Papillomatosis is a disease of most of the domesticated and wild life species characterized by the formation of warts on the affected skin surface causing cutaneous papillomatosis or cutaneous warts. Cutaneous papillomas are common skin tumors in animals and papillomavirus infection is the most common cause of papillomatosis or warts. Their forms were likewise various; some were found flat, upon a broad basis; some were filiform or rice grain like; others were conical, growing on a pedicle and some others appeared cauliflower like on the surface of the skin. Numerous descriptions of unwanted and or abnormal epithelial outgrowths in the skin of many species have been reported (Karstad and Kaminjolo, 1978; Theilen et al., 1985; Hayward et al., 1993; Le Net et al., 1997; Uzal et al., 2000; Sundberg et al., 2000, 2001; Schulmann et al., 2001; Literak et al., 2006; Nespeca et al., 2006; Tobler et al., 2006; Munday et al., 2007; Rector et al., 2008; Stevens et al., 2008; Lange et al., 2009; Silvestre et al., 2009; Bam et al., 2012) and such lesions were associated with papilloma viruses of different genera under the family papilloma viridae according to the species concern (Alfieri and Khazaeil, 2000). Conspicuous amount of fibrous connective tissue at the base forming cores upon which neoplastic epithelial cells are massed, are the common characteristic feature associated with infection with a papilloma virus (PV). Members of this genus are known from humans, deer, dog (Tobler et al., 2006, 2008), cat (Felis domesticus) (Sunderberg et al., 2000, Tachezy et al., 2002), horse (Angelos et al., 1991), donkey, mules, sheep (Hayward et al., 1993), elephant, European bison (Literak et al., 2006; Tomita et al., 2007), elk, opossum, tapirs (Kidney and Berrocal, 2008), multi-mammate, giraffe (van Dyk et al., 2011), zebra (Equus zebra) (van Dyk et al., 2009), captive zebra (Equus burchellii) (Lohr et al., 2005) and European harvest mice, turtle, chaffinch and parrot etc.

Bovine cutaneous papillomatisos is a common viral disease of cattle, manifested as benign tumors or warts on skin surface and are caused by infection with the contagious Bovine papilloma virus (BPV) (Almeida and Waterson, 1969; Barthold et al., 1974; Olson, 1993). Bovine papillomatisos has emerged to be an important disease leading to economic depreciation of animals, deterioration of the appearance and of the leather. Different BPV types preferentially infect either cornified stratified squamous epithelium of skin or uncornified mucous membranes. The appearance of the lesions are influenced not only by viral type but also by environmental and host factors. The benign and malignant tumors caused by BPV are of emergent disease importance for beef and dairy cattle in the world. In the present investigation our efforts were directed to study the incidence of the disease, its prevalence, epidemiology, determination of the cause of the disease and its therapeutics in respect to West Bengal in India.
BPVs are a heterogeneous group of epitheliotropic viruses that recognize bovines as its classical host. Although twelve BPV types have been characterized and classified into three genera in global context, two of the viral types under the genera delta papillomavirus (BPV-1 and BPV-2) cause most of the warts found on the animal body irrespective of breed and sexes, with a high incidence in young bovines as compared to adult ones in Indian context. Of course very recently BPV-10 has been detected in cattle teat wart cases in UP (India) by SISPA DNASE technique (Rai et al., 2010).

It has been established that both BPV-1 and BPV-2 are the etiologic agents of cutaneous and genital fibro-papillomas in cattle (Campo, 2002). However, it has been demonstrated that both BPV-1 and BPV-2 can jump species and infect bison and equids. Since no systemic studies or investigation was made on this disease and prevalent virus types was not detected earlier in West Bengal, present investigation was proposed to undertake on studies of bovine and bubaline cutaneous papillomatosis with special reference to the detection of prevalent virus types as etiology, epidemiology of the disease, it’s clinico-pathology and therapeutics as it’s remedy.

Cutaneous warts in bovine and bubaline species are benign tumors induced by host-specific papillomaviruses which are entered through any skin abrasions. Papillomas were small solid outgrowths those appeared on the skin, udder and teats of both cattle and buffaloes were screened and recorded. Surveys of BOCP and BUCP were conducted randomly in dairies and in rural areas, during the period from December- 2008 to January-2013, in various districts of West Bengal covering at least one GP of a Block of the respective District or at least one Ward of the respective Municipality of the said district. Prevalence of BOCP was almost 14 fold higher than BUCP. In all three different modes of clinical survey it was obvious that both male and female cattle and buffalo had suffered from BOCP and BUCP. It was observed that females had the maximum affection as compared to males. Whereas in an earlier study it was observed that only females had suffered from the disease as recognized by Maeda et al., (2007). In contrast, other studies had demonstrated the presence of papilloma in both female and male bovines (Bagdonas and Olson, 1953; Oz soy et al., 2011) and buffaloes (Nooruddin et al., 1989, Chelapati Rao, 2010) causing BOCP and BUCP. Our study also goes with the findings of earlier workers (Leishangthem et al., 2008, a, b; Singh et al., 2009; Pangty et al., 2010; Kumar, 2012). Total wart case recorded was one thousand seven hundred sixty nine (n = 1769), of which BOCP was one thousand six hundred forty (n = 1640) and BUCP was one hundred twenty nine (n = 129). Such a hues occurrence of papillomatosis as recorded in both cattle and buffalo during the present investigation would probably be the highest one ever reported. The cutaneous and para-genital lesions as recorded in buffaloes during the present investigation were characterized by multiple gray-white papillary growths
of tough consistency. Surface showed sessile vegetative outgrowths. These findings were in agreement with the findings documented by Silvestre et al. (2009) in buffaloes suffering from bubaline papillomatosis in Italy.

Disease occurrence in the present investigation was categorized as: not detected (no incidence), 2: intermittent and 3: permanent and accordingly the endemic nature of the disease were proved in certain organized dairy farms in Bengal. The trend of the disease occurrence could be characterized as: 1: declining; 2: stagnating or 3: increasing and all three categories were noted in individual farms. With the adoption of strict precautionary measures and health care like proper deworming, good plan of nutrition and supplementation of minerals and vitamins, the trend of the disease has been declining in organized farms, as studied.

Warts recorded in random field survey in both cattle and buffalo was nine hundred thirty nine (n = 939) and in institutional search it was five hundred fourteen (n = 514) only, based on day book inspection and questioner survey. This study clearly reflects that although the clinical cases of cutaneous warts were highly prevalent among the susceptible bovine population, all the cases were not brought to the clinics for quick cure by treatment or surgical excision.

In organized farm survey total warts recorded was two hundred thirty two which revealed that warts not only occurred in rural unorganized dairy sector but also it equally occurred in organized dairies. Special survey intended to record cattle teat papillomatosis in organized farm, revealed forty three CTP cases and for bubaline teat papillomatosis; BTP was recorded in unorganized dairy sector numbering forty one (BTP, n = 41). Although BOCP was observed in almost all the districts of West Bengal both in farm and field condition prevalence of BUCP was meager and of course it was not recorded in all nineteen districts of West Bengal. This could be due to very minimal buffalo population in those districts. However the present study revealed that the disease was prevalent in West Bengal and could take the shape of an emerging disease entity. BUCP was almost unknown entity in WB before this investigation was initiated.

Incidence of papillomatosis or papilloma was recorded either as questionnaire survey or by day book inspection in the Veterinary clinics (BAHC, ABAHC and SAHC) of Animal Resources Development Department under the Directorate of Animal Husbandry and Veterinary Services, West Bengal and above all clinically affirmative cases were recorded by physical inspection of different herds during field survey both in farm and field. Prevalence of BOCP in farm condition was recorded in Government Dairy farms like State Livestock Farm (SLF, Kalyani, Nadia, 0.72%-7.40%), Haringhata Farm, (Mohonpur, Nadia, Government Dairy, 0.52%-0.90%), Bull Mother Farm (Paschimbanga Go Sampad Bikash Sangtsha : PBGSBS, Haringhata, Nadia, 0.33%-1.06%), Institute Dairy Farm (National Dairy Research Institute, India, 0.50%-0.90%), and in State Livestock Farm (SLF, Motipur, Nadia, 0.72%-7.40%) and Haringhata Farm, (Mohonpur, Nadia, Government Dairy, 0.52%-0.90%).
Institute, Eastern Regional Station/NDRI, ERS, Kalyani, Nadia, 1.44%-2.2%), organized dairy farms of different Goshala(s) namely Calcutta Pinjrapole Society Dairy Farms (1. CPS Dairy, Anandanagar, Nadia District, 0.33%-0.73% 2, CPS Dairy, Uluberia of Howrah District, 0.28%-0.66% 3, CPS Dairy, Sodepur of Kolkata, 0.23%-0.49%), organized private Dairy (R. K. Mission Dairy, South 24 Parganas, 4.12%-8.5%) whereas prevalence of BUCP other than rural sector, was observed in Government Dairy farms like State Livestock Farm (SLF, Kalyani, Nadia, 4.16%-7.86%), Private Dairies in Kanchrapara and Halisahar Municipality area, Naihati Municipality, of N-24 Pargana District, Bansberia Municipality area, Jhulonia, of Hooghly District, Kalyani Municipality area, Hainghata Gram Panchayet area, of Nadia District.

In the field survey, during standard clinical examination, clusters of papillomas as well as individual (solitary) papilloma of several millimeters in size (size of a grain to that of cauliflower, 6-8cm) were found elsewhere on the animals body at varying anatomical locations and represented in tables showing gross clinico-pathological observations. The ultimate size which a papilloma attains and the time of regression of a wart commences is the two developmental features exhibiting the greatest variability in bovine and bubaline cutaneous papillomatosis. It is well known that in some animals warts could become very large and persist for a much longer time and such observation was also made during the present investigation. This was in agreement to Olson and Skidmore (1959). The papillomas were dry, rough and lightly colored. The extent and duration of papillomatous lesions were variable and could have depended on the type of virus involved, area affected and degree of susceptibility and above all immune status of the afflicted animal (Radostitis et al., 1989). A form of persistent cutaneous papillomatosis with smaller numbers of papillomas occurred in herds of older cattle were also noted. Morphology of the papillomas recorded in this study corresponds with growing or developing as well as regressing phases.

The findings of prevalence and pathology of CWTs in the present study were more or less in accordance with earlier report from UP and Uttarakhand state of Indian subcontinent as documented by earlier workers (Somvanshi et al., 1986; Leishangthem, 2006; Singh, 2007; Leishangthem et al., 2008; Singh et al., 2009; Pangty et al., 2009, 2010) in which 37 natural cases of CWTs in cattle were studied where three types of growth patterns viz. exophytic fibropapilloma (cauliflower like 19 and dome shaped 13) and occult/ fibroblastic papilloma (n = 5) were recorded (Singh, 2007). Further, 20 spontaneous cases of CWTs in Indian water buffaloes were studied histopathologically; among those four cases were exophytic fibropapilloma (cauliflower like) and four exophytic fibropapilloma (dome shaped) and three were endophytic papilloma (cauliflower like 2 and papillary growth 1; Singh, 2007). This study however supports the earlier studies and has generated additional data regarding histological typing of CWTs in cattle and buffaloes.
Four buffaloes and six indigenous cattle from Makkuva, Andhra Pradesh, suffering from moderate grade cutaneous papillomatosis bearing cutaneous lesions on face, neck, antero-ventral parts of the body, legs and teats and also at many other parts of the body were reported and therapeutic management of the disease, by autohemotherapy was reported by Chelapati Rao (2010). The present study however supports the clinical observations as reported by the earlier workers.

In the field survey (including abattoir survey) total eight hundred ninety two numbers of BOCP i.e. (CCWT+CTP = 892) and forty seven numbers of BUCP, i.e. (BCWT+BTP = 47) cases were recorded reflecting percentile recorded incidence as 94.99% BOCP and 5.01% BUCP in field condition. This study documents the presence of cutaneous and para-genital fibro-papillomas including teat papillomas both in cattle and buffaloes in West Bengal. Overall field survey on BOCP revealed percentile affection of CCWT was (n = 810) 90.80% and CTP was (n = 82) 9.19%. Whereas overall field survey on BUCP revealed percentile affection of BCWT as (n = 27) 57.44% and BTP (n = 20) 42.55%. These findings are in accordance with earlier reports of Nair and Sastry, (1955) and Thilakrajan, (1980) who reported an incidence of 6.7% (126 cases) and 6.8% (44 cases) of BOCP in Madras state during 1940-51 and 1952-62, respectively. Paul Gupta et al., (1984) observed an outbreak of papillomas in dairy cattle in Haryana. Since there is no available report on the study of bovine and bubaline papillomatosis in Bengal, present findings could not be correlated or compared.

The macroscopic aspects of the pedunculated and plane papillomas were similar to the description of Gerdes and Van der Lugtz, (1991), Santin and Brito, (2003), Leishangthem et al., (2008) and Marins and Ferreira, (2011). Cattle and buffaloes of both sexes were the bearers of pedunculated or sessile cutaneous round, elevated, flat and or mixed papillomas, reflected cutaneotropic tropism as described by Souto et al., (2005). The tumours were exophytic located preferentially at the anterior part of the body followed by caudal affinity of affliction. Warts with irregular morphology and typical cauliflower like appearance and nodular shape were common clinical observations. In regards to division wise wart incidence in field survey, highest incidence was recorded in Presidency(PDN) division (n = 443, BOCP = 420 and BUCP = 23, i.e. BOCP, 94.80% and BUCP, 5.20%) and lowest in Jalpaiguri (JPG) Division (n = 106, BOCP = 100 and BUCP = 6, i.e. BOCP, 94.33% and BUCP5.66%). Whereas in Burdwan Division (BDN) a sum total of 390 cases was recorded of which BOCP was 372 (95.38%) and BUCP was only 18 (4.61%). In BDN Division among the BOCP, CCWT was 93.54% and CTP was 6.45%, whereas among the BUCP, BCWT was 66.66% and BTP was 33.33%. In PDN Division out of recorded 420 cases of BOCP, CCWT was 86.45% and CTP was 8.35%. Surveys of CCWTs were conducted in various organized dairy farms/ rural/ urban areas of certain regions of Northern India especially in UP, UK, HP,
West Bengal and Tamilnadu (Leishangthem, 2006; Singh, 2007; Pangty, 2009; Hajra, 2009; Nagarajan, 2011; Pathania, 2010; Kumar, 2012). Those surveys revealed that CCWTs were widely prevalent in dairy and rural cattle of the said states of India. Present investigation also revealed that both BOCP and BUCP are widely prevalent in Bengal.

In the Institutional survey i.e. survey in Veterinary clinics, 497 numbers of BOCP and 17 numbers of BUCP cases were recorded which clearly reflects higher incidence of BOCP and least incidence of BUCP as recorded in Veterinary Clinics. Overall institutional survey on BOCP revealed incidence of CCWT was \((n = 415)\) 83.50% and CTP was \((n = 82)\) 16.49%. Whereas overall survey in clinics on BUCP revealed BCWT \((n = 16)\) 94.11% and BTP \((n = 01)\) 5.88%. The study revealed the highest occurrence of papillomatosis of udder and teats were in pluriparous animals. These findings go along with Sharma et al., (2005) who reported that the papillomas of udder and teat were higher in pluriparous buffaloes.

