BPV like biomolecules.

Virus particles were found in some nuclei of the keratinocytes. Virions exhibited icosahedral symmetry, were approximately 35-45 nm in diameter and did not have an envelope. Capsomer arrangement was readily seen in electron micrographs.
BPV-specific DNA was detected from all cutaneous specimens irrespective of cattle and buffalo by PCR analysis. Besides cutaneous specimens in teat specimens of both cattle and buffalo BPV specific DNA was also detected. Of course in few TP samples BPV specific DNA could not be detected which could be due to the lack of viral DNA present in the sample or could be due to the presence of some other type of BPV other than BPV-1,-2,-5,-9 & -10. BPV-Type 1 and Type-2 were prevalent in WB and played important role in developing teat papillomatosis in Bengal. Due to the virulence of both viruses, widespread and long-lasting teat papilloma cases might have induced and emerged in Bengal. Persistently infected cutaneous BPVs harboured in the herds might induce teat papillomas too.

PCR based detection of prevalent BPV types in entire Bengal was done that revealed Both BPV-1 and BPV-2 types and co-infection or mixed infection of both the types present in ailing animals. Positive bands (301bp and 165bp) were found by using BPV-1 and BPV-2 specific primer sets targeting L1 gene. All of the amplified fragments were confirmed to be either BPV-1 or BPV-2-specific DNA by sequence analysis. On the other hand BPV-specific DNA to other BPV types as tested was not detected from any of the specimens.

The amplification and dissociation plot of real time PCR assay of CCWT samples revealed that eleven samples were BPV-1 positive, sixteen samples including two healthy skin samples were BPV-2 positive and five were positive for both the types of viruses. On the other hand the amplification and dissociation plot of real time PCR assay of BTP samples revealed that seven samples were BPV-1 positive and six samples were BPV-2 positive. No healthy teat skin sample harboured any one of the BPV type. BPV types as determined by PCR based study were also subjected to cloning and sequencing of some of the amplified products of both the types. Molecular phylogenetic tree of the BPV isolates of West Bengal and representative BPV types (both BPV-1 & -2) of Indian origin was generated by neighbour joining and those revealed close phylogenetic lineage in case of BPV-2, L1 with higher bootstrap value, and lower bootstrap support in case of BPV-1, L1. BPV-1,L1 WB origin was found in a monophyletic branch and BPV-1, E proteins evolutionary tree, clustered with high bootstrap support for BPV-1, Indian origin.

Comparative sequence analysis of both BPV-1 and -2 types of West Bengal with that of Indian types as previously reported revealed that both the types had closely homologous genetic variants. On analysis of the sequences, 98% homology was seen in partial sequence of L1 gene of BPV-2 with that of the published sequences of BPV-2, L1 of Indian origin. Phylogenetic study revealed close lineage with the published sequences of BPV-1, L1 and BPV-2, L1 of Indian origin. Although BPV-2 WB origin had close resemblance to Indian isolates BPV-2 (showing 98% sequence homology), BPV-1 WB origin was distantly related in terms of sequence homology as compared to previously published sequences of BPV-1, L1 Indian origin.
In the present study, some homeopathic preparations available in the market (like sulphur and Thuja-mother tincture and Thuja 200X) and their use by per-oral route and Thuja 200X by S/C route was sought to be used as an alternative of surgical intervention and other cost effective therapy for treating warts. It could give relief to the owner from discharging the cumbersome job of daily antiseptic dressing, cost of antibiotic administration, meticulous cleanliness if be got it treated by surgical method. Thuja mother tincture per oral route of administration in treated group of ailing animals brought complete warts cure in 76.66% cases whereas Thuja 200 X potency in the same route of administration caused 80% complete recovery in affected animals. Thuja 200X when administered parenterally caused sixty percent complete clinical cure and thirty percent partial to moderate regression of warts in animals. Comparatively lesser effectiveness as achieved in parenteral therapy of Thuja 200X could be due to the treated animals bearing larger and broad based sessile type of warts. Effectiveness of Thuja 200X potency was better than Thuja mother tincture. Although homeopathic remedy took somewhat long duration in making complete clinical cure, they were found safe, cheap and economic. Regression of warts was also histologically evaluated and those were characterized by moderate kariolisis, progressive degeneration of the verrucous tissue and formation of epithelial tissue close to normality.

The host response to papillomavirus infection is a complex process of skin barrier protection, innate immunity, and acquired immunity. Warts usually will regress over time and after six months of infection, 30% of warts will clear on their own (Messing and Epstein, 1963). Warts usually shrink off after a few months. Multiple injections of autogenous vaccines were credited for being an effective cure. This study provided strong evidence of the effectiveness of formalinized autogenous vaccine in clinical cure of BP with a success rate of 55% (in formalinized autogenous vaccine diluted with glycerol saline), 60% (in formalinized autogenous vaccine diluted with PBS) and 85% (in formalinized autogenous vaccine diluted with normal saline). Whereas 70% alcohol inactivated autogenous vaccine diluted in PBS caused 70% complete cure of BP.

Auto-haemotherapy or self-blood therapy as administered to the clinically affirmative cases of cutaneous warts, revealed signs of regression after fourth administration. Auto-hemotherapy alone had brought 90% recovery in ailing animals whereas auto-hemotherapy along with Levamisole brought about 100% complete recoveries. The papillomatous growth gradually dried up and there was gradual regression in size of the warts, followed by their sloughing, leaving either scar marks or cutaneous sores. The study revealed that auto-hemotherapy were found to be one of the effective conventional therapy to cure papillomatosis of cattle and buffalo and the animals get rid of papillomatosis with in four weeks of last administration. Hence without using any chemical agent, only auto-
hemotherapy can also be employed to treat cutaneous papillomatosis (CP). Hundred percent clinical cures was obtained in combined levamisole and self- blood therapy (auto-hemotherapy) reflecting most efficacious therapeutic approach in treating BP.

Having treated bovine papillomatosis with levamisole at a dose of 2.5 mg/kg/day on days 1,3,5,7,9 and 16, by per oral route, 82% recovery was achieved and somewhat superior result was obtained (88% recovery) in levamisole parenteral therapy with the same dose rate and same spells of administration. Non-specific stimulation of the immune system by oral or parenteral formulation and administration of levamisole might have brought about the clinical recovery with the shedding of warts.

Single shot S/C administration of Ivermectin resulted in complete recovery in 70% cases with partial recovery in 23.33% and no response in 6.66% cases. Oral Ivermectin therapy was found less efficacious as compared to injectable Ivermectin and Levamisole therapy. Cure rate of Ivermectin therapy on bovine papillomatosis as studied revealed complete clinical cure 53.33% in oral therapy, 70% in single shot S/C Ivermectin therapy and 86.67% in double shot S/C Ivermectin therapy. Partial to moderate cure was seen in 26.67% cases in oral therapy, 16.67% in single shot S/C Ivermectin therapy and 13.33% in double shot S/C Ivermectin therapy. No clinical alteration or recovery was noted in 20% cases in oral Ivermectin therapy and 13.33% in single shot S/C Ivermectin therapy. Effect of three different regime of Ivermectin therapy (per oral, single S/C and double shot S/C Ivermectin) clearly reflects the significant effect on recovery from clinical papillomatosis where double shot S/C Ivermectin was found superior to two other regime of Ivermectin therapy.

Having treated with antiomalone alone, caused 77.77% complete wart recovery in infected animals and with antiomalone in conjunction with S/C Ivermectin therapy caused 82.22% clinical recovery showing better therapeutic response and or effectiveness in combined therapy.