India has vast resource of livestock and poultry, which plays a vital role in improving the socio-economic conditions of the rural masses. India ranks first in respect of buffaloes (having 57% of world’s buffalo population), second in cattle and goats, third in sheep, fourth in ducks, fifth in chickens and sixth in camel population in the world (according to the Annual Report 2009-10, Department of Animal Husbandry, Dairying and Fisheries, Government of India). India possesses one of the largest livestock population in the world, which is more than 529.698 million with top position in buffalo (105.343 million), second position in cattle (199.075 million) and goat (140.537 million), third in sheep (71.558 million), and pig (11.134 million) according to 18th livestock census and the total population of cattle and buffalo has been increased in the tune of 6.98 per cent and 7.04 per cent respectively as compared to previous livestock census. Cattle husbandry is an important activity of villages of our country for livelihood and employment since centuries. Dairy raisers received many valuable animal products like milk, meat, hide as well as manure from their dairy animals. In spite of such prospects, few diseases are the stumble blocks to achieve the success and incurring 31.3% economic losses due to animal disease. Bovine papillomatosis is an important disease in bovine population in few state of the country leading to considerable economic depreciation of animal due to decline in value of skin and hide.

Bovine papillomatosis is a common viral infection of cattle and buffalo caused by Bovine papillomaviruses (BPV) belonging to the Papillomavirus (PV) genus of Papillomaviridae (Van Regenmortel et al., 2000) causing benign hyper proliferative lesions of both mucosal and cutaneous epithelia (Borzacchiello and Roperto, 2008). BPVs are a heterogeneous group of epitheliotropic viruses that recognize bovines as its classical host. Twelve different BPV types have been characterized and classified into three genera: Delta papillomavirus (BPV-1 and -2), Epsilon papillomavirus (BPV-5 and -8) and Xi papillomavirus (BPV-3, -4, -6, -9, -10, -11 and -12), and an as yet unassigned PV genus (BPV-7) (Bernard et al., 2010; Hatama et al., 2011; Zhu et al., 2011). In cattle BPV type-2 and -3 produce cutaneous lesions or squamous papilloma; however, type-3 has a geographical restriction for Australia. Bovine papilloma virus (BPV) type-1, produce fibropapillomas (teat and penile fibro-papillomas), BPV-2 is associated with cutaneous warts, alimentary fibro-papillomas and urinary bladder tumours, BPV-3 causes cutaneous papillomas, BPV-4 is associated with pure epithelial papillomas of the upper gastrointestinal tract, BPV-5 induces fibro-papillomas of the udder, BPV-6 causes papillomas of the teats, BPV-8 causes cutaneous papillomas and BPV-9/10 are associated with epithelial squamous papillomas of the udder (Borzacchiello and Roperto 2008). All the BPV types recognized so far worldwide are said to
be strictly species specific but BPV type-1 (BPV-1) and BPV type-2 (BPV-2) are the exceptional viruses that can jump species and they can infect equids (horses, donkeys and mules), where it causes fibroblastic tumours called sarcoids at their infection sites (Nasir and Campo, 2008). BPV-1/-2 was also found co-infecting giraffe (van Dyk et al., 2011), zebra (van Dyk et al., 2009), buffaloes (Silvestre et al., 2009; Singh and Somvanshi, 2010) and yaks (Bam et al., 2012) and to produce cutaneous warts. It is a non-enveloped double-stranded DNA virus and its genomes comprise nearly 8 Kb, which includes a long control region (LCR), early (E) and Late (L) genes. The LCR (about 500-1000 nucleotides) contains transcriptional regulatory sequences and the replication origin (Munger and Howley, 2002).

BPV in most of the cases is chronically maintained in the animal’s body in a quiescent state or can cause neoplasia or persist asymptotically. Keratinocyte stem cells in the epithelial basement layer can maintain papillomavirus genomes for decades. However, it has been reported that these viruses can be detected in healthy skins of animals as commensals and in latent infections or subclinical infections too (Astori et al., 1998; Antonsson and Hansson 2002; Ogawa et al., 2004).

The primary source and natural carrier of the virus is cattle. The contagion enters the body through scratches or other defects. Although the infection occurs through both direct and indirect contact, other factors may also play a significant role in the occurrence of the disease. Contaminated materials, milking machines, tuberculosis injections, malnutrition and hormonal imbalances as well as semen, mutations and long term exposure to sunlight and immunodeficiency also can help in establishing the disease in susceptible animals (Olson, 1993; Araibi et al., 2004; Lindsey et al., 2009).

BPV infection has been associated with four main clinical presentations linked with specific viral types, caused by a heterogeneous group of epitheliotropic viruses that recognize bovines as its classical host, causing 1. Cutaneous fibro papillomas (BPV-1and BPV-2); 2. Cutaneous papillomas (BPV-3), 3. Gastro-intestinal papillomas (BPV-4) and 4. cutaneous teat papillomas (BPV-6) and teat fibro-papilloma (BPV-5), (Ogawa et al., 2004; Wosiacki et al., 2006; Claus et al., 2007).