The present investigation revealed cutaneous warts (CWT) as well as teat warts (TWT) were prevalent in almost all districts of West Bengal. Buffaloes being reared along with cattle that suffered from CCWT had also got infected and suffered from warts. The risk of cross-infection of bovine papillomatosis among other species can lead to a state of alert for the economy and public health. Serological studies performed by Buff et al., (1988) confirm the presence of mammary papilloma in a female dog due to cross-contamination with bovine fibropapillomavirus. Also Nasir and Campo (2008) showed that the BPV-1 type infects horse and at times leading to death. Although papillomavirus DNA is consistently found in the sarcoid lesions of horse (Amtmann et al., 1980; Lancaster 1981) and have been found in European bison, buffaloes and zebra. In the present study BPV DNA (BPV-1 &-2) was detected in water buffaloes.

In regards to division wise wart incidence in Institutional survey i.e. survey in Veterinary clinics, highest incidence was recorded in Burdwan (BDN) division \((n = 276)\), of which BOCP was, \(n = 265\) and BUCP, \(n = 11\), i.e. BOCP, 96.01% and BUCP, 3.98%) and lowest in Presidency (PDN) division \((n = 116)\), of which BOCP, \(n = 113\) and BUCP, \(n = 03\), i.e. BOCP,97.41% and BUCP 2.58%). Whereas in Jalpaiguri division (JPG) a total of 122 cases was recorded of which BOCP was \(n = 119\), (97.54%) and BUCP was only \(n = 03\)(2.45%). In JPG Division among the BOCP, CCWT was \((n = 100)\) 84.03% and CTP was 15.96%, whereas among the BUCP, BCWT was \((n = 03)\) 100% and BTP was 0%. In PDN Division out of recorded 113 cases of BOCP, CCWT was 84.07% and CTP was 15.92% and out of total 3 BUCP 100% was BCWT. In regards to overall wart cases in PDN Division, CCWT was 81.89%, CTP was 15.5%, BCWT was 2.58% and BTP was 0%. In BDN Division out of recorded 276 cases of wart, CCWT was 79.7%; CTP was 16.30%, BCWT 3.62% and BTP 0.36%. In regards to BOCP \((n = 265)\), CCWT was 83.01% and CTP was 16.98% and in regards to BUCP \((n = 11)\), BCWT was 90.90% and BTP was 9.09%.
Day book inspection based institutional survey also revealed wide prevalence of the disease throughout West Bengal. As compared to field survey, recorded prevalence of the disease in Veterinary clinics is less and this could be due to the fact that all clinical cases of warts were not brought to the clinics. Since most of the clinical cases did produce little harm to the animals, only severely infected animals and complicated clinical cases (warts with hemorrhagic sores, magotized warts) papillomatosis had been presented in the clinics.

Survey in organized farms was conducted in both Government Dairy Farms (GDF) and private dairies (Goshalas) including institutional Dairy Farms and altogether two hundred thirty two numbers of wart cases (n = 232) was recorded in both cattle and buffalo, of which CCWT was two hundred eight (n = 208, 89.65%), BCWT was sixteen (n = 16, 6.89%) and BTP was eight (n = 8, 3.44%). Higher incidence of warts (n = 170) was recorded in Nadia district (in NDRI, Kalyani, n = 14, PBGSBS, Haringhata, n = 24, GDHF, Haringhata, n = 23, SLF, Kalyani, n = 85 and CPS, Anandanagar, n = 24) followed by Kolkata (n = 23) (CPS, Sodepur), Howrah (n = 22) (CPS, Lilua) and South 24 Parganas (n = 17) (RKM Dairy, Narendraapur). In regards to year wise incidence, higher incidence of wart cases was recorded during year- 2009 (n = 75), followed by year-2010 (n = 56), year-2011(n = 47), year-2012 (n = 46) and year-2008 (n = 5). Results of field survey, farm survey and questionnaire survey (institutional survey) revealed that the disease was highly prevalent in WB but it was neither studied nor reported earlier from WB.

Patho-morphological and epidemiological features of infection as obtained, confirmed the presence of both CWT and TP in both cattle and buffalo populations in West Bengal and closely resembled the patho-morphological features of the infections of cattle and buffaloes as reported earlier from various parts of UP and Uttarakhand (UK) of Indian sub-continent. Such findings as noted in buffaloes also goes in proximity of close resemblance to the observations laid down by Silvestre et al., (2009) from Campania, South Italy. Many infected areas were identified for the first time where cutaneous as well as teat papillomatosis were prevalent but not reported earlier. However results of this study indicate that papillomatosis in cattle and buffaloes proved to be an emerging disease entity in WB not studied or reported earlier. The endemic and geographically confined distribution of bovine and bubaline fibro-papillomatosis established by the present study would claim that host related genetic and above all environmental factors may have an influence on the susceptibility of bovine and bubaline populations to BPV infection. An extensive literature search and results of survey of Indian animal disease databases revealed that the endemic presence of cattle and buffalo fibro-papillomatosis was not detected earlier. Results of the questionnaire survey confirmed the endemic presence of fibro-papillomatosis in various organized dairies of West Bengal. Of course the Indian studies had thrown light on cutaneous
warts in cattle (Leishangthem et al., 2008; Singh et al., 2009; Pangty et al., 2010), buffaloes (Pangty et al., 2010; Singh and Somvanshi, 2010, Nagarajan, 2011) and Yak (Bam et al., 2012) no such study or virological investigation was undertaken in West Bengal. To our believe it would be worth mention that WB could be represented as the hot seat of papillomatosis based on the presently recorded hues wart cases both in cattle and buffaloes.

Cutaneous papillomatosis (CP) is a specific infectious disease in the species of animal in which it spontaneously occurs. Infectious papillomatosis although most often seen in cattle, may also be seen in buffalo. The present investigation also has confirmed this claim. CP occurring in cattle called cattle cutaneous papillomatosis and in buffaloes it could be regarded as bubaline papillomatosis or buffalo warts or bubaline papilloma. Most warts in both the species appeared as epidermal proliferations that had a keratotic surface resembling a cauliflower known as verruca vulgaris. The lesions in cattle and buffalo were encountered on almost any part of the body. Some papillomae are topographically specific and caused by distinct viruses having different antigenic reactions and DNA compositions. Wart lesions were generally regarded as hyperplastic to a form of benign neoplasia as they did not metastasize and kill the affected host (Lancaster and Olson 1982).

Bovine cutaneous papillomatosis (BOCP) and above all bubaline cutaneous papillomatosis (BUCP) including bubaline teat papillomatosis (BUTP) of both the species concern were found to be a common viral neoplastic disease of the skin in West Bengal, being manifested as benign tumors (benign neoplasms) or warts. The clinically affected animals had mostly multiple, hard, typical pedunculated, benign finger like projection or cauliflower like or dome shaped growth on the skin or non-pedunculated, keratinized or non-keratinized horny benign warts of varying shape and size on head, eyes, neck, dewlap, shoulder, brisket, barrel, back, limbs, para-genital region, teats and udder. These sites were usual as also reported by Miller and West, (1972) as on nostrils, mouth, eyelids, head, neck, and dewlap, shoulder, along the lower line of abdomen, udder and teats. These warts were chronic in nature. Etiology of this disease is connecting to papillomaviruses, and cattle are found to be the natural carriers of the virus (Hargis, 1995; Smith 1996; Campo 2002). Bovine papillomatosis has been investigated in different parts of the world. The clinical signs, microscopic alterations on gross lesions observed in this study are similar to those described by different workers (Butler, 1960; Amstutz, 1978; Olson et al., 1982; Abu-Samra et al., 1982; Elzein et al., 1991; Dawlat et al., 1997; Goldschmidt and Hendrick 2002; Atasever et al., 2005; Jelinek and Tachezy 2005; Salib and Fargail 2011). The epidemiology and clinical behavior of BP are suggestive of an infectious and contagious origin. Although BP is a self-limiting disease, occasionally persist and in the presence of additional critical genetic or environmental factors, could progress to malignancy. The clinical warts in our study took long time to regress and animal to recover.
Bovine cutaneous papillomas are observed mainly on the head and neck, but in some animals localization in other parts of body has also been reported (Abu-Samra et al., 1982; Atasever et al., 2005 and Cimtay et al., 2004). A similar pattern of wart distribution with low to moderate number of cutaneous lesions was seen in our study. Maeda et al., (2007) reported warts to have found only in the teats of heifers. In contrast to Maeda et al., (2007) other workers had observed warts on the abdomen, shoulder, chest, flank, base of the horns, eyes, ears, dewlap, neck, jaw, hip, thigh, buttocks, vulva, rectum, knees and posterior back of cattle and buffaloes (Tweddle and white, 1977; Campellaro et al., 1978; Lancaster and Olson, 1982; Nooruddin et al., 1989, Jelinek and Tachezy, 2005, Turk et al., 2005; Wosiacki et al., 2002; Claus et al., 2007; Borzacchiello et al., 2009; Ozsoy et al., 2011; Catroxo et al., 2013; Lunardi et al., 2013). Present study also supports the earlier literatures regarding the appearance of lesions on animal body. Clinically numerous number of warts emerging from cutaneous surface affecting larger areas of the body was also noticed which clearly reflects generalized and or whole body papillomatosis not reported earlier either from West Bengal or from any other provinces of India by earlier workers. Although affected cattle are usually younger than two years of age, cattle of all ages may be affected (Smith, 1996). In this study papillomas were recorded only in animals aged between six months and sixty months which are in agreement with the data reported in the previous studies (Atasever et al., 2005; Cimtay et al., 2003).

Clinically, CWTs (n = 1769) were observed in both organized (CWTs, n = 275) and unorganized farms as well as in rural livestock population (CWTs, n = 1493) in both cattle and buffaloes. In the present investigation it was found that the papillomatous lesions were found elsewhere on the animal body in cattle but in case of buffaloes there had some limitations as compared to cattle. Among total recorded one thousand seven hundred sixty nine (n = 1769) number of spontaneous cases of clinical warts, one thousand six hundred forty (n = 1640) cases were recorded in cattle and rest one hundred twenty nine (n = 129) cases were recorded in buffaloes, reflecting cattle as the prime victim or most vulnerable species for BPV as compared to buffaloes.

Clinical evaluation of CCWTs revealed uniform as well as tumors with different morphologies and or mixed tumoral lesions with varying morphology, some circumscribed and mostly exophytic outgrowths as nodular, dome like, club-shaped, conical or cauliflower shaped with or without pedicles. Of course flat, lobulated, globoid, cylindrical, mulberry-like, ovoid and or spherical lesions with hard horny or smooth textured lesions were recognized. Tumours were of tough consistency and the surface mostly showed flattish vegetative outgrowths and some revealed as if towel surface rough tumour topography. Macroscopically, the papillomatous lesions in great majority of BOCP cases exhibited circumscribed aspects,
pedunculated, wrinkly surface, cornified, of grayish and lacking hair. These data are similar to other literature (Tweddle and White, 1977; Jelinek and Tachezy 2005; Turk et al., 2005; Maeda et al., 2007; Borzacchiello et al., 2008). The gross morphology of the lesions in cattle and buffaloes thus supports the findings documented by earlier Indian workers (Somvanshi et al., 1986; Leishangthem, 2006; Singh, 2007; Leishangthem et al., 2008; Singh et al., 2009; Pangty et al., 2009).

Exirpated tumours were mostly grey white and occasionally brown or black owing to ulceration or necrosis. On transverse section the excised tumours were seen to consist of homogeneous, grey white, glossy connective tissue covered by hyperplastic epidermis. The macroscopic aspects of the pedunculated and plane papillomas were similar to the description of Gerdes and Van der Lugtz, (1991) and Santin and Brito, (2003).

The BOCP cases (n = 1640) as recorded in the present investigation revealed highest incidence through field survey (n = 892) by physical inspection on door to door visit, followed by recorded incidence in Veterinary Clinics (n = 497) through questioner survey, organized farm survey (n = 208) by thorough inspection and specialized farm survey for CTP in farms (n = 43) by physical examination. Among the recorded BOCP cases CCWT was higher (n = 1433) than CTP (n = 207) cases. In regards to gender affinity of infection if any it revealed higher incidence in female (n = 1280) than the male (n = 360). The role of sex in the infection may return to the female cattle usually under stress factors such as gestation, lactation and progression in age. On the other hand, male cattle are usually directed to fattening and meat production and mostly slaughtered at age of 2-3 years. Of course screening of male animals was lesser than the females which were suggestive that there was hardly a gender preference for BPV infection.

In regards to gross regional distribution of wart, highest number of wart cases was recorded in anterior region (n = 850) showing anterior regional specificity of infection, followed by posterior region (as caudal affection, n = 531), anterior and posterior (cranio-caudal) regional affection (n = 196) and whole body or generalized distribution (n = 63). Detail studies on such regional distribution of warts were not undertaken by the earlier workers and this would probably the first attempt to establish the gross regional affection of the disease. In regards to year wise incidence of BOCP, highest incidence was recorded in year-2010 (n = 582), followed by year-2012 (n = 412), year-2011 (n = 392), year-2009 (n = 242), year-2008 (n = 9) and year-2013 (n = 3).

The CWTS are known to be transmitted from cattle to buffaloes and vice versa (Singh and Somvanshi, 2010). In the organized Dairy Farms, CCWT cases were higher (n = 208) than CTP cases (n = 43). CCWTs were mostly confined to cranial parts (anterior body parts, n = 122) and most interestingly involvement of the ear (n = 48, 39.34%) ( flap of the ear, ear
base and or ear pinna) was recorded in 48 number of clinical cases which could be due to the fact that these were transmitted from the infected animal to healthy one by tattooing machine. Association of tattooing with papilloma development on the ear of calves were reported earlier (Studdert et al., 1988). Grossly it was observed that in few CWT cases of cattle spread extensively to the adjacent areas forming large cauliflower like masses but in buffaloes, almost all case showed solitary growths. This finding was observed earlier too (Pangty et al, 2010; Singh and Somvanshi, 2010a; Kumar, 2012). Here in the present study, the severity of cutaneous papillomatosis was observed higher in cattle than the buffaloes, which could be due to the higher viral load or more susceptibility or for other reasons. This is in agreement to Kumar, (2012). As per age wise distribution of wart incidence was concerned, highest incidence of CWTs were observed in tender aged / sub adult( juveniles), followed by young calves and least in adult and aged animals. These findings are in accordance with Singh and Somvanshi (2010b) who noticed more cases in calves than the adults.

BPV may affect all ages of cattle; however, affected cattle were usually younger than 3 years of age (Smith 1996). Congenital tumors associated with papillomavirus infection are extremely rare in cattle (Desrochers et al., 1994; Misdorp, 2002, and Morris et al., 2011). In our study not a single case of congenital papillomatosis was recorded in West Bengal. This finding goes with the findings observed by Roperto et al., (2012). In this study, cutaneous papillomatosis was detected in animals aged between 6 months and up to 4 years, teat papillomatosis up to 8 years. Even though bovine papillomas are generally located on the head and neck, besides on thorax and inter scapular region, there have been reports that in some animals they also form in other regional and or anatomical location on the skin of the body (Abu-Samra et al., 1982; Blood and Radostits, 1989; Jones et al., 1997; Goldschmidt and Hendrick, 2002; Atasever et al., 2005; Jelinek and Tachezy, 2005; Borku et al., 2007). In our study most of the lesions were found to localize on anterior anatomic region (n = 857) as compared to caudal or posterior anatomic region (n = 326) of the animal body. Grossly, it was observed that many CWT cases (n = 63) of cattle spread extensively to the adjacent areas forming large cauliflower like masses but in buffaloes, almost all cases showed solitary growths. These observations are partially in accordance with Somvanshi et al., (1986). Papillomatosis may become a significant herd problem when a large group of young, susceptible cattle become infected. The percentile incidence of the disease could rise as high as 20-25%. In this study, the percentile incidence of bovine papillomatosis was detected between 0.34- 15.2 % in different herds.

