Cutaneous papillomatosis or in other word cutaneous (skin) warts have been recognized in man and animals for centuries. The name papilloma originates from the Latin word papilla (nipple) and the Greek suffix oma (tumour). In this regard papilloma refers to the teat like nodule created by the tumour or a tumour restricted on the teat skin (Hatama-2012). A viral aetiology in the development of papilloma was first recognized in 1907. Bovine papillomavirus (BPV) was first described to associate with papilloma lesions in 1955; transforming capacity of the BPV was revealed in 1963 and the BPV type-1 genome was identified in 1980.

In humans and in animals, papillomaviruses (PVs) are widespread and successful sub microscopic pathogens associated with a variety of benign epithelial neoplasms called papilloma, fibropapilloma or warts and even malignant neoplasia. The genus consists of a number of antigenically distinct papilloma viruses and can infect mucous and cutaneous epithelia inducing hyperplastic, papillomatous, and verrucous squamous epithelial lesions in humans, and in a wide range of domestic and wild animals and birds. To date more than a hundred human PV (HPV) types have been partially identified and a wide variety of PV types have also been detected in mammals and birds (de Villiers et al., 2004). Phylogenetic comparisons have classified human and non-human papillomaviruses into 16 genera within the Papilloma viridae family. With the advent of PCR and molecular cloning, significant advances have been made in understanding of PVs of man and animals. The PVs isolated from the same species are sub classified into types according to nucleotide sequence homology. Types are largely distinguished by the characteristic restriction endonuclease cleavage of their genome. However, it is established fact that PVs have got ubiquitous distribution and have invariably been detected in healthy skin from both humans and animals as commensal agents (Antonsson et al., 2000; Antonsson and Hansson, 2002). Susceptible animals become infected by PVs from in apparent carriers as a result of micro trauma of skin or mucosae. This viral family is complex and includes 18 genera and more than a hundred PV types. In recent years, the strategy of partial amplification of the L 1 gene using a polymerase chain reaction (PCR) assay with degenerate primers followed by sequencing has allowed the identification of a broad range of new and putative new PV types in both human and animal hosts. Bovine papillomavirus induces diseases of considerable veterinary importance in farm animals, but has also an enormous value as an in vivo model for HPV. The bovine papillomatosis is an important disease leading to economic depreciation of animals, deterioration of the appearance and of the animal leather. The lesions may progress to cancer due to the synergistic action of genetic or environmental co-factors (Leal et al., 2003;
Borzacchiello and Roperto, 2008). Recent insights into BPV biology has opened up new fields of discussion about co-infection, cross-species infection, and transmission of these viruses.

2.1 Papilloma Viruses

PVs are a diverse group of small, non-enveloped (naked) and double stranded circular DNA viruses that occur in a broad range of distantly related animal species belonging to the amniotes, including humans (Antonsson and Hansson 2002; de Villiers et al., 2004). All these are having icosahedral symmetry and are only 55 to 60 nm in diameter with two capsomeres. The genome of PVs is double stranded, covalently closed circular DNA molecule of 7.2-8.0 kb in size (Bujard, 1967; Crawford, 1969). PVs are defined by genomic properties, rather than serology, and are therefore described as PV genotypes, not serotypes. Least serological cross reactivity among major capsid proteins was detected in PVs of different species (Koller and Olson, 1972) and no DNA sequence homology was detected between members of the group (Lancaster and Olson, 1978) with standard assays. The specificity of most PV types for a single host species suggests the postulate that PVs evolved together with their host i.e. host link evolution (Chan et al., 1995 and 1997). Papilloma viruses are assumed to be ancient viruses that have co-evolved and co-speciated with their host species, a hypothesis which is supported by the fact that PVs of closely related host species are generally closely related themselves and cluster together in the PV, phylogenetic tree, with dating of PV divergence largely coinciding with the host species divergence (Van Ranst et al., 1995; Tachezy et al., 2002a). The genome of PVs can be functionally divided into two domains on the basis of transforming capability and the two domains are E (Early) and L (late) region (Danos et al., 1983; Lazo, 1988; Wilson et al., 2002). PV genotypes are classified by analysis of only part of the viral genome that encompasses the combined nucleotide sequences of the E6, E7 and L1 open reading frames (Chan et al., 1995). Recently phylogenetic comparisons of the L1 ORF nucleotide sequences of 96 HPVs and 22 animal PVs have further classified PV types into genera and species. Higher order phylogenetic clusters i.e. major branches of PV types have now been classified into genera, sharing less than 60% identity in the L1 ORF nucleotide sequences. Each gene within the 16 genera identified and defined by biological properties and genome organization (Chan et al., 1995).