BPVs can induce papillomatosis of the skin, the genital and para genital area, the eye and ear, the teats and udder, the upper gastro intestinal tract and the urinary bladder. Any animal runs the risk of getting PV infection and developing warts but the risk is generally increased in young animals that have poorly developed immune system and animals those are crowded in close quarters with infected animals and above all those are sick and stressed. However, BPV infection is a unique pathological situation developed as a result of virus exposure to single or multiple lesions of the dermal or mucosal epithelia. The virus usually appears to infect the basal cells of the epithelium, causing hyperplasia with hydropic
ballooning of the cytoplasm, large eosinophilic keratohyaline granules and vesicular nuclei. Some cells degenerate while others are stimulated to excessive growth and formation of warts (papillomata) but most of the warts are benign and do not proliferate indefinitely. Benign warts in immune-competent animals almost never undergo malignant transformation.

Warts are spread usually by direct contact or indirectly via fomites left on surfaces. Many factors such as contaminated food and equipment viz halters, nose leads, grooming and ear marking objects etc., inheritance, nutritional imbalance, hormonal imbalance that suppressing the immune system, may also play vital roles in the spread of the disease (Campo et al., 1994a; Dinc 1995; Nicholls and Stanley 2000; Otter and Leonard 2003). It is known that confined populations are more vulnerable because of the virus dissemination by both direct (skin to skin or animal to animal) or indirect (contaminated objects) contact (Hama et al., 1988; Nasir and Campo, 2008). Besides the established skin-skin pathway, another via like arthropod vector and vertical transmission has been suggested (Freitas et al., 2003; Finlay et al., 2009). However, these alternatives via of transmission might be less efficient (Bravo et al., 2010). Of late the detection of BPV DNA sequence in reproductive tissues and cells (oocytes, ovary, uterus, cumulus cells and uterine lavage) strongly suggests that these sequences could be an important alternative of viral transmission that could contribute to the widespread incidence of bovine papillomatosis and its complex pathology (Yaguiu et al., 2006). To date, the BPV 2 genome has also been detected in lymphocytes during latent papilloma virus infection in cattle (Campo et al., 1994; Stocco dos Santos et al., 1998). In addition, the occurrence of horizontal transmission of BPV 2 has been reported in the healthy cattle experimentally inoculated with peripheral blood from hematuric animals (Stocco dos Santos et. al. 1998). Recent findings of BPV in epidermis and formation of L1 capsomers of equine sarcoid and active BPV in normal skin of equine (Bogaert et al., 2008; 2010a, b; Brandt et al., 2011) could help explaining the occurrence of equine sarcoid in animals kept far away from any bovine virus source, especially when living in close contact with other affected equids (Brandt et al., 2011). It is believed that flies can be a vector for BPV and transmit the virus between bovine and horses. The mechanism of transmission of BPV in a cattle herd and to other placental mammals should be most studied since BPVs are disseminated infecting and co-infecting these animals due to its plasticity.

Papilloma is common in animals of young age and adult animals are more or less resistant to infection probably due to acquired immunity derived from apparent or in apparent infection during their young age (Radostits et al. 1994). Pathogenesis of papilloma virus infection (papillomatosis) in cattle is related to decay of immunological response associated with the presence of BPV (Knowels et al., 1996; Hansen 2002). The incubation period ranges from 2 to six months depending on factors such as the specificity of virus, dose, and route of
exposure and immune status of the host animals. In cattle, warts occur on almost any part of the body, but when a number of animals in a group are affected predilection site observed was common to all affected animals. Usually, the severity of infection has been observed to be higher in the cattle than buffaloes, which may be due to the higher viral load or more susceptibility or any other reason.

Cutaneous warts are the morbid and solid outgrowth of the skin with a complex etiology and pathogenesis and these are of different external appearance according to their coating. When they are covered by the epidermis, they appear dry, hard and horny. Most warts appear as epidermal proliferations that have a keratotic surface resembling a cauliflower known as verruca vulgaris. Their forms are likewise various; some are flat, upon a broad basis; could be discrete, low, flat and circular in appearance, pedunculated or non pedunculated, pea-sized; filiform, or rice grain like appearance; others are conical, growing on a pedicle. However, in majority of the cases these are of pedunculated form with cauliflower-like appearance on the surface of the skin.

Duration of warts varies considerably and may persist for long periods and is then, in a proportion of cases, rejected, leaving the host immune to reinfection (Jarrett 1985). The immune system of an infected animal plays an important role in the outcome of PV infection as an immunosuppression and physical trauma can reactivate latent asymptomatic PV infections in animals and signs appear. The disease infectious bovine papillomatosis is a chronic contagious viral malady of cattle caused by BPVs and is characterized by the presence of warts or papillomas of various sizes on the skin, localized to teat or generalized leading to excessive papillomatosis affecting different anatomic locations and involving large areas on animal body. Extensive lesions leads to depreciation in both aesthetic and economic value of the animal due to progressive loss of body condition, hides value, increased risk of conditions like repeated trauma, knocking and bleeding, flyblown condition ormyiasis of traumatized warts, subsequent bacterial infections leading to necrotic dermatitis, mastitis, interference in suckling, milking and hindrance in coitus.