The distribution of tumoral lesions as a whole both in cattle and buffaloes was more concentrated in the anterior portions than the posterior region, which clearly suggested that the virus had got cutaneous tropism with anterior specificity than the caudal affection. The
findings of prevalence, gross morphological appearance of lesions and pathology were more or less similar in accordance with earlier report from Indian subcontinent (Somvanshi et al., 1986; Leishangthem, 2006; Singh, 2007; Leishangthem et al., 2008; Singh et al., 2009; Pangty et al., 2009). Papillomas appearing on the surface of the skin as well as on teats were found benign and none of the affected animal died except one severely infected bovine heifer that suffered from whole body papillomatosis, with concurrent cutaneous acarasis, myiasis and endo-parasitism. Consequently in few cases, the warts did not regress and abnormally spread on cutaneous surfaces and those forms of warts were found problematic and of most economic significance. Economic impact of the disease is clearly observed in loss of animal condition, secondary bacterial infection, skin myiasis, occasional bleeding from lesions being knocked down, interfering lactation process and lastly reducing animal price and above all hindering sales.

The study revealed that the highest occurrence of cutaneous papillomatosis were in the younger animals whereas major occurrence of papillomatosis of teats or of udder and teats (n = 277), were recorded in pluriparous animals. It was evident from earlier studies that Bovine papilloma could be occurring in all ages (Blood and Radostits, 1989; Olson et al., 1992; Olson 1993; Campo et al., 1994), but it commonly occurred in young animals (Campo et al., 1994; Smith, 1996; Hatama, 2011). Higher incidence of CCWT in tender or young age groups could be due to the fact that young ages were more susceptible to the infection than the adult as described by Otter and Leonard, (2003); they recorded an outbreak of fibro-papillomas in calves. It is thought to be due to ill-developed immune system, alkaline pH of the skin of young ages. Most of these animals have been weaned, lost their maternally derived antibodies and are in the process of building their own immunities while being challenged by many pathogens; that may facilitate virus infection and also young ages are more susceptible to parasitic infestation and exposure to stress factors.

A high tick infestation rate (66.73%) was recorded and or observed among the infected animals. It is believed that ticks have two inducing roles for BP viz piercing the skin and their by making entry points where viruses enter the cutaneous tissue, infect basal keratinocytes and replicate their genomic materials in the differentiating spinous and granular layers of the epidermis and cause development of excessively grown warts (Radostitis et al., 2007). The ticks suck a large volume of host blood where they insert their hypostome into the skin and secrete a cementing substance from the salivary glands to secure the hypostome in place. They damage the skin barrier while feeding on host blood; secrete saliva that prevent clotting of blood and has got immune suppressive effects. Jitka et al., (2004) confirmed that Th2 cytokines; IL-6 and IL-10 were down regulated by salivary gland extracts of *Ixodes ricinus*. The immune suppression thus enhances papilloma virus infection (Lesnik et al., 1999; Brady et al., 1999; Koski and Scott, 2003).
Besides tick’s infestation, endoparasitic loads in animals caused by worm infestation as detected in more than 65% of CWT infected animals especially GI nematodes and liver flukes could exert possible immunosuppressive effect on animals for facilitating virus infection. Parasitic gastroenteritis was the common findings in the young calves, caused by PGE nematodes and they had an immunosuppressive effect as recorded by earlier workers (Koski and Scott, 2003). They stated that deficiencies of iron, copper, zinc and molybdenum, had been associated with higher worm burdens that consequently affected immune response. Besides PGE nematodes, liver flukes infestation as recorded in 45% CWT infected animals would throw light on the possible immunosuppressive effect of the flukes that induced BPV infection in younger animals. Brady et al., (1999) had mentioned that Th1 response to B. pertussis antigens was markedly suppressed and the bacterial infection was exacerbated following infection with liver flukes.

In the present study it was observed that warts occurred predominantly in young animals and the lack of susceptibility of adults to natural infection being ascribed to immunity acquired by apparent or in apparent infection when young (Radostits et al., 2007). It is clear that the young ages are more susceptible to the infection than the adult as described by Otter and Leonard, (2003). Our study also revealed most susceptibility of the disease found in one to two years old age group and the incidence rate decreased by higher age group which was in agreement to Hatama, (2011). It is thought to be due to ill developed immune system, alkaline ph of the skin of young ages, more susceptibility to parasitic infestation and exposure to stress factors, forced weaning and thereby loosing maternally derived antibodies and in the process of building their own immunities while being challenged by many of the opportunistic pathogens, facilitate virus infection in young age group mainly in calves and heifers. Concurrent ecto-parasitism either ticks infestation or acariasis were also observed among most of the infected animals. It is believed that ticks have certain roles in inducing papillomatosis in bovines.

Although teat warts affected cattle were mostly the younger ones, recorded in the age group 3 years to 8 years, all age classes may be concerned. In this study papillomas were recorded only in 2-8 years old animals and this is in agreement with data previously reported. Whereas Sharma et al., (2005) reported that occurrence of papillomas (teats and udder warts) in buffaloes were higher in advanced stage of lactation and the maximum lesions were observed in winter season (37.78%) followed by autumn (33.33%), summer (20.00%) and spring (8.89%).

Papillomatous lesions harvested from the skin of cattle and buffaloes contained BPV type-1 and type-2 DNA and were morphologically similar to previously described papilloma lesions of both cattle and buffalo. Lump lesions were either flat and sessile or pedunculated
and distributed elsewhere on animal body. Conclusion could not be made about the stage of infection based on the gross appearance of the lesions. Until recently PV studies dealt mainly with transmission experiments, virus ultrastructure and chemical composition and pathological description of lesions. The main impediment for the analysis of these viruses has been the lack of a reproducible cell culture system permissive for the replication (Lancaster and Olson, 1982). However, PV field has advanced recently, especially in the areas of molecular biological techniques and molecular cloning, which have made the viruses more amenable to study (Lancaster and Olson, 1982). While electron microscopic evaluation did not provide genetic information about the type of PV involved.

Papillomatosis in buffalo is comparatively less common than in cattle but recently, the disease is established in buffaloes from Northern India (Singh and Somvanshi, 2010a; Somvanshi, 2011) and in Italy (Silvestre et al., 2009). With a view to these findings field survey of buffalo papillomatosis was undertaken in organized dairies (GDF, Haringhata Farm and SLF, Kalyani, Nadia), unorganized private dairies in and around Kanchrapara, Naihati, Halisahar of N 24 Pargana, Kalyani and Gayespur Municipality area of Nadia district and also in large animal slaughter house (Kolkata). Besides field survey, questionnaire survey was made to record the incidence of buffalo warts by day book inspection of at least one Block level Veterinary clinic in each district of WB.

Bubaline papillomatosis in the present investigation was found to occur in cutaneous form causing both cutaneous as well as teat papillomas. Gross morphological feature of the lesions were more or less similar to that of cattle warts (CCWT and CTP). A case of teat wart was also observed in buffalo (Singh and Somvanshi, 2010a). In the present investigation all together seventy numbers of BTP (n = 70) cases were recorded which clearly reflects that BTP has been emerging as a significantly important clinical entity in Bengal. From the survey all together one hundred twenty nine cases of bubaline papillomatosis was recorded and all showed macroscopic lesions consistent with skin fibro-papillomatosis. Fibro-papilloma of the buffaloes was found to be firm, single or multiple skin tumours of varying size. On average the lesions were 1-5 cm in diameter but sometimes they reached to 6-8 cm. Although the numbers of fibro-papillomas on animals were usually less than ten, individuals carrying more than 10 tumours were also found. Fibro-papillomas were typically localized on the head, ears, neck, back, belly, legs and at perineum of the infected animals. In the buffalo, fibropapillomas were found on the back, in the gluteal region and in the vulvar and perivulvar regions, all common sites of scratching and scratching. It is known that papillomavirus tumours tend to manifest themselves at site of trauma (Campo, 2002; Campo et al., 1994). This could probably due to re-activation of latent virus, due to release of inflammatory cytokines and stimulation of cell proliferation leading to tumour development (Campo, 2002; Campo et al.,
1994). Some tumour surfaces exhibited moderate and transient papillary structure but the typical appearance was of a firm, hairless mass covered with verrucous epidermis. As per age wise distribution was concerned, BCWTs were observed least numbers in young buffalo calves, higher in adolescent and maximum in adults. These findings were in accordance with Pangty, (2009), Singh and Somvanshi, (2010b) and Pathania, (2010) where they noticed more cases in adults than calves. It was not established earlier until Leishangthem, (2006) had found BPV that can infect buffaloes but in literature a few cases were reported from Indian sub -continent. In the present investigation good prevalence of BCWT and BTP was recorded which clearly reflects its emergence in West Bengal.

Prevalence of cutaneous warts in buffaloes was recently reviewed (Somvanshi, 2011). Singh and Mouli, (1990) reported a case of fibro-papilloma in right eye of buffalo in Andhra Pradesh. Joshi et al., (1994) reported a case of fibro-papilloma of mammary gland skin in a buffalo calf in Gujrat. Degloorkar et al., (1992) reported 0.5% prevalence of bubaline papilloma in Parbhani, Maharashtra. Wangikar et al., (2001) recorded few cases of papilloma from Maharashtra. Sood et al., (2006) described a case of fibropapilloma in buffalo from Jammu and Kashmir. Leishangthem, (2006) observed two cases of fibropapilloma in buffalo calves from Mathura. Pangty, (2009) recorded 5 cases of CWTs in buffaloes and these were diagnosed as fibropapilloma and papilloma. Jana and Ghosh, (2010) from West Bengal reported a case of fibropapilloma in a male buffalo calf and its surgical management. Singh and Somvanshi, (2010 b) reported 20 cases of CWTs in water buffaloes. Pathania, (2010) recorded 3 cases of CWTs of which two in Murrah and one in non- descript buffalo calf. In the present study total 129 cases of clinical wart cases were recorded in West Bengal, of which, BCWT was 59 and BTP/ buffalo teat warts were 70. These studies indicated that CWTs were prevalent in buffaloes in organized dairy as well as in rural areas. Cutaneous papillomatosis as well as teat papillomatosis has been established as separate disease entity in buffaloes. In the present study it was obvious that teat papillomatosis in buffaloes was higher than that of the cutaneous wart cases.

On clinical studies of cutaneous warts in buffaloes showed tumours of varying shape and sizes, rough, grayish with irregular morphological appearance elevated and attached by a broad base to the underlying skin with moderate blood supply. In the buffalo, besides warts (fibropapillomas) on head, neck, limbs and eyes, fibropapillomas were found on the back, in the gluteal region and at peri vulvar and peri anal regions which were the common sites of scrapping and scratching. It is known that the papillomavirus tumours tend to manifest themselves at the site of trauma (Campo, 1997; Campo et al., 1994), probably due to re-activation of latent virus, due to the release of inflammatory cytokines and stimulation of cell proliferation leading to tumor development (Campo, 1997; Campo et al., 1994a). These
findings were also partially in accordance with Singh and Somvanshi, (2010b) who recorded 20 cases of CWTs in buffaloes. In our study typical cauliflower like white masses on peri-genital region and at perineum were observed in few buffaloes reflecting posterior or caudal regional distribution, not recorded by earlier workers from UP and UK of India. Of course like other workers from UP and UK, in WB also cauliflower like warts with numerous horny papillae, dome shaped with smooth outer surface and papilliferous type with single long papillae like projections on the skin were also recorded. On young buffaloes warts usually appeared on the head, neck and shoulders and they had spread over adjacent areas of the body. However it was comparatively lesser than the CCWT that had disseminated rapidly afflicting greater areas of the body. Many small warts or a few large ones were found to weaken the animals and slowed their growth. Like CCWT, BCWT had occurred singly or in clusters, but in great majority of BCWT cases clustered occurrences were very few only as compared to CCWT cases. Many warts in cattle in the present study were small and rounded, others were broad, thin, long or club-shaped and some took the shape of cauliflower like growths, but in our study in buffaloes the warts were mostly cauliflower like having grey white coloration and hard and tough consistency. Warts on ear flap and at the perineum and at para-genital area were exceptionally white cauliflower-like as compared to other lesions studied and recorded in buffaloes. Incidence of warts at peri-genital region was in accordance to the report of Silvestre et al., (2009), around eyes as peri-orbital warts as recorded was in accordance to Singh and Mouli, (1990). Most of the warts in buffaloes received a plentiful blood supply as revealed on surgical excision.

The preliminary and or tentative diagnosis of bovine and bubaline papillomatosis during survey or animal screening was based on the presence of variable sized wart like cutaneous lesions on animal skin. Diagnosis of infection was otherwise based on histopathological findings and the use of an electron microscope and above all the PCR based detection of the type of virus involved for the causation of the disease or neoplasia. The pathology of different types of BP (BPV-1,-2, -3, -4,-5 &-6) was discussed in literature. The more classical papillomas with only a modest connective tissue support are caused by BPV-3 (skin) and BPV type-5 (teat). These tend to be smaller and less pedunculated (Lancaster and Olson, 1982). Lesions over the udder and naval flap were papillomatous but on ears were often of digitative type. Rice grain papilloma and sessile fibroma were frequent on neck, trunk, limb and udder. Jarrett et al., (1984) classified the lesion into round, flat and rice grain type with specific etiological virus sub groups. Lindholm et al., (1984) stated that rice grain papilloma were the intermediary stage in development of papilloma i.e. fibroma, plaque or papilloma. In the present investigation all classical morphological appearance of CWTs and TPs were recorded and or observed in both cattle and buffaloes. The findings of prevalence.
and gross pathology of CWTs are more or less in accordance with earlier Indian workers report, as investigated in Northern India (Singh, 2007; Leishangthem et al., 2008b; Pangty et al., 2010 and Pathania, 2010).

Haematological studies of BOCP and BUCP cases showed significant increase in values of TLC and lymphocytes count and decrease in Hb and PCV values. Some of these findings like increase in values of TLC and decrease in PCV were similar to the findings of Abu-Samra et al., (1982). Earlier Somvanshi et al., (1986) also got marked lymphocytosis and neutropenia in BP affected animals. Serum biochemical studies revealed decrease in value of BUN, glucose and SGPT and increase in value of cholesterol and SGOT, in infected cattle as compared to control animals. Whereas in infected buffaloes there was increase in values of blood glucose and BUN and decrease in values of total serum protein, globulin, cholesterol and both SGOT and SGPT, as compared to the control animals. BP affected buffaloes showed increase in TLC and eosinophil values which cannot be explained properly. Leucocytosis can occur in BP as warts got secondarily infected. These findings were confirmed in some of the cases on histopathological examination as massive neutrophilic infiltration and suppuration was observed in epidermis. Lymphocytosis was recorded in regressing cases of warts (Barthold and Olson, 1974). Serum biochemical studies revealed fluctuations in the values of cholesterol, SGOT and BUN level in infected animals. Cholesterol was increased in case of BP affected cattle, whereas in BP affected buffaloes there were decrease in the value of cholesterol. Low cholesterol values could be the indication debility of the animals. Total serum protein and albumin concentrations were low in both cattle and buffaloes those had mild to moderate infections, which was again in agreement to Abu-Samra et al., (1982). These findings are also in agreement with the findings laid down by Leishangthem (2006).