2.2.1. PV Types

Papilloma viridae is an ancient and diverse taxonomic family of non-enveloped DNA viruses, collectively known as papilloma viruses (PVs) and can infect a large range of hosts in nature, yet each virus species is generally species-specific (Nasir and Campo, 2008). Members of the genus (PV) are known from humans (> 100 types), cattle (Jelinek and Tachezy, 2005), deer, dog, horse, European bison (Literak et al., 2006) sheep, elephant, tapirs
(Kidney and Berrocal, 2008), elk, opossum, multi-mammate and European harvest mice, turtle, chaffinch and parrot. As there are roughly 15,000 species of mammals and birds, all likely host species, one can speculate that there could exist more than thousand different animal PV types (Bernard and Chan, 1994).

The species specific PVs so far studied have been claimed to be evolved together with their host i.e. host linked evolution (Chan et al., 1997; Sundberg et al., 1997). Each virus is named first according to its natural host, such as cotton tail rabbit papillomavirus (CRPV or shope virus), bovine papillomavirus (BPV), deer papillomavirus (DPV), human papillomavirus (HPV), etc. The PV types in brief are designated by the abbreviation ‘PV’ which is preceded by one or two letters indicating the host species followed by a number whenever multiple PV types are found in the host species. Accordingly the animal PVs (isolated from different animals) were, Colobus monkey PV (Colobus guereza); CgPV1 (O’Banion et al., 1987) and CgPV2 (Kloster et al., 1988), a neotropical primate, howler monkey (Alouatta fusca) PV (HMPV), PsPV (Phocoena spinipinnisi.e. seal PV, ChPV-common chimpanzee PV, pygmy chimpanzee (Pan paniscus) PV (PCPV) (van Ranst et al.,1991), Rhesus monkey (Macaca mulatta) PV (RhPV), Canine oral PV (COPV) (Bregman et al., 1987), Feline PV (FePV) (Sundberg et al., 2000;Tachezy et al., 2002; Terai and Burk, 2002), Cotton tail rabbit PV (CRPV), Rabbit oral PV (ROPV), Multimammate PV (MnPV) i.e rat PV, Equine PV (EQPV) (Angelos et al., 1991), Reindeer PV (RPV) (Eriksson et al.,1994), Deer PV (DPV), Porcine genital papillomavirus, Ovine papillomavirus, Fringilla papillomavirus (FPV). Physically DPV exhibits a higher degree of relationship with BPV-1 than has been found between any other PVs isolated from different species. Other PVs are European elk PV (EEPV), Finch PV (FPV), Psittacus erithacus timneh (African grey parrot PV), PePV, Ovine PVs types 1&2 (OvPV), Bovine Papilloma Virus types 1 to 13 (BPVs) and Human PVs (HPVS) (Bedell et al., 1991; Bernard, 1994; Bernard et al., 1994). The European bison, related to cattle belonging to the Bovidae family, has been reported to suffer from cutaneous papillomatosis and the causative virus has been identified to that of BAPV-2 bovine papilloma virus (Litera’k et al., 2006). Most of the animal PVs have been detected and characterized by PCR with consensus primers designed from genes encoding L-1, L-2, E-6 and E-7 proteins (Manos et al., 1989; Forslund et al., 1999; Ogawa et al., 2004; Ambrose and Clewley, 2006; Ogawa et al., 2007; Lindsey et al., 2009).

2.2.2 Classification of Papilloma Virus

Papillomaviruses were originally grouped together with the polyoma viruses in the old family papova viridae based on their small size, non-enveloped capsids, and circular, double stranded genomes. However, a lack of overall homology of particle size, genome organization, and sequence similarities between the two viral genomes led to the recognition
of two separate families, polyoma viridae and papilloma viridae (Howley and Lowy, 2001). After being assorted in the old family papova viridae, PVs were re-designated as a distinct family, papilloma viridae (van Regenmortel et al., 2000; Bernard, 2006). Viruses within the papilloma viridae family are defined by genomic properties, rather than serology, and are therefore described as PV genotypes, not serotypes (Pfister, 1987). Papillomavirus genotypes are classified by analysis of only part of viral genome that encompasses the combined nucleotide sequences of the E6, E7, and L1 open reading frames (ORFs) (Chan et al., 1995).

The PV family is formed by 18 genera (alpha Papilloma virus to Sigma Papilloma virus), based on nucleotide sequence diversity verified in the L1 open reading frame (ORF) (de Villiers et al., 2004). PVS can also be categorized to their tissue tropism and the histologic character of the lesions they cause: group 1 (BPVs types 3 and 6 and cottontail rabbit papillomavirus) induce cutaneous neoplasia; group 2 (BPV type 4) induce hyperplasia of non-stratified squamous epithelium (Bloch et al., 1995); group 3 (BPV type 1, 2 and 5) induce subcutaneous fibromas in addition to cutaneous papillomas; and group 4 (deer papilloma virus) induce primarily fibromas with minimal cutaneous hyperplasia. Virtually all mammalian species are hosts for one or more PVs and may have wild pro links. More than 100 human PVs have been isolated, cloned and sequenced completely and many more partially (de Villiers et al., 2014). New molecular tools such as broad range PCR assays as well as interest in animal PVs have permitted the detection and characterization of several new animal PVs.