Bovine warts are hardly a health threat, unless they become malignant or cancerous which do occur in mucosal epithelia forming serious lesions i.e. mucosal papillomas which tend to run a prolonged course and or persistence and exposure to environmental carcinogens and immuno-suppressants. The bovine papillomatosis is an important disease leading to economic depreciation of animals, deterioration of the appearance and of the animal leather. Papilloma virus infection in cattle can result in weight loss and retarded growth. The lesions are often associated with the mammary gland and interfere with milking. It can lead to reduction in milk yield. Some types of warts may involve the venereal regions where they can cause pain, disfigurement, infection of epithelia of the prepuce and penis of young bulls.
making coitus difficult and dystocia when vaginal mucosae of the heifer is affected (Fitzpatrick 1998). The tumours can spread along the perineum and even up toward the back; they can become necrotic and cause loss of reproductive functions (Borzacchiello 2007). The disease affects young animals often and more severely, but may affect cattle of all ages (Olson 1993; Campo and Jarret 1994; Smith 1996; Nicholls and Stanley 2000; Jelinek and Tachezy 2005) and can cause dramatic economic losses in cattle through benign and malignant lesions (Diniz et al., 2009). Thus the disease can lead to a serious economic loss if not diagnosed and treated promptly. Besides serious economic impact, the zoonotic potential and the medical implications for the corresponding transmission route are most important (Bravo et al., 2010).

In India bovine papillomatosis has been reported sporadically by various workers. Cutaneous warts (CWTs) are widely prevalent in both organized and unorganized dairy farms of urban as well as rural sector in UP, Uttarakhand, Himachal Pradesh and Tamilnadu, as revealed on the surveys of CWTs conducted since 2006 (Leishangthem, 2006; Singh, 2007; Pangty, 2009; Pathania, 2010; Nagarajan, 2011; Kumar, 2012). In West Bengal the disease has been clinically reported by few workers and only a preliminary survey was made in the hill tract of Darjeeling district by Hajra (2009). In the field condition many clinical cases has been recorded in both cross bred and indigenous cattle but systemic approaches for studying the epidemiological aspect of the disease as well as the genotype of the viral pathogens, has not yet conducted. This basic approach is very much needful for strategic state planning for effective control measures to save the dairy raisers from such losses.

Diagnosis, treatment and prophylaxis are the three key components for effective control of the diseases in field condition. Traditionally the disease could be diagnosed by typical morphological characteristics of the cutaneous lesions followed by histopathology and immunohistochemistry, which is very much time consuming and less sensitive for bio typing of the viruses. There is no efficient in vitro system of studying the virus due to its notorious character for not growing in cell culture and bio-typing of the prevalent virus types is not possible by the serological tests. Information on papillomavirus type can be utilized to develop methods of prevention or treatment. Therefore molecular approach is the best and most convenient way of virus bio typing, confirmatory diagnosis as well as molecular epidemiological studies.

The lack of any permissive culture and animal models for papilloma viruses replication and disease (Campo et al., 1990) as well as limited effective topical treatments have been two significant challenges for the discovery and development of effective therapeutics. At the moment, there is no vaccine or effective treatment for the control of bovine papillomatosis. No agreement has been achieved on the methods used for the
treatment of the disease. There is few treatment modalities for BPV infection available with varried level of success ranged between 15-50%. Many different therapeutic approaches and modalities to the treatment of warts in cattle and buffaloes irrespective of CWT and TP have been tried and suggested by various workers and it is fact that no single one is universally effective. A wide range of homeopathic, allopathic or other alternative approaches like cauterization, excision, cryotherapy, autologous or heterologous vaccination and auto-hemotherapy etc. are also reported in the literature. Vaccine therapy for cutaneous warts in cattle has been a common veterinary practice for decades (Olson and Skidmore, 1959). A formalinized suspension of bovine warts with inactivated virus provides a vaccine for effective treatment and prophylaxis of bovine papillomatosis (Lesnik et al., 1999; Borzacchiello 2008). The lack of any permissive culture and animal models for papillomaviruses replication and disease as well as limited effective topical treatments have been two significant challenges for the discovery and development of effective therapeutics. Therefore, a suitable, easy to use, effective and non-invasive and cost effective therapeutic measure needs to be assessed in field condition. And since no strategic research work have been conducted for identification of types of BPV prevalent in West Bengal and further, no epidemiological studies has been conducted on Bovine and Bubaline papillomatosis till date in Bengal, the present investigation is proposed to undertake strategic research on bovine cutaneous papillomatosis with special reference to pathological natures of prevalent BPV genotypes associated with cutaneous papillomatoses, molecular characterization of the pathogens and therapeutic approaches with the following objectives:

1. To investigate the epidemiological aspect of Bovine and Bubaline cutaneous Papillomatosis in West Bengal
2. To study the patho- morphological changes of spontaneous cases of different cutaneous papillomatous lesions in cattle and buffalo
3. To identify the virus using molecular tools and electron microscopy
4. To study the virus types prevalent in West Bengal
5. To access different therapeutic measures for clinical cure