Diagnosis of cutaneous papillomatosis, which is commonly observed in cattle and less frequently in buffaloes, is based on clinical symptoms, histo-pathological findings and the use of an electron microscope (Gerdes et al., 1991; Cheville, 1994; Turk et al., 2005). In the present study diagnosis of bovine and bubaline papillomatosis was confirmed by the presence of variable sized cutaneous warts, histopathology, EM studies (SEM and TEM) and PCR based detection of virus.

BPV infection starts in epithelial basal layers, with the virus undergoing differentiation throughout the activation of early and late genes to produce virions on the superior external layer. The penetration of a non- enveloped virus into the target cell involved two steps (Campo, 1995): 1) attachment and interaction with the cell membrane through binding to a surface receptor molecule and 2) penetration of the viral particle. Papilloma virus probably attaches to a conserved receptor widely expressed on the cell surface. Recent studies have suggested that the entrance of papillomavirus involves interaction with cell surface molecules that act as receptors.
Earlier CWTs were microscopically characterized by fibro-papillomas with acanthosis, hyperkeratosis and down-growth of rete pegs. The virus infects the basal cells of the epithelium, causing hyperplasia with hydropic ballooning of their cytoplasm, presence of prominent keratohyaline granules and vesicular nuclei. Some degenerated cells were found whereas others were stimulated to excessive proliferation and formation of warts (Lancaster and Olson, 1982). Microscopic changes typical of fibropapillomas were observed in all cases of cutaneous papillomas revealing epidermal hyperplasia, acanthosis and hyperkeratosis along with elongated growth of papillary projections extending into dermis. The dermis was also hyperplastic due to marked proliferation of connective tissue elements. Similar changes were also observed by Abu-Samra et al., (1982); Lancaster and Olson (1982); Gupta et al., (1989); William et al., (1992); Wadhwa et al., (1996) and Shukriti Sharma et al., (2003).

Microscopic examination of the wart tissues in our study showed hyperkeratotic areas on the surface of the epidermis, hyperplasia in the keratinocytes in the stratum spinosum, vaculolic and balloon like degeneration in the cells and a granular appearance in the keratinocytes. Epidermal acanthosis and hydropic changes were also observed in the keratinocytes of the epidermis. These findings were in agreement to Tan et al., (2012).

Histologically, in the present study, the tumours were characterized by fibroblastic proliferation with overlying acanthosis, ortho-keratotic hyperkeratosis and occasional areas of parakeratosis which were also observed by Hayward et al., (1993). The tumor cells exhibited an infiltrative growth at the interface with normal tissue, expanding the dermis and surrounding moderate hyperplasia, as well as vacuous cytoplasm in the stratum granulosum, with presence of large and irregular keratohyalin-like granules. Most cutaneous lesions were relatively characterized by productively infected keratinocytes, degenerating into koilocytes, represented by clear cytoplasm around nuclei. These features were characteristic of the cytopathic effect of PV infections. Follicular polyps were also observed in hyper-plastics derms characterizing the plane form of the papilloma. These characteristic features as observed in the present study was in accordance to Marins and Ferreira, (2011). Histology pathology of CCWT biopsy samples showed epidermal hyperplasia and rete pegs extension in fibrous tissue. Cutaneous warts on histo-micrograph also revealed finger like projections covered by hyperkeratinized layers along with hyperplasia of the epidermis where stratum spinosum showed degeneration and prominent vacuolations. According to reports of previous workers (Lancaster and Olson, 1982), CCWT is microscopically characterized by fibropapillomatosis with acanthosis, hyper keratosis and down-grown rete ridges. According to Jelinek and Tachezy, (2005) histologically the CCWTs of linea ulba microscopically revealed characteristic pathognomonic changes of fibropapilloma with many koilocytes along with presence of intra nuclear inclusions in few cells. The dermis revealed neoplastic fibroblasts.
and structureless intracellular matrix and non-purulent vasculitis. Histopathological examination of papilloma showed that the lesions were solely epithelial, the presence of acanthosis, hyperkeratosis and the pathognomonic koilocytes (Tsirimonaki et al., 2003).

Histopathological investigation of buffalo cutaneous wart samples revealed marked hyperkeratosis and fibromatosis. Besides hyperplasia of epidermis, there was presence of numerous vacuolated cells and few mitotic figures. Fibromatosis was pronounced in those BCWT cases. All these cases were diagnosed as fibropapilloma. The dermal papillae were elongated with irregular rete ridge formation covered by acanthotic epidermis. In the prickle cell layer, single cells or small groups of cells with vacuolated cytoplasm were seen as like as cattle CWT. Others showed variable degrees of ballooning degeneration with presence of clumped, pleomorphic keratohyaline granules. Koilocytosis was the most prominent feature in CCWT as compared to BCWT. In the dermal layer the proliferating cells were large, plumb fibroblasts arranged in haphazard whorls and fascicles. In others the connective tissue was mature, hyalinized and poor in cells as compared to histopoiesis of CCWTs. Neutrophilic exocytosis into dermis and epidermis were seen in cases with secondary infection. In BCWTs on histological investigation epidermal proliferation was found minimal and characterized as slight acanthosis and accentuation of rete pegs.

Histopathologically among 20 (n = 20) studied buffalo wart cases 17 were fibropapilloma, one was fibroma and two were occult type of papilloma. All were of exophytic type and not a single case was identified or diagnosed to be of endophytic type. Whereas in the present investigation one CCWT case was diagnosed as typical endophytic papilloma. Pathania, (2010) reported three BCWT cases of which two cases were found to be endophytic type fibropapilloma and one case diagnosed as fibroma. Pangty, (2009) detected one case each of endophytic papilloma and fibropapilloma and one case of fibroma. Singh and Somvanshi, (2010b) carried out histopathological investigation of 11 cases of BCWTs among those, four cases each was diagnosed as exophytic fibro-papilloma (cauliflower-like) and exophytic fibropapilloma (dome shaped) and three were endophytic papilloma (two cauliflower like and one as single papillary growth). Present study although supports the views of earlier workers in great majority, in regards to endophytic type of warts our views differ in case of BCWT, since no such histopathological findings were noted in all the BTP and BCWT samples studied histopathologically.

In histopathological examination of CCWTs, uniform as well as irregular epidermal hyperplasia and expansion of the stratum spinosum were determined. Microscopically occasional areas of parakeratosis were also observed by Hayward et al., (1993), as well as the density of the stratified scaly epithelium (hyperkeratosis) and the proliferation of the thorny stratum (acanthosis) that were registered by Eisa et al., (2000). The koilocytosis was described
initially as cells with nuclei picnotics, moderately irregular, outlined by extensive clear halos with superior volume than the cytoplasm (Silveria et al., 2005). Other authors affirmed that the koilocytosis constituted a sign of pathognomonic infection by papillomavirus (Xavier et al., 2005). Oliveira et al., (2005) and Sundberg et al., (2000), observed in the benign lesions the presence of great clear cells, displastic, with vacuous nuclei and prominent cytoplasm with granules of keratohyalin characteristic of the viral cytopathic effect. Acanthosis was seen in stratum spinosum in majority of the cases. Stratum spinosum cells were swollen and their cytoplasm contained basophilic granules of various sizes. Exophytic papillomatous proliferations were the most common form of cutaneous infection by papilloma virus but endophytic papillomas were also observed (Le Net et al., 1997). In the present study, histopathology revealed that fibro-papilloma (exophytic) was the most common histopathological type observed in CWTS of both cattle and buffaloes. Papilloma occult and fibroblastic type and papilloma of other types (endophytic papilloma) found in CWTS. In no case of buffalo, endophytic fibropapilloma was recorded. Similar histopathological types were recorded by earlier workers in the CWTS (Pangty et al., 2010). Kumar et al., (2013) found few endophytic fibropapilloma cases. In some cases there were capillary dilatation, hyperemia and lymphocytic infiltration in the propria. The histopathologic examinations confirmed the clinical pathognomonic findings of the tumor papillomatosis. The viral cytopathic effect was demonstrated in papilliform lesions independent of this morphology.

Papillomavirus was readily detected in the cutaneous lesions and the presence of keratinocytes with nuclear atypia, cytoplasmic vacuolization, and keratohyaline granule abnormalities (koilocytes) was characteristic. Koilocytes occur only within maturing keratinocytes in the superficial or intermediate layers of the epidermis and must be distinguished from keratinocytes with cytoplasmic vacuolar change due to other causes, such as glycogen accumulation (Shah and Howley, 1996). The presence of changed keratinocytes known as koilocyte in the present investigation suggested that the papilloma was of viral origin. Exophytic papillomatous proliferations are the most common form of cutaneous infection by papillomavirus but endophytic papillomas are also observed (Le Net et al., 1997). In the present investigation one endophytic papilloma was also detected in CCWT which supports the claim of the earlier worker (Marins and Ferreira, 2011).

The etiological agent of bovine cutaneous fibropapillomatosis is thought to be BPV-1 and BPV-2 (Bagdonas and Olson, 1953; Huck, 1965; Jarrett, 1985; Theilen ad Madewell, 1987; Amin et al., 1997; Campo, 1997; Jelinek and Tachezy 2005) and in the present investigation both BPV-1 and BPV-2 have been consistently found to be the etiologic agent of bovine and bubaline cutaneous as well as teat papillomatosis occurring and circulating in Bengal, based on PCR based detection of BPV-1 and BPV-2 DNA. The macroscopic and
microscopic findings of the tumours observed in the present study was similar to those described before (Goldschmidt and Hendrick, 2002; Atasever et al., 2005; Jelinek and Tachezy 2005; Ozsoy et al., 2011).

Histopathological investigation revealed that the fibropapilloma (exophytic) was the most common histopathological type observed in CWTS of both cattle and buffaloes. Papilloma occult type of samples revealed a marked ortho or parakeratotic hyperkeratosis with long, thick, hair like cornified surface projections and papillate epidermal hyperplasia. The underlying dermal papillae had a mild to moderate infiltration of neutrophils and fewer lymphocytes. In great majority of the BCP microscopically it revealed hyperkeratotic areas on the surface of the epidermis, hyperplasia in the keratinocytes in the stratum spinosum, vacuolic and ballon like degeneration in the cells and a granular appearance in the keratinocytes. Epidermal acanthosis and vacuolic changes were common features as observed in the keratinocytes of the epidermis. The dermal papillae were elongated with irregular rete ridge formation covered by acanthotic epidermis. In the prickle cell layer, single cells or small groups of cells with vacuolated cytoplasm were seen. Others showed variable degrees of ballooning degeneration (koilocytes) with presence of abundant clumped, pleomorphic keratohyaline granules. Comparable to cattle (with abundance of koilocytes), buffalo warts showed only few koilocytes in the upper layer of stratum spinosum which was in agreement to Kumar et al., (2013). The histopathologic exams confirmed the clinical pathognomonic findings of the tumour papillomatosis.

Ultra-structural pathology of bovine and bubaline skin in clinical cutaneous papillomatosis revealed pathognomonic signs of BPV induced ultra-structural features of CWT. Both scanning electron microscopy (SEM) and transmission electron microscopic (TEM) studies of the tumour tissues were undertaken which revealed pathognomonic changes confirming the tumours as virus induced papillomas and or fibropapillomas. This was for the first time SEM studies were made on CCWT and BCWT in Indian context which revealed a deep insight of the papilloma on ultra-structural topographic conformation of the warts. SEM studies revealed numerous uneven longitudinal folds(on lateral view), multiple projections and multiple pores over the surface of the warts (epical view), multiple grooves inside the pores (on higher magnification), multiple grooves and fissures and sponge or towel surfaced surface topography of the tumours. These findings affirmed the tumours as warts or papillomas too. This was for the first time SEM studies were undertaken in CCWT and BCWT cases in Indian context revealing deep insights of tumour surface topography and the pathognomonic fine surface structural alterations.

The presence of papillomavirus in cattle and buffalo herds suffering from cutaneous papillomatosis in Bengal was documented in this study under transmission electron
microscopic (TEM) techniques. On the other hand, microscopic analysis of the lesions by negative staining revealed papilloma virus particles of spherical shape, characterized as full and empty particles and containing capsomers of icosahedral symmetry. Similar results were also found in other studies with cattle (Brobst and Hinsman, 1966; Tajima et al., 1968; Ford et al., 1982; Zhou et al., 1993; Liu, 1998; Cartroxo et al., 2005; Turk et al., 2005; Mendonca and Netto, 2005; Xu et al., 2006; Leishangthem et al., 2008; Silvestre et al., 2009), deer (Sundeberg et al., 1985a, b), felines (Sundeberg et al., 2000) and humans (Buck et al., 2005).

It was not possible to observe through the negative staining technique variants and tubular particles described by Cheville and Olson (1964) and Doane and Anderson (1987). Groups of rounded, viral particles with a 35 nm in diameter were found intranuclearly in sections of ultrathin fragments, which conferred the literature related to bovine (Tajima et al., 1968; Zhou et al., 1993; Jelinek and Tachezy, 2005; Maeda et al., 2007, Roperto et al., dogs (Buff et al., 1988), deer (Sundberg et al., 1985b), cats (Sundberg et al., 1985a, b) and poultry (Daoust et al., 2000). In this study, the virus particles as demonstrated ultra-structurally in both cattle and buffalo cutaneous warts, exhibited 30-35 nm in diameter, with typical non-enveloped icosahedral structure which form para-crystalline arrays in the nucleus of infected cells. Negative staining of the BP sample under TEM showed scanty BPV-like particles. These findings were more or less similar to those of Turk et al. (2005) and Cartroxo et al. (2005) who demonstrated virions that were very similar to BPV by EM examination of tumour tissue by the negative staining technique. In the present investigation diameter of the virions as measured was smaller than the diameter reported from earlier workers from Indian sub-continent. Only sporadic virions were reported to occur in the hyper-keratotic surface layer, while large clusters were formed in the deep layers. Both solitary destroyed and coreless particles with electron core space were reported in the aggregates.

During acute virus infection, replication of the genomes strictly linked to the state of differentiation of the PV infected cells. In BP the virus initially infects the basal keratinocytes. The early region genes are expressed in the undifferentiated basal and supra-basal layers. Viral DNA replicates in the differentiating spinous and granular layers and expression of the structural proteins is limited to the terminally differentiated cells of the squamous layer, where the new virus particles are en-capsidated and released into the environment as the cells die. In the present study by TEM productive phase of virus replication and or multiple virions were detected in the infected tissue.

Infected nuclei displayed numerous irregular clumps of condensed chromatin and that correspond to the description by Doane and Anderson (1987). Electron microscopically, virus particles have also been demonstrated in skin papillomas in cattle in Indian subcontinent (Leishangthem et al., 2008; Pangty 2009, kumar et al., 2010). Intra-cytoplasmic viral
particles, cited by Jelinek and Tachezy, (2005) in cattle and by Sundberg et al., (1985) in cats were not observed in this study, even the crystalline arrangements described by Elzein et al., (1991) in cattle and by Sundberg et al., (1985b) in deer. However, cylindrical or tubular intranuclear structures were observed, according to Daoust et al., (2000), who studied lesions in birds.

In the water buffalo (*Bubalus bubalis*) (Silvestre et al., 2009) and the bison (*Bison bonasus*) (Literak et al., 2006) virus has also been demonstrated electron microscopically, and following its sequencing it was claimed to be homologous to the long control region of BPV-1 (prototype sequence, accession number X02346) in the water buffalo (Silvestre et al., 2009) while the DNA sequence of 413 bp amplicon derived from the European bison compared to the consensus sequence of BPV-2 (Gene Bank accession number AY300818) (Literak et al., 2006). In the present study the virus was also demonstrated ultra-structurally in Murrah buffaloes suffering from cutaneous wart.

Ultra-structurally, neoplastic cells had elongated nuclei containing peripheralized heterochromatin and central eu-chromatin, few intra-cytoplasmic organelles mainly comprised of rough endoplasmic reticulum (RER) and mitochondria and few cytoplasmic processes. Numerous virions, consistent with those of papillomaviruses, were also detected when a scraping of the surface layer of a wart lesion was re-suspended in chilled distilled water and examined electron microscopically after negative staining with phosphotungstic acid (PTA). TEM studies revealed small intra-nuclear aggregates of virus particles in few epidermocytes but alteration of ultra-structural changes like damage to desmosomes and disorganization of cytokeratin filaments were observed in many cells. The most conspicuous changes consisted of alteration of the arrangement of cytokeratin filaments and transformation of desmosomes in cytoplasmic tags, which could probably have produced the spongiosis under light microscopy. Cell junction defects were considered to be typical of neoplastic cells, while the number of desmosomes could be reduced or variable (Cheville, 1994). Desmosomes which generally provide strong intracellular coupling were found as anchoring sites for cytoskeletal intermediate filaments. The direct mediators of cell to cell adhesion are claimed to be the transmembrane glycoproteins.

In the present investigation, TEM studies of CCWT and BCWT revealed cornified, more or less structureless tumour stroma. Few basal cells and scanty cytoplasmic organelles like mitochondria and RER were observed. Secretory vesicles and keratinocytes were also prominent. TEM showing elongated or somewhat elliptical with plenty of cytoplasm with nuclei having uneven borders with marked peri-nuclear zone of heterochromatin was evident. Such findings are in agreement with the observations laid down by earlier Indian workers. Junctional complex between cells were normal in most of the cases. Marked mitochondrial
degeneration was observed in few cases. In addition to extensive and diffuse vesiculations in mitochondria, RER cisterene underwent moderate changes. In this study, certain ultra-structural features such as bizarre-shaped nuclei, margination of nucleoli, margination and condensation of heterochromatin, electron dense cytoplasm and certain organelle could be suggestive of apoptosis. TEM study also showed a chromatin margination, followed by its compactness towards the nuclear periphery. The nucleus appeared markedly rearranged as compared to the normal one, which revealed a perinuclear and perinucleolar dense heterochromatin, clearly distinguishable from the diffuse inter-chromatin.

According to Lancaster and Olson (1982), productive BPV infected cells showed an increase in the size and number of desmosomes and tono-fibrils, whereas the other epithelial cells showed degenerative changes with loss of tono-fibril, detachment of desmosomes, focal nuclear atypia and cytoplasmic vacuolization. Towards the upper layers of the epithelium, such changes were reported to be more pronounced. In the granular layer, nuclear degenerations along with margination and condensation of chromatin were evident. EM analysis revealed the presence of virions in crystalline array in nuclei of degenerated cells in the keratinizing layer ((Lancaster and Olson, 1982). Small intra-nuclear aggregates of virus particles in epidermocytes and aggregates of virions in fibroblasts in the dermis were observed under EM (Jelinek and Tachezy, 2005). In the present study we demonstrated good many numbers of virions in CCWT and BCWT. Of course, we could not demonstrate virus particles in all CCWT and BCWT ultrathin section under TEM study and that could possibly because of failure of selection of right type of cells.

Vascular endothelial cells were found as targets for the action of pro-inflammatory cytokines viz tumour necrosis factor (TNF), which would elicit signaling pathways leading to activation or apoptosis of the endothelial cell. TNF alone could cause activation but in combination with other agents that release ceramide, apoptosis is induced. In the present study, the swelling of the endothelium and margination of lymphocytes in the blood capillaries were probably due to the effect of TNF and undoubtedly caused ischemia of the tumours. This ischemia probably contributed to regression of the tumour due nutritional and oxygen deprivation.

Both light and electron microscopy revealed cells with vacuolated cytoplasm in the prickle cell and keratohyaline layers of all papillomas. Cell vacuolation was most pronounced in the keratohyaline layer. If cell vacuolation is caused by immature PVs, the quantity of virus could be the factor in producing cell alterations. As the cell progresses towards the skin surface an amount of virus may accumulate which is capable of causing cell damage.

In the present study two BPV types, BPV-1 and BPV-2 and mixed infection of both the types were detected (by various PCR based assays) in papilloma specimens from various
districts of West Bengal. In Indian studies, BPV-1 and BPV-2 were detected in the cutaneous warts in cattle (Leishangthem et al., 2008; Singh et al., 2009, Pangty et al., 2010), buffaloes (Pangty et al., 2010; Singh and Somvanshi, 2010; Nagarajan, 2011) and yak (Bam et al., 2012). BPV-2 DNA was also demonstrated/quantified in urine and urinary bladder tumours of EBH–affected hill cattle (Leishangthem et al., 2006; Pathania et al., 2011). The simultaneous presence of BPV-1 and BPV-2 viruses could be related to different sources, as undetected infected tissues, considering that the virus can spread via a hematogenous route throughout the host body. In cattle, viral DNA has been found in blood (Campo et al., 1994; Stocco dos Santos et al., 1993, 1998; Wosiacki et al., 2006) and in plasma (Freitas et al., 2007). However, no significant difference between gross morphologicl appearance, pathology and outcome of the disease and disease progression based on the presence of either BPV-1 and -2 or both. This was in agreement to Pangty et al., (2010). There is a report of detection of BPV-1 and BPV-2 mixed infections in sarcoid affected zebras (Lohr et al., 2005, van Dyk et al., 2009), like that of cattle and buffaloes. DNA was obtained from healthy skin biopsies of nine sarcoid affected zebra from the Gariep Dam Nature Reserve, South Africa and all tested positive for the presence of BPV-DNA using real-time PCR assay. Among the nine HSS samples of zebra tested, 33% were positive for BPV-1, 11% for BPV-2 and 56% had both BPV-1 and -2DNA (van Dyk et al., 2009). Virus co-infection can involve the presence of different types of the same virus and or different viruses related to different diseases (Yaguiu et al., 2008). Mixed infection of both BPV-1 and BPV-2 was also detected by the earlier workers from Northern India (UP and UK). Study conducted by Pangty et al., (2010) revealed the presence of mixed infection in 11 CWTs (9 CCWT and 2 BCWT) cases for the first time in India. Pathania (2010) detected BPV-2 in 3 CWT cases in buffaloes. Leishangthem et al., (2008a) for the first time in India, detected BPV-2 DNA in cutaneous warts in cattle and buffaloes by PCR technique. Further Singh et al., (2009) detected BPV-2 DNA in 19 CWTs samples of BPV affected buffaloes at various dairy farms in Northern India.

BPV DNA is detected by a variety of PCR-based techniques. These PCRs are based frequently on the detection of one or two BPV types using degenerated or type specific primers. In the present investigation both BPV-1 and –2 types including mixed type infection of both the types were detected by PCR-based techniques. Genotyping is performed either by real-time detection (Rai et al., 2011) or by sequence analysis (Brandit et al., 2008) or restriction fragment length polymorphism analysis (Carr et al., 2001) of the generated PCR fragments. Consensus primers capable of identifying potentially more than two BPV types have also been described (Ogawa et al., 2004). PCR assays using degenerate primers that amplify partial fragments of the L1 gene, followed by sequencing, have suggested the existence of numerous yet uncharacterized BPV types in cattle herds from diverse geographical regions.
In this study, papilloma virus infection was confirmed by identifying BPV-1 and BPV-2 nucleic acid with PCR based assays, preceded by clinical, histopathological as well as electron microscopical findings respectively. CCWT biopsies as tested by conventional PCR revealed thirty one numbers positive to BPV-1 and twenty nine numbers positive to BPV-2 types and thirty three numbers positive to both the types, as reflected by the specific yield of amplicons corresponds to 301bp and 165 bp product size respectively affirmed on 1.5% agarose gel visualized by GelRed. Healthy skin biopsy materials as tested fifteen in numbers by PCR detected BPV-2 DNA in four cases reflecting the presence of BPV in healthy animals or clinically wart free animals too. In case of CTP biopsied samples as tested by PCR based technique detected BPV-1 DNA in 15 cases, BPV-2 in three and mixed types in five cases. CCWT DNA samples showed either 301bp or 165bp amplified product corresponding to the expected L1 gene fragment of BPV-1 and BPV-2 segment.

Likewise in BCWT biopsies PCR assays using type specific primers of BPV-1 and BPV-2 targeting L1gene amplified partial fragments of the L1 gene of BPV-1 DNA in seven cases, BPV-2 DNA in eight and both the types in three cases. In case of BTP biopsied samples PCR based detection revealed BPV-1 DNA in seven cases, BPV-2 in six and BPV-1 and BPV-2 DNA in three cases. No BPV type was detected in healthy teat skin samples and even in three clinically positive BTP samples.

The detection of BPV-1 and -2 mixed infections by PCR are in agreement with that of earlier report of simultaneous detection of BPVs and bovine leukemia virus by PCR and in situ hybridization (Yaguiu et al., 2008). These authors observed BPV-1, -2, and -4 DNA sequences in three females of the herd and in their offspring. They also demonstrated the simultaneous presence of these three BPV types, not only in their target wart epithelium cells but also in blood. Besides, there is a report of detection of BPV-1 and -2 mixed infections in sarcoid affected zebra (van Dyk et al., 2009). DNA was obtained from healthy skin biopsies of nine sarcoid – affected zebra from the Gariep Dam Nature Reserve, South Africa and all tested positive for the presence of BPV-DNA using the real-time PCR assay. Of the nine samples tested, 33% were positive for BPV-1, 115 for BPV-2 and 56% had both BPV-1 and -2 DNA (van Dyk et al., 2009). Virus co-infection can involve the presence of different types of the same virus and or different viruses related to different diseases (Yaguiu et al., 2008).

BPV-1 and -2 were predominately reported to be associated with paragenital lesions and cutaneous fibro-papillomas. In a survey of ten individual fibro-papillomas occurring on the head, neck and flank, four were identified as BPV-2 and the remainder as BPV-1 (Jarrett, 1985). In another study, similar results of both BPV-1 and -2 in fibro-papillomas of the udder were recorded (Pfister, 1980). The DNA of different types of BPV involved in CWTs were also detected by PCR analysis of various tissues, tissue fluids and further confirmed by
nucleotide sequencing of PCR amplicons (Stocco dos Santos et al., 1998; Antonio Carlos de Frietas et al., 2003; Borzachiello et al., 2003; Claudemir de Carvalho et al., 2003; Tsirimonaki et al., 2003; Leishangthem, 2006; Pangty et al., 2009).

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In our investigation, both the BPV types (BPV-1& -2) were proved to have the potential for causing cutaneous as well as teat lesions in both cattle and buffaloes. Our findings are in agreement to (Leishangthm et al., 2008a, Singh et al., 2009, Pathania, 2010 and Pangty et al., 2010). BPV DNA was detected both by conventional and real time polymerase chain reaction (PCR) based techniques using type specific primers. However, analysis of samples from healthy skin also reveals the presence of BPV DNA or the said viral types as commensal form. The severity of papillomatosis as observed in the present study were higher in cattle than buffaloes, which could be due to higher load or more susceptibility or any other reason irrespective of the type of virus involved in wart development.

Infection of buffalo by BPV-1 and BPV-2 and mixed infection of both the types as detected was based on several pieces of evidence: first, the histologic appearance of buffalo fibro-papillomas was reminiscent of that of cattle fibro-papillomas, second, papillomavirus-like particles could be detected in the fibro-papillomas, third, the BPV-1 and BPV-2 DNA were present in tumors and fourth, the viral late protein (L1) was abundantly expressed. These observations proved that BPV-1 and BPV-2 were the two active agents of fibro-papillomatosis also in the water buffaloes. The detection of BPV-1 and -2 mixed infections by PCR are in partial agreement with that of earlier report of simultaneous detection of BPVs and bovine leukaemia virus by PCR and in situ hybridization (Yaguiu et al., 2008). The authors observed BPV-1, -2 and -4 DNA sequences in three females of the herd and in their offspring. They also demonstrated the simultaneous presence of these three BPV types, not only in their target wart epithelium cells but also in blood. The etiological agent of bovine cutaneous fibro-papillomatosis is thought to be BPV-1 and BPV-2 (Bagdonas and Olson, 1953; Huck, 1965; Theilen and Madewell, 1987; Jelinek ; Campo 1997; Tachezy, 2005) and in the present investigation both BPV-1 and BPV-2 have been consistently found to be the
The etiologic agent of bovine and bubaline cutaneous as well as teat papillomatosis occurring and circulating in Bengal. The macroscopic and microscopic findings of the tumours observed in the present study was similar to described before (Goldschmidt and Hendrick, 2002; Atasever et al., 2005; Jelinek and Tachezy 2005; Ozsoy et al., 2011).

Two types of BPV sequences were found in cutaneous wart and teat wart biopsy specimens using both conventional and real-time PCR. The PCR amplifications using specific primers for BPV-1 and -2 yielded products with the expected sizes (summarized in tables). No other types of BPV were detected in spite of using specific primers for BPV-5,-6, 9 and -10. Detection of BPV DNA sequences in different samples clearly reflects only BPV-1 and BPV-2 was prevalent in West Bengal.

The amplification and dissociation plot of real time PCR assay done on CCWT samples revealed eleven (n = 11) samples were BPV-1 positive, fifteen (n = 15) samples were BPV-2 positive and five (n = 5) samples were positive for both the types. Of course in one healthy skin sample BPV-1 was detected. Real-time PCR results of both cattle and buffalo demonstrated BPV-1 and BPV-2 DNA and showed increase in fluorescence at 80.4±1.24 °c and 80.2±1.20 °c respectively. Both CCWT and BCWT samples as tested by Real-time PCR based assay demonstrated almost similar pattern through different graphs fluorescing at 75-80°C. Earlier testing of BPV in bovines by real time PCR was done by Pangty et al. (2010). Fragments, 301bp and 165bp in size of the L1 ORF of the CCWT and BCWT samples were amplified, cloned and sequenced to confirm that the amplicons obtained were indeed related to BPV (BPV-1 & -2). Blast search showed the highest similarity (98%) with the BPV-2 L1 encoding genes found in cattle and buffalo and alignment of the said sequence with the published BPV-2 L1 sequences of Indian origin revealed only two mismatch or two SNP at 36 (A to G) and 82 (T to C). Phylogenetic analysis showed that BPV-2 L1 protein of WB origin formed one cluster with all the published BPV-2 L1 protein of Indian origin with 99% bootstrap support. Whereas the phylogenetic tree as constructed with BPV-1, WB origin was concurrent the BLAST results obtained (With BPV-1 L1 Indian origin) and formed two separate clads/clusters showing close phylogenetic lineage with low bootstrap support. Results of the neighbor-joining tree of BPV-1 (WB origin) L1 encoding gene showed the phylogenetic lineage and relationship of sequences compared to published sequences of BPV-1 (Indian origin), revealed 74% bootstrap support with BPV-1, PKC-16, L1 whereas other BPV-1 types of Indian origin formed another cluster and showed 100% bootstrap support among them.

Earlier, the DNA of different types of BPV involved in BP were detected by PCR analysis of various tissues and tissue fluids and further confirmed by nucleotide sequencing of PCR amplicons or by Southern Blotting (Stoccos dos Santos et al., 1998; Antonio Carlos de
Frietas et al., 2003; Borzacheillo et al., 2003; Claudemir de Carvalho et al., 2003 and Tsirimonaki et al., 2003). DNA can also be detected by in situ hybridization methods (Jelinek and Tachezy, 2005). Further, BPV-2 has been detected in reproductive tract tissues, fluids and oocytes from slaughtered bovine females not afflicted by cutaneous papillomatosis. BPV-2 DNA sequences were found in ovarian and uterine tissues as well as in oocytes, cumulus cells and uterine flushing (Claudemir de Carvalho et al., 2003). Antonio Carlos de Frietas et al., (2003) detected BPV-1 DNA sequences in peripheral blood, warts and plasma samples of bovines affected by cutaneous papillomatosis. BPV-1 has also been detected in warts, blood, placenta and amniotic fluid obtained from a bovine and her calf thus showing the evidence of vertical transmission of BPV-1. Stocco dos Santos et al., (1998) detected BPV-2 DNA in peripheral blood samples used for inoculation, as well as in the calves by Southern blot and PCR analysis.

The presence of BPV-1 and BPV-2 in water buffalo tumors raises the question of the provenance of infection. Buffalo are closely related to cattle, both species belonging to the Bovidae family, and very often cattle and buffalo herds are kept together. These two facts would explain cross-species infection from cattle to buffalo. Moreover, buffalo fibropapillomas contain virus and are therefore productive for virus, presumably infectious. Thus, once established in a buffalo, infection could spread from buffalo to buffalo, without cattle intermediary (Silvestre et al., 2009).

The occurrence of multiple BPV types in a specific anatomical location and the detection of same viral type in distinct body sites were the outcome of the present study. Besides a specific viral type also determined cutaneous lesions with diverse gross aspects and diverse viral types were being able to cause skin lesions with similar morphological characteristics. Owing to the absence of cross reactive immunity of BPV types and the occurrence of multiple infections (co-infection with multiple BPV types) there could have persistence of skin warts. Regional studies adopted in depth aiming to establish and demonstrate the most prevalent BPV types occurring in the cutaneous warts and that would demonstrate the future vaccine design where one should consider multiple characters of BPV infections.

Phylogenetic study of BPV-1,L1 and BPV-2,L1(WB origin) partial sequences with those of the published partial sequences of BPV-1,L1 and BPV-2, L1 and few other E proteins of Indian origin revealed close lineage in case of BPV-2 and distant lineage with BPV-1, L1. The West Bengal isolate of BPV-2 L1 showed the sequence divergence at position 9 base pair corresponding to 1320 of Indian standard BPV-2 L1, and 60 base pair corresponding to 1371. While rest of the sequence (base pair) showed homology with stated Indian type BPV-2 L1. Hence in conclusion it can be opined that there was a sequence
variation or diversity in WB type as compared to Indian type as studied and published in database. Within India also there was sequence diversity and the sequence diversity obtained from this result will have immense implication in designing PCR primers for the WB isolate. So, it is essential to characterize genetically all the isolates infected with in different agro-climatic zones of India for the purpose of diagnosis.

Infectious papillomatosis although generally regarded as a self-limiting disease, in our study spontaneous regression or clinical recovery was noted in very few animals only. In neglected cases, the lesions did not regress of their own and even in few cases the disease reached serious proportions and the spread of tumours was so extensive that the animals had ill thrift, health threat, stunted growth and aggravation owing to myiasis and secondary bacterial infection. Economic losses for farmers were mostly evident in animals with extensive lesions that seriously interfered with the health and vigor of the animals. Secondary bacterial infection also occurred in animals with severe grades of infection affecting extensively covering wide areas of the body and had altered the body condition of the animal. Warts on valuable pedigree animals interfere with sale value and warts on teats affect milk production. Animals with large and extensive lesions would be the ones likely to be treated and, thus, it is important that treatment should be effective once warts have fully developed. Despite a reluctance to treat warts, farmers might use a product which is injectable and relatively cheap. Moreover, a new therapy would be attractive in cases of extensive or mammary warts. Solid immunity prevails following regression of warts (Susaneck 1989).

Warts usually shrink and drop off within a year (Bagdonas and Olson, 1953; Messing and Epstein, 1963; Huck, 1965; Theilen and Madewell, 1987; Studdert et al., 1988)). The spontaneous recovery has probably been the basis for the alleged effectiveness of many regimes of treatment including several kinds of oil, toothpaste of various brands, wart pinching, or twisting off close to the base. Any of these appear to be successful if the warts regress spontaneously.

Treatments are varied and include topical medication with podophyllin, autogenous vaccines general immune stimulants such as arsphenamine, cauterization and surgery (Lassauzet and Salamin, 1993). There are several treatment options like antimony preparations viz. potassium antimony thiomalate (Rajguru et al., 1988; Wadhwa et al., 1992; Khasatiya et al., 2008; Dileep kumar and Ansari, 2012); autogenous vaccine (Pearson et al., 1958; Butler, 1960; Theiler and Madewell, 1979; Prasad et al., 1980; Gupta et al., 1984; Wadhwa et al., 1995; Inayat et al., 1999; Lesnik et al., 1999; Vahid and Jayakumar, 1999; Venugopalan, 2000; Turk et al., 2005; Shelar et al., 2007; Sreeparvathy et al., 2011; Dileep kumar and Ansari, 2012; Hamad et al., 2012); homeopathy (Soni and Parekh, 1977; Somvanshi and Sharma, 1986; Rai et al., 1991; Dighe, 1992; Maclead, 1992; Prakash, 1993;
Discussion

Veena, 2001; Bhaskar Rao et al., 2005; Hossain et al., 2005; Beoricke, 2008; Shakoor et al., 2012; Umadevi and Umakanthan, 2013; cryotherapy (Paithanpagare and Tank, 2009); Levamisole (Cihan et al., 2004); ivermectin therapy (Borku et al., 2007; Ghassan et al., 2011); and autohemotherapy (Bajric et al., 1983; Pattanayak, 2004; Mitra, 2005; Jana and Jana, 2009; Hegde, 2011; Kumar, 2011, Ramrisha and Sundaravinayakim, 2013; Ranjan et al., 2013). There is no single treatment that is 100% effective and different types of treatment may be combined. The research into efficacy of treatment must take into account the possibility of spontaneous regression. It is a valid management option to leave warts untreated if it is not going to harm the animal health and vigor. The ideal aims of treatment of warts (1) to remove the wart with no recurrence; (2) to produce no scars, and (3) to induce lifelong immunity. Affected animals should be isolated from susceptible ones to reduce the chance of spread but with long incubation period, many would likely to get exposed before the problem would be recognized. Mustering, yarding, tattooing, dipping and other husbandry practices should be kept to a minimum during any noticeable outbreak of warts to avoid stress. Disinfection with formaldehyde of stalls, fence posts and other environmental virus reservoirs could prevent transmission.

The clinical wart removal treatment as adopted in the present study was cauterization (thermo-cautery), cryotherapy or excision to get rid of those warts. Surgery and cryosurgery has been recommended for complete cutaneous wart cure (Venugopalan, 2000; O’Conor, 2001; Valentine, 2004). In our study the choice of treatment in both mild and few moderate grade infection (viz. situations like animals bearing warts 1-20 and of big sizes) was surgical excision and or cryotherapy, although, sometimes attempt could also be made with chemical cautery. In the present study, signs of pain were not evinced by the animals during or after cryotherapy due to the local analgesic effect in cryogen (Podkonjak, 1982). If warts are removed at mature or regressing stage, incidence of recurrence of the disease is very less. It could also be concluded that surgical excision of warts is a better option for curing BOCP than curetting of warts, use of autogenous vaccines and administration of Levamisole and Ivermectin, provided the warts were old, large and not so extensive. In the present study the surgical excision of large warts were done using a scalpel and bleeding was controlled by thermo-cautery or potassium permanganate cauterization. The virus particles circulating in the blood could act as auto-vaccine. The second regime including curetting the warts aimed to reintroduce the virus to circulation which was considered as autogenous vaccination, topical application of trichloro-acetic acid was done and the injection of immune stimulant (Levamisole) were used for the treatment of animals. Tri-chloroacetic acid was used with the aim to kill wart cells. Warts regression and time elapsed until recovery of the infected animals was used to measure the efficacy of the treatment mode or regime.
Venugopalan, (2000) and O’Conor, (2011) have suggested remedial measures for removal of warts such as use of autogenous vaccine, wart enucleation, burning with hot iron or eraser, ligation and surgical removal of wart (excision) with surgical knife, application of salicylic acid ointment, dimethyl-sulfoxide ointment and potential caustics etc. Surgical removal of one or two warts was proposed but surgical intervention may increase in size of the residual warts and prolong the course of the disease (Radostits et al., 1971). In our study except in one case no such incidence was noted. If warts are removed at mature or regressing stage, incidence of recurrence of the disease is very less. If done too early, chances of recurrence is more. Solitary large sessile warts were removed surgically and for pedunculated, medium and small size solitary warts cryosurgical treatment was done.

Cryotherapy has been used to treat a variety of benign tumours including papilloma with high recovery rate (Paithanpagare and Tank, 2009). Cryotherapy could be made using various cryogen like nitrous oxide, liquid nitrogen etc. In the present investigation liquid nitrogen was used as the coolest cryogen and surgical ablation of warts with liquid nitrogen as cryogen was found as an effective method for treatment of papillomas or warts. Application of liquid nitrogen was done to ensure all wart tissue was killed from the site. During or after cryotherapy in no case, signs of pain in animals were evinced by the affected animals due to the local analgesic effect in cryogen (Podkonjak, 1982). Advantage of minimal bleeding episode associated with cryotherapy of papillomas was evident when compared to that occurring with surgical excision. No recurrence of the growth in and around the cryo-treated area was observed within 30 days of treatment. During the follow-up observations of liquid nitrogen cryotherapy to multiple papillomatous growths of treated animals, it was interesting to note that freezing a few of the papillomatous lesions caused the other peripheral lesions to regress. Neel, (1980) opined that the liquid nitrogen cryotherapy might have stimulated an immune response by bio-absorption of the papillomatous tissues. This was considered to be potentially beneficial to the wart inflicted animals.

Cauterization was nothing but the burning of the skin growth in conjunction with electric current called electro-surgery whereas cryotherapy involved freezing of the warts for easy removal or extirpation of warts with the help of forceps or metallic tweezers and instantly applying liquid nitrogen and freezing the warts almost instantaneously. But our study revealed there were risks of suffering from skin burns or dyschromic wounds for these methods.

Depending upon the size and extent of the lesions and its persistence if any surgical excision or extirpation were done. Besides the cases where all treatment modalities failed, there only option found left and that was surgical intervention to get rid of the warts. Surgical removal was recommended provided the warts were sufficiently objectionable and the best
time for surgical removal was when the warts were near maximum size or had started regressing. In surgical intervention, excision by a pair of scissors or scalpel under local anaesthesia, although found easy way of treatment could not be adopted for extensive papillomatosis or whole body papillomatosis cases. Even for few large warts removal study by surgical intervention, bleeding occurred profusely and would become a messy sight for the animal owner and that needed prompt care to check bleeding. However the bleeding was relatively harmless and aluminum chloride was often applied on the affected area to alleviate it. Since aluminum chloride was not easily available and the cost of the compound was high, cheap and better alternative was established using potassium permanganate for cauterization after excision of warts which caused instant bleed check and prevented further growth or reappearance of warts as it burnt the residual wart tissue as well as the virus involved. Our study revealed there was also risk of reappearance of new warts or neo-formations at the site of excision in few cases in simple surgical extirpation but recurrence was prevented by raw potassium permanganate cauterization method. Surgical resection was found obligatory for the persistent and exceptionally large warts which did not respond to other modes of treatment. Of course if warts be got treated by surgical method it warrants the animal owner to discharge the cumbersome job of daily antiseptic dressing, cost of antibiotics administration and meticulous cleanliness.

Clinical methods although found fast and took only a few hours to complete, the full recovery from the wound could take weeks. Permanent scarring and damage of hides could be a repercussion to the animal owner. Tanned hides in such cases revealed rough, weak spots where from warts were removed. Seriously affected hides often contained pits or holes and looked moth eaten.

Surgical intervention may not be possible if a large is involved and sometimes aggravates the condition. Depending on the size and extent of the lesions, surgical resection, ligation if pedunculated (on a stalk), cryosurgery or thermo-cautery could be recommended. Warts excised surgically did not recur in older wart cases and the excision did not cause spontaneous regression of other left out warts. The results of other workers on surgical treatment are not unanimous. Surgical removal of one or two warts was proposed but surgical intervention could increase the size of the residual warts and prolong the course of the disease (Radostits et al., 1971). Hedrich and Renk, (1969) reported spontaneous regression of the rest of the warts after surgical removal of few warts. Olson and Skidmore, (1959) did not observe recurrence of older warts but such treatment applied at early stage could not change the course. Lenter, (1949) observed regrowth of wart after excision and had recommended the use of 10% oil of Thuja locally after excision. Nagaraju et al., (2013) documented successful management of cutaneous wart by surgical excision and thermo-cautery. In our investigation
following surgical excision instead of thermo cautery cheap alternative way out was raw potassium permanganate application at the bleeding point to check the blood oozing and cauterization of the left out tumour tissue that prevented recurrence or neo formation at the operation site.

In the present study, the surgical excision of large warts was done using a scalpel and bleeding was controlled either by thermo-cautery or by potassium permanganate cauterization as and when required. The infective virus particles circulating in the blood, acted as auto vaccine. The second regime including curetting the warts aimed to reintroduce the virus to circulation which was considered as autogenous vaccination, topical application of trichloroacetic acid was done aiming to kill wart cells and the injection of immune stimulant (Levamizole) were used for the treatment of animals. Warts regression and times elapsed until recovery of the infected animals were used to measure efficiency. The regression of warts and healing were observed 15-90 days post treatment. Surgical excision was superior to regime 2 considering mean 21 days required for regression of warts and healing compared to the 43 days for regime-2. The rate of success in the regimes, 1 and 2 was 100%. It can also be concluded that surgical excision of warts is better treatment option for bovine and bubaline papillomatosis than curetting, autogenous vaccine and administration of levamisole.

The correct choice of treatment method is critical and the successful treatment still remains challenging. The best possible method should be used for each individual lesion taking into account type, anatomical location, duration, and previous treatment history and owners resources. Each factor would influence the decision but no method is universally effective. There are various modalities in the treatment of papillomas and majority of the treatments have been advocated without agreement on the efficacy (Or E and Bakirel, 2002, Cimtay et al., 2003 and Hemmatzadeh et al., 2003). The best possible treatment should be given at the earliest available opportunity. Inappropriate treatment of BPV can result in either further aggravation or in an increased rate of infection among other animals and may even result in death. Infection in cattle and buffalo must be dealt with promptly using full veterinary support.

Successful treatment of papillomatosis has been a great challenge for field practitioners. Commercially available vaccines are less efficacious. Reports of bovine papillomatosis treatment with vaccine produced from formalinized suspension of wart tissue indicate variable results. Besides, there were difference in the treatment in autogenous vaccination relate to nature of diluent used for preparation of autogenous vaccine. By large, autogenous vaccines were used for therapy in the present study gave variable response and this was in agreement to Sreeparvathy et al., (2011). Glycerol saline was recommended by Pearson et al., (1958) and Amstutz, (1978). Instead of glycerol saline by utilizing distilled
water recovery from clinical papillomatosis was also achieved by Wadhwa et al., (1995). In the present study in a trial PBS was used as diluent for the preparation of autogenous vaccine and similar type of clinical recovery was achieved as obtained by the previous worker. Therefore under field conditions where glycerol-saline solution is not readily available, distilled water and PBS can be used in the preparation of autogenous papilloma vaccine for cattle and buffalo. The results of using the auto-genous vaccine irrespective of the diluent used gave promising clinical cure and were in agreement with earlier workers (Pearson et al., 1958; Olson and Skidmore, 1959; Amstutz, 1978; Prasad et al., 1980; Moulton, 1990; Wadhwa et al., 1995; Inayat et al., 1999; Lesnik et al., 1999; Suveges and Schmidt, 2003; Hamad et al., 2012). Our results have shown that effective therapeutic approach for treating papillomatosis of cattle and buffalo could be produced by the formalization of wart tissue or by use of 70% alcohol inactivated wart tissue extracted autogenous vaccine. Alcohol inactivated autogenous vaccine gave a better response within a shorter period compared with treatment by formalinized autogenous vaccine.

The role of the immune response in determining the out-come of BPV infections was well known. It has long been assumed that papilloma regression is mediated by immunological mechanisms which are probably cellular in nature. In most cases, regression of lesions occurred following activation of the host immune response. However several immune evasion mechanisms that could contribute to persistence and malignant progression of the disease had been described (O’Brien and Campo, 2002).

There is a significant relationship between the development of warts and immunity. The disease mostly occurred in individuals which were immunocompromised (Lutzner, 1985). In humans generalized warts have been observed in patients with inherited immunodeficiency (Hausen, 1998). Multiple injections of vaccines have been credited for being an effective cure. Autogenous wart vaccines have been claimed to be one of the most important efficacious alternative mode of treatment of cutaneous papillomatosis. In the present study adopting autogenous wart vaccine (formalinized vaccine, vaccine prepared in PBS, vaccine prepared in glycerol saline, vaccine prepared in 70% alcohol) there was remarkable recovery and recovery was characterized by shedding of old warts and stoppage in the occurrence of fresh warts. However complete regression was not seen in large or very big warts. In those warts there was partial regression. The longer recovery period in the present study could be due to less frequent use of autogenous vaccine. Wadhwa et al., (1995) got faster recovery with the use of six injections of autogenous vaccine. Autogenous wart vaccine has been successfully used in sessile and pedunculated warts in cattle (Theiler and Madewell, 1979 and Gupta et al., 1984), in buffaloes (Singh and Christopher, 1976) and in caprine papillomatosis (Rajuru et al., 1988). Actually autogenous wart vaccine activates the immune system of the body and prevents further occurrence.
Of course failures have been noted (Smith, 1990). Treatment with such vaccine showed 93.5% efficiency. Two injection of 1-2 week apart autogenous vaccine is recommended in which recovery period is 3-6 weeks with 80-85% recovery rate, but only 33% recovery rate was noticed when warts on teat and response of low, flat, sessile warts to vaccination has always poor (Radostits et al., 1994). Similarly Wadhwa et al., (1995) reported 71% efficacy of autogenous vaccine against cutaneous papillomatosis. Chandran (1961), Singh and Christopher, (1976) and Prasad et al., (1980) had also reported autogenous vaccine to be highly effective in treating both cutaneous and teat papillomas. The reason for lower efficacy of autogenous vaccine against teat papillomas as compared to cutaneous papillomas could be that typical fibropapillomas occurring commonly on head and neck is seen rarely on the teats. Viral capsid antigen which might be responsible for inducing immunity is present in lower concentration in atypical teat papillomas as compared to typical fibropapillomas. However according to Lindholm et al., (1984), it is not known, whether shedding of papillomas induced by autogenous vaccine is caused by viral antigens or other antigens expressed in or on tumour cells.

Autogenous vaccination made from sterile homogenized tumour tissue which was administered twice, prevented new cases and with sick animals recovering after vaccination (Turk et al, 2005). Lesnik et al., (1999) also reported that treatment with autologous vaccine showed 93.5% efficiency with no difference in the used vaccine after 105 days of vaccination. Suveges 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. Smith, (1990) reported treatment with autogenous wart vaccine sometimes failed. Commercial vaccines for cattle rarely seem to effectively promote regression of existing warts to prevent malignant regression, 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 the present study, no difference in the treatment of animals was observed in relation to the nature of diluent used for preparation of autogenous vaccine. Glycerol-saline as recommended by Pearson et al., (1958) and Amstutz, (1978) was used in Group -1 whereas in Group 2 sterile normal saline solution replaced glycerol-saline. All the same vaccine prepared by utilizing NSS also affected recovery. Indian worker (Wadhwa et al., 1995) has reported a successful use of bovine papillomatosis autogenous vaccine prepared with saline solution as diluent. Therefore, under field conditions where glycerol-saline solution is not readily available, NSS probably can be used in the preparation of autogenous papilloma vaccine for cattle.

In the present study, treatment with 70% alcohol inactivated vaccine was found superior than the formalinised autogenous vaccine. A marked reduction or regression of the
warts was observed after a week of treatment with alcohol inactivated autogenous vaccine and these progressively degenerated after three weeks of the last vaccination. Then only scar was visible. Here wart rejection could have involved a cell mediated immune response, with infiltration of the site by large numbers of lymphocytes and macrophages. Autogenous vaccines were found the most efficacious treatments for cutaneous warts but expensive and cumbersome in preparing the same. In addition, they can fail against atypical warts (Barthold et al., 1974) or in the case of large confluent lesions (Ssenyonga et al., 1990). Such findings were in agreement to (Olson et al., 1960; Jarrett, 1985).

Not only for CCWT cases but also the animals having warts on teats and udder also showed marked improvement. Some remnants to the extent of 5% were left out on the udder and teats of one cow even after 60 days. Remaining warts completely degenerated within three weeks following 2ndslot of treatment with autogenous vaccine comprising two injection of 5ml each given subcutaneously at 4 days interval. Successful vaccination against papillomavirus infection could probably also have induced neutralizing antibodies and specific T cells directed against early viral proteins (viz. E7). It was in agreement to Nico et al. (2002).

In the present study, results showed the efficacy of bovine papillomatosis treatment with the autogenous vaccine inactivated in 70% alcohol induced in the manner of earlier regression of papillomas. No adverse reaction of vaccination was observed in any animal. Data reported previously based on the treatment only with autogenous vaccine showed a longer period necessary for animal recovery than our results obtained (Scot and Anderson, 1992; Lesnik et al., 1999). It could be considered appropriate in all stage of papilloma and early stage the disease (growing stage of warts) when surgical intervention is contraindicated due to possibility of recurrence of papillomas. Similarly Wadhwa et al. (1995) reported 71 percent efficacy of autogenous vaccine against cutaneous papillomatosis. Chandran (1961), Singh and Christopher (1976) and Prasad et al., (1980) had also reported autogenous vaccine to be highly successful in treating cutaneous and teat papillomas.

The autogenous vaccines stimulate the immune system against the papilloma viruses. The variation of response could be attributed to type of virus involved, developmental stages of papillomas, method of collection of papilloma tissues and preparation of vaccine, schedule of administration and immune function of the patient. Papillomatosis is noticed commonly in immune deficient animals and supplementation of minerals and vitamins, proper deworming, use of immune-modulators, supplementation of para immunity inducer along with autogenous vaccination can enhance the faster recovery. When the disease is a herd problem, it can be controlled by vaccination with a suspension of ground wart tissue in which the virus has been killed with formalin. It would be necessary to begin vaccination in calves as early as four to
six weeks of age. The vaccination should be repeated within four to six weeks and at one year age. Immunity develops in a few weeks but is unrelated to whatever mechanism is involved in spontaneous regression. If the animal was exposed to the virus before vaccination, immunity may develop too late to prevent warts. A vaccination program must be in effect three to six months before its preventive value will be evident.

It is generally accepted that regression of papillomas or fibropapillomas is mediated by cellular immunity. Paulik et al., (2001) found decreased blastogenic activity of lymphocytes in bulls with cutaneous papillomatosis persisting more than one year. Surgical extirpation of a large proportion of the papillomas and the use of autologous vaccine led to continual regression of the tumours and gradual elevation of lymphocytic activity.

Some papillomas are topographically specific and caused by distinct viruses having different antigenic reactions and DNA compositions. Therefore a vaccine providing immunity to one of them does not confer immunity to other. By large, autogenous vaccines stimulate the immune system against the papilloma viruses. The variation of response may be attributed to type of virus involved, developmental stages of papillomas, method of collection of papilloma tissues and preparation of vaccine, schedule of administration and immune function of the patient.

Antimony preparations with varying efficacy was in use in treating bovine and bubaline papillomatosis (Rajguru et al., 1988 and Wadhwa et al., 1992). To our knowledge the usage of antimony preparations for the treatment of bovine teat warts or bovine teat papillomatosis has been poorly studied. However, it was reported that antimony preparation does work well in bovine cutaneous warts where the lesions are small, nodular and cauliflower like and it has least or poor efficacy on treating large fibropapillomas bearing wide base and big sized flat warts. The efficacy of antimony preparation in the present study revealed complete clinical cure in seven clinical cases of teat papillomatosis bearing typical filiform warts with rough surface texture (in 4 animals), small pedunculated warts (in 2 animals) and sessile type warts (in 1 animal) and no effect on typical flat wide base teat papilloma and large frond type warts as recorded in three animals. Our study differed from the findings of Shukriti Sharma et al., (2003) who found no response of treatment by six injections of lithium antimony thiomalate to a severely infected case of udder wart in a cow. The same line of treatment as adopted for treating bovine teat papillomatosis by Hafiz et al., (2009) in Jammu and Kashmir resulted in 100% clinical cure in ten cross bred lactating cows those suffered from teat warts characterized by round elongated rice grain like lesions on teat skin. The present study was carried out to see the effect of lithium antimony thiomalate on bovine teat papillomatosis and was found to be an effective therapy for curing CTP.
Teat warts appear in several forms, such as frond type, a flat round type and elongated rice grain structure. They are usually multiple, always sessile and are up to 2 cm in diameter. The frond forms have filliform projections on them (Blood and Radostits, 1989). In the present study, the warts were grossly classified as filiform, large frond type and typical flat elevated, round type warts and histologically frond epithelial type as well as characteristic to frond fibro-papilloma type.

Surgical intervention and vaccination would not have been the right choice for treating teat papillomatosis since it was evident that surgical intervention and vaccination could increase the size of residual warts and prolong the course of the disease or aggravated the condition (Radostits, 1989). Besides cauterization, excision, cryotherapy, administration of local anaesthesia, autologous or heterologous vaccination, and auto-hemotherapy; use of drugs for the non-specific stimulation of the immune system can be employed for treating bovine papillomatosis.

Another treatment method employed for treating warts is the administration of drugs or chemical substances to stimulate the nonspecific immune system that can induce regression of wart lesions (Silva et al., 1998; Amin et al., 1997; Shah and Howley 1996; Dinc, 1995; Bajric et al., 1983). Having treated bovine papillomatosis with levamisole at a dose of 2.5 mg/kg/day on days 1, 3, 5, 7, 9 and 16, Chian et al., (2004) have reported 100% recovery within 7-12 days after the completion of treatment. In the present investigation comparative studies were also made with using Ivermectin orally twice at 15 days interval, parenterally single S/C administration of Ivermectin and two shots of S/C administration of Ivermectin. Results on overall response to such therapy were 53.33%, 70% and 86.66% respectively. Partial to moderate recovery was also noted in 26.66% , 23.33% and 13.33% cases with no response to such therapy was noted in 20% and 6.66% cases respectively. Gradual loss in size of wart after the therapy instituted was noted with loosening from the site of attachment and sloughing of the dead tissue and or dropping of the degraded warts which occurred after treatment indicated clinical cure. This clearly reflects two shots S/C administration of injectable form of Ivermectin was superior to single shot administration followed by two shots of oral administration. These results could have occurred mainly for three reasons, the first could be due to the cytotoxic effect of Ivermectin on carcinic cell of the wart as the same inhibiting effect of calvatic acid which is extracted from Calvatia craniformis fungus on the carcinoma (Hamao et al., 1975), also the ivermectin extracted from soil fungus and may be possessing this ability. Thus the therapy resulting in gradual loss in size after the administration of the drug and warts loosing vitality were obvious and that could have happened due to the cytotoxic effect of the drug on the carcinic cell of the warts.
Kim et al., (1992) observed that protein-bound polysaccharides extracted from cultured *C. craniformis* mycelium suppressed the growth of sarcoma in mice by up to 74.1%. The anti-tumor activity of at least one of extract fraction referred to as calvatane was believed to be as a result of immune potentiation rather than cytotoxicity. The second reason is may be due to the ivermectin effect on the cellular immune response of the animal and this effect could be due to significant elevations in total count of white blood cells and in percentage in lymphocyte cells after treatment by ivermectin were be share in raising the immunity of the infected animals and cause the degradation of wart tissue. One of the components of the lymphocytes is T-cell, it can be distinguished from others by presence of special receptor on its surface called T cell receptor (TCR) and play a central role in cell mediated immunity as virally infected cells and tumor cells (Schwarz and Bhandoola, 2006). There is other T-cell called natural killer T-cells (NKT-cell), is able to recognize and eliminate some tumour cells (Barton et al., 2000). Effectiveness of Ivermectin in treating bovine papillomatosis was a superior treatment modality as reported by Borku et al., (2007) and our study thus confirmed the previous reports. It was reported that ivermectin, an anthelmintic drug, increased antibody production, T lymphocyte and macrophage-dependent response, and therefore had an immunomodulator effect (Blakley and Rousseaux-1991; Uhlir 1991; Rao et al., 1987, Sajid et al., 2007). Ivermectin could also influence the immune system but mechanism involved in immunopotentiating effect of ivermectin is unknown. Borku et al., (2007) reported that ivermectin was effective treatment of bovine cutaneous papillomas. Our study confirms the findings in agreement to Borku et al., (2007).

It is clear that the immune system plays an important role in modulating the severity of papillomas. It is suggested that the regression of lesions is probably affected by cellular rather than humoral immunity (Nicholls and Stanley, 2000). Ivermectin stimulates both humoral and cellular immunity (Blakley and Rousseaux, 1991; Rao et al., 1987; Uhlir, 1991). In the present study, the high rate of clinical improvement and or cure rate obtained upon both oral as well as subcutaneous administration of Ivermectin for the treatment of papillomatosis might have occurred due to immunostimulatory effect of Ivermectin, as earlier workers had previously reported (Borku et al., 2007). In general beneficial effects from the use of Ivermectin mediated by the immune system have also been observed in pronounced antitumour effect (Drinyaev et al., 2004; Korystov et al., 2004). Most interestingly it was observed that antibody production, T lymphocytes and macrophages dependent response was enhanced by ivermectin treatment in mice (Blakley and Rousseaux 1991). Serum specific antibody activity was increased after ivermectin treatment (Rao et al., 1987; Uhlir, 1991). In the present study, having treated bovine papillomatosis with both oral and parenteral ivermectin, 53.33% and 70-80% recovery (complete clinical cure) were observed with in
This study clearly demonstrated ivermectin treatment to be an effective treatment mode on bovine papillomatosis. Compared to other treatment methods, the administration of ivermectin either per os or by S/C route was determined practical.

Although bovine papillomatosis is a self-limiting disease, majority of the animals in our study had long lasting multiple papillomas without any sign of regression for months. Levamisole, an isomer of Tetramisole, has been used over a period of 20 years as an immunomodulator and treatment of tumours and several infectious disease of viral etiology of man and animals. The role of Levamizole as non-specific immune stimulant is very clear in many infections as reported by Cam et al., (2007). They evaluated it for treatment of bovine cutaneous papillomatosis. Amery and Butterworth (1983) found that Levamisole had good effects as an immune-modulator for blood disorders, renal failure, vasculitis and photosensitivity. In spite of that the immune-stimulating effect of Levamisole against BOCP was non obvious where the levamizole may promote the general immune response but it could not help in eliminating the warts in a rapid manner. That was disagreeing with the past studies of the role of non-specific immunomodulator (parapoxvirus) against BP. The inactivated parapox ovis viruses had a complex genetic structure and thereby they were considered as non-specific strong immune-modulator, which induced host immune reaction. There was evidence that such immune reactions resulted in more than elimination of the virus (Fachinger et al., 2000). In the present investigation for studying the effectiveness of Levamisole both oral as well as injectable form were used for treating bovine papillomatosis and the outcome of such study revealed complete clinical cure in 82% and 88% infected cases respectively. In both the cases regression started as early as 15 DPT but complete shedding or regression in oral mode started at 30 DPT whereas in parenteral mode of therapy complete regression started as early as 20 DPT. Healing and or regression was evaluated by macroscopic examination and or visual assessment (i.e. decrease in diameter, changes in colour, shrinkage, dryness and dropping of dead tissue). In earlier studies by Cihan et al., (2004), a 100% success rate was obtained from the treatment of viral papillomatosis. Total healing as they found to occur during 7-12th days post treatment but as in the present study complete clinical cure needed little more time as shown earlier. Our findings were consistent with the results of other studies where levamisole had been used against viral infections for prevention or medication (Murchay and Quinn, 1986 and Gokce et al., 1997). Cell mediated immune response in viral infections during the recovery period and prevention appears more important than humoral response. Long-term usage of anthelmintic doses of levamisole could depress the immune system. For this reason, it was advocated that therapeutic and preventive doses only for three days with three days intervals and 1/3rd or 1/4th doses were more effective (Brunner and Muskoplat, 1980; Gokce et al., 1997). In this study a total of six applications,
during 1, 3, 5, 7, 9 and 16th days, @ 2.5 mg/kg of Levamisole IM or oral doses were used and no difference in the healing process was observed between IM and oral applications. Levamisole has been reported to enhance immune response to viral antigens (Babuik and Misra, 1981). It increases the antibody response to vaccination through cell-mediated response to vaccination through cell-mediated response (Giambrone and Klesius, 1985). Peroral levamisole treatment in mice showed an enhanced production of Interleukin-1 (IL-1) in isolated peritoneal macrophages (Vojtic, 1998). Its greatest immunostimulating effect has been observed in immunosuppressed animals (Brunner and Muscoplat, 1980). Usage of Levamisole was found safe and cheap alternative treatment option for papillomatosis in cattle in place of surgical or cryosurgical approaches (Olson, 1993; Smith, 1996 and Stedham, 1984). Nonetheless the immune-stimulating effect of levamizole against CWT was obvious in this study which hastened clinical recovery or complete clinical cure.

Auto-hemotherapy for treating bovine cutaneous papillomatosis has nowadays been gaining importance (Bajric et al., 1983; Pattanayak, 2004; Mitra, 2005; Jana and Jana, 2009 and Kumar, 2011) and it may serve as a useful adjunct to autogenous vaccine (Ranjan et al., 2013). Nonspecific stimulation of the immune system by so called self-blood therapy or autohemotherapy for treating bovine and bubaline papillomatosis have also been reported with varying efficacy (Bajric et al., 1983; Jana and Jana, 2009; Chelapati Rao, 2010). In autohaemotherapy after fourth injection, the papilloma growths showed signs of regression. The findings were in accordance with those of Chelapati Rao, 2010; Chetan Kumar, (2011); Ganesh Hegde, (2011) and Kavitha et al, (2014). Bajric et al., (1983) had reported papillomas to regress completely in the majority of cattle undergoing administered autohemotherapy, 3-4 times at intervals of 7-10 days. In our study, auto-haemotherapy as administered revealed signs of regression after fourth administration. The abnormally grown epithelial and fibrous tissues were shed by animals due to better immune-surveillance that developed after the autohemotherapy which was not noticed earlier. In our study autohemotherapy revealed higher rate of success in clinical recovery; 90% complete cure observed in autohemotherapy alone and 100% cure rate seen in combined autohemotherapy and levaisole therapy. The treated animals showed far better clinical improvement with the disappearance of lesions. 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. Overall clinical cure was noted in 90% cases at DPT-90 and complete regression started as early as DPT-45 (in 15% cases), at DPT-60 (in 18% cases), at DPT-75 (in 12% cases) and at DPT-90 (35% cases). By the end of six to seven weeks papillomatous growths were completely reduced in 15% cases and only light black coloured scars were seen at the sight of the growths. By the end of 12 weeks all the papilloma growths were completely regressed. 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). The present study revealed that the nodular pedunculated, small pea shaped warts, cauliflower like pedunculated growths responded well while warts which were very large, sessile type, or smooth, flat or round circumscribed, did not respond that well with auto-hemotherapy. The warts which were abnormally grown with epithelial and fibrous tissue proliferation were shed by the animals due to better immune-surveillance that developed after the injection of its own blood (auto-hemotherapy) which was not noticed earlier. The virus infects the basal cells of the epithelium causing the excessive growth which is characteristic of wart formation. The tumour contains epithelial and connective tissue. In the present study the abnormally grown epithelial and fibrous tissue die after injection of self-blood of the sick animal. It may be the case that auto-hemotherapy helped the immune system of the affected animals to know and identify the abnormality of the virus infected cells. No adverse reaction of auto-hemotherapy was observed in any animal. Withdrawing blood from the vein and the immediate application in the muscle stimulates an increase in macrophages. The standard rate of macrophage is 5% in the blood and in therapy the rate could increase to 20-22% and this could result in powerful immune stimulus and multiplies the body defense. It is known that macrophages carryout a cleansing of everything; they could eliminate the viruses. Macrophages eliminate the fibrin which is the clotted blood. Blood in the muscle, works as the foreign body and to eliminate the same there was increase in production of macrophages by the RE system. RES being activated produce more macrophages and its action finishes or drops at the 5th to 7th days, since the blood in the muscle is coming to an end. Auto-hemotherapy in the present study showed its wart therapeutic effect on clinical cure of warts by way of indirect and direct stimulus on immune function and above all by increasing macrophage activities.

Auto-hemotherapy for treatment of chronic diseases in human beings is an age old practice, but has gained limited public acceptability (Wallis, 1947; Olwin et al., 1997). However, there seems paucity of reports available on use of auto-hemotherapy in veterinary practice. Auto hemotherapy was reported to cause complete cure of papillomatosis in cattle (Pattanayak, 2004; Mitra, 2005; Hegde, 2011 and Kumar, 2011), and in buffaloes (Jana and Jana, 2009). The findings of the study revealed that without using any chemical agent, auto haemotherapy can be effectively employed to treat papilomatosis and get rid of papillomatosis within four weeks. In the present study it was obvious that the abnormally grown epithelial and fibrous tissue died after injection of self-blood of the infected animal. It may be the case that the auto-hemotherapy may help the immune system of the affected animals to know and
identify the abnormality of the virus induced cells. Auto-hemotherapy is believed stimulate the reticulo-endothelial system and to increase population of macrophages in circulating blood, which might be responsible for enhancing regression rate of the papillomas. Concurrent administration of paraimmunity inducers support rapid regression of warts and enhancing efficacy of autogenous vaccines (Inayat et al., 1999; Turk et al., 2005). From the present study, it can be concluded that auto-hemotherapy is effective in treating bovine papillomatosis.

On contrary to surgical excision, cryosurgery, thermocautery, autohemotherapy, immune modulation by Levamisole therapy, drug therapy like Antimony compound and ivermectin modulation or application of caustic agents, warts were treated by homeopathic treatment alternative using sulphur and Thuja. In this study, Thuja was used successfully against cutaneous papilloma in cattle. Such preparations were used in cattle for cutaneous warts and for venereal warts in man through various routes (Prakash, 1993; Madrewar, 1996; Veena, 2001; Umadevi and Umakanthan, 2013) and orally against oral papilloma in dogs (Umakanthan, 2002) and claiming complete recovery of the disease. Though the authors were not aware of the exact mechanisms how the homeopathic preparations under reference acted on papillomas but it was believed that the preparations could play an important role in cutting down the blood supply of the papillomatous growth.

*Thuja occidentalis* (Mor Punkh, vernacular name in Urdu) is a common small doom shaped plant found in orchards or gardens. Thuja-30 potency drug is prepared from the mother tincture of its leaves. This drug is quite popular in human being for removing warts in general and black cauliflower like lesions in particular (Hussain, 1988). It acts on skin, blood and genitourinary organs (Maclead, 1992). This is of real importance in the treatment of skin conditions accompanied by the development of wart like growths which bleed easily (Madrewar, 1999). In the present therapeutic trial application and or use of Thuja mother tincture, Thuja-200x per oral route and S/C use of Thuja-200x gave wonderful results in curing warts in cattle. The homeopathic treatment although took little more time to exert its effect but the definite results on regression or on clinical recovery was observed. During the study it was observed that shrinking, sloughing and disappearance of warts happened in treatment group of animals except in the control animals where rather than regression intermediate neo formations were also noted. This wonderful effect could be ascribed to its various chemical constituents, i.e. Dextro-pinene, Dextro-thujone, thujine, levo-fenchone and pinpierin (Felter, 1904). Thuja, a homeopathic medicine derived from plants was found safe and cheap and could be given safely either by oral route or by percutaneous route (Beoricke, 2008).
In the present investigation selection of homeopathic medicine like sulphur and Thuja proved its worth within six weeks. It is in line with the findings of Madrewar, (1999) who described 90%, 95% even 100% cure in various diseases (skin diseases like pityriasis, hyperkeratosis, parakeratosis, pachyderma, urticarial and warts) which had been declared incurable by the other system of treatment. In the present investigation Thuja mother tincture per oral route (74.33% clinical cure) was more efficacious than Thuja 200x potency per os (70% clinical cure) and Thuja 200x S/C (60% clinical cure).

The one of the probable mode of actions is antagonism of gama amino butric acid (GABA) receptor system and the convulsant action is caused by modulating the GA-0BS-gated chloride channel (Umadevi and Umakanthan, 2013). Thuja containing alpha thujone which acts on the non-competitive blocker site of GABA, A receptor (Grieve, 1931). The vital chemical constituents present in Thuja could be responsible for exerting regression of warts in man and animals but the exact mechanism of action is yet to explore or to establish. As papillomatosis is known to be a self- regressing disease taking 5-6 months or even 18 months (Radostits et al., 1989) and as the regression or sloughing of warts occurred pretty earlier (within 45-90 DPT) there are reasons to believe that the regression and sloughing occurred due to action of drug not by self-regression. Therefore this line of treatment can be practiced for the treatment of papillomatosis.

The successful therapeutic approach as adopted with the homeopathic medicines on bovine cutaneous papillomatosis including teat papilloma had its worth for being considered as cheap and best alternative non-surgical remedial measure.

Hundred percent clinical cures was obtained in combined levamisole and self- blood therapy (autohemotherapy) reflecting most efficacious therapeutic approach in treating BP. In autohemotherapy after fourth injection, the papilloma growths showed signs of regression. The findings were in accordance with those of Hegde, (2011) who treated cutaneous papillomatosis in a non-descript cow by autohemotherapy and Chetan Kumar, (2011) that used autohemotherapy for the treatment of BP. The findings of this study revealed that without using any chemical agent, autohemotherapy can be effectively employed to treat cutaneous papillomatosis.

Autohemotherapy alone was found also to be a very good therapeutic approach in curing BP giving 90% clinical cure and 99% overall response to such therapy. Parenteral mode of immunomodulation therapy by Levamisole S/C and Ivermectin double shot S/C administration was also found most effective giving 88% and 86.66% clinical recovery of BP respectively. Levamisole oral therapy alone was also found effective in clinical recovery of BP resulting in 82% clinical cure, partial recovery in 12% and no response in 6% cases. The warts which are abnormally grown epithelial and fibrous tissue were shed by the animals due
to better immune surveillance that developed after the injection of its own blood (autohemotherapy) and synergestic action with the administration of either levamisole or Ivermectin, which was not noticed earlier.

Formalinized autogenous vaccines prepared in different diluents as studied revealed 85% clinical cures where NSS was used as the diluent, 70% clinical cure in 70% alcohol inactivated autogenous vaccine, 60% clinical cure in formalinised autogenous vaccine where PBS was used as the diluent and 55% recovery where glycerol saline was used as the diluent. Autogenous vaccines either formaline inactivated or 70% alcohol inactivated and irrespective of the diluent used gave 55%-85% clinical recovery.

Injectable Antimony compound (Lithium Antimony Thiomalate, Injection Anthiomaline) along with oral or injectable Ivermectin therapy gave almost identical clinical cures (82.22%) and partial to moderate regression 13.33% and 11.11% respectively. On the other hand Injection Anthiomaline alone was also found as an effective drug for bringing clinical cure in 77.77% cases. 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. In the present study, the high rate of improvement and or clinical cure obtained upon the application of single or double injections of Ivermectin for the treatment of papillomatosis might have occurred due to immune-stimulatory effect of Ivermectin as well as due to cytotoxic effect of ivermectin on carcinic cells of the wart, as other authors have also previously reported (Borku et al., 2007; Ghassan et al, 2011). Absence of recovery in 20% cases in oral Ivermectin therapy and 13.33% cases in single S/C Ivermectin therapy led us to consider that Ivermectin did not stimulate the immune system of these animals sufficiently as suggested by Nicholls and Stanley, (2000). The results of this study revealed ivermectin as an effective agent for the treatment of bovine cutaneous papillomatosis and it provides a strong evidence base for the treatment of this chronic disease.
Regression study conducted in the field by clinical and histological comparative analyzing of the cutaneous wart samples collected before and after the therapy (at DPT45 and DPT60) instituted by Thuja oral and parenteral therapy and by the administration of antimony compound, Injection Anthiomaline revealed similar type of observations, macroscopically showing the indication of degeneration, necrosis, drying, shrinking and shedding of warts. Microscopic examination of regressive papilloma collected at sixth and eighth week of treatment revealed picnotic nuclei and reduction in size of the cells (at DPT-45) followed by cellular necrosis at DPT-60. These findings were in agreement to Hossain et al., (2005). Regression of lesions were observed clinically and histologically. Microscopic finding of clumping of nuclear chromatin, reduction in size of cells and extensive thickening of keratin layer simulates the characters of necrotic cells (Jones et al., 1997). As papillomatosis is a self-regressing disease taking 5-6 months or even 18 months for complete regression (Radostits et al., 1998), and as the necrotic changes took place at 6-8 weeks of treatment with either Anthiomaline, levamisole, ivermectin, autohemotherapy (alone) or in combination, homeopathic medication etc. (by Thuja of different potency and routes), there are reasons to believe that these changes occurred due to the action of the drugs or the therapeutic agents and not by self-regression.

It is very important that the adoption of prophylaxis and prevention measures, such as rigorous inspection when introducing animals in the farm, isolation of sick animals, vaccination, sterilization of milking equipment, use of disposables such as needles and syringes, control of flies and ticks through the use of insecticides and to maintain good hygiene and biosecurity measures (Campo and Jarret, 1994; Wadhwa et al., 1996; Maeda et al., 2007). Proper disinfection of tack, tagging pliers and tattooing instruments would prevent the spread of the wart virus. The infected animals may not have visible warts, but they may still contaminate equipment. Tattoo or tagging pliers can be disinfected between uses on calves, with a 2 to 4% solution of formaldehyde. Tack that has been in contact with infected calves can also be disinfected with formaldehyde.