Historically, PVs had been classified according to their tissue tropism, and grouping was supported later on the basis of phylogenetic analysis of PV sequence data (Meyers, 1994; Chan et al., 1995; de Villiers, 2001). PV phylogenies typically subdivide into mucosal or genital HPVs, cutaneous HPVs and three main animal PV clades: the artiodactyls ruminant PVs, the distant avian PV group and a group containing canine, feline, rabbit, and rodent PV types. However, sequence analysis has highlighted some significant exceptions. Bovine papilloma virus types i.e. BPV-3, BPV-4 and BPV-6 do not group with the artiodactyl PVs, but instead form an isolated Taxon (Jarret et al., 1984) and HPV-1, HPV-41, and HPV-63 are most closely related to the canine and feline PVs sharing little similarity to HPVs in either the mucosal or the cutaneous groups (Egawa et al., 1993). HPVs and animals PVs have been divided into five subgroups (A to E) and at least two other clusters based on sequence relatedness (Antonsson and Hansson, 2002; Ogawa et al., 2004).

2.2.3 Genomic and biological idiosyncrasies of animal PVs

The existence of seven homologous genes in every animal and human PV suggests similar structures and functions of the encoded proteins. LCR is the region between the early and late ORFs and contains several PV replication regulatory elements. Virus encoded E1 and
E2 proteins are essential for efficient initiation of viral DNA replication and bind cooperatively to adjacent sites in the virus replication origin in the LCR. All animal PVs have the same genomic organizations of ORFs and inter-genic regions that are more or less homologous: E6-E7-E1-E2/E4-E5-SIR-L2-L1-LCR. With the exception of E5, all of these genes of different PVs are homologous to one another, as evidenced by the extensive similarities in inter type alignments. Although the E5 genes of both super groups encode highly hydrophobic membrane proteins with similar molecular properties, there is no indication from sequence studies that these two protein families are homologous. Furthermore, BPV-1 and BPV-2 contain ORF E5 localized in the early and late regions of genome (Schiller et al., 1986), and BPV-3, BPV-4, BPV-6 contain ORF E5 localized in the E6 region in the genome but lack ORF E6 (Jackson et al., 1996; Morgan and Campo, 2000).

BPV-4 (and the relatives BPV3 and BPV6) constitutes the only major exception to the standard gene layout of PVs by lacking the homologue of an E6 gene. In place of E6 their genomes contain an ORF termed E8, which encodes a hydrophobic membrane protein with functional similarities to E5 proteins (Faccini et al., 1996). However; there is no indication that the BPV4 E8 gene may be homologous to either the E5 gene of genital HPVs, or to the E5 gene of fibro papillomaviruses.

Another major idiosyncrasy has been detected in the canine oral PV (COPV), which has a 1.5 kb non coding region between E2 and L2 in the same position where all other PVs have a more than 10 fold shorter non coding region (Delius et al., 1994). No function is known to be associated with this sequence, except transcription termination signals of the early genes similar to those of all other PVs. As, COPV is remotely related to HPV-1 and HPV-63, which do not have such a large inter-genic region, one may speculate that this sequence is not a prerequisite for idiosyncrasies of the COPV lifecycle, but may have originated by a recombination event, being retained as it was not detrimental.

2.2.4 PV Genome

All Papilloma viruses have a closed circular, double stranded DNA genome with a nucleotide length of about 7.9 kilo base pairs and a molecular weight of about 5.2 x 10^6 Daltons. The viral DNA is combined with histones derived from the cellular pool of the natural host and condensed into nucleosomes. The papillomavirus particles (52 to 55 nm in diameter) contain the viral genome within a spherical capsid composed of 72 capsomers. All ORFs are located on one strand, indicating that transcription occurs on only one strand. Transcription is regulated by the differentiation state of the infected cells and is complex, due to the presence of multiple promoters, alternate and multiple slice patterns, and differential production of mRNA in different cells (Howley and Lowy, 2001).
All the PVs have the same genomic organizations of open reading frames (ORFs) and inter-genic regions that are apparently homologous: E6-E7-E1-E2/E4-(E5)-SIR–L2-L1-LCR. Unusual ORFs have also been described in several types of PVs (Tachezy et al., 2002). The genomes of European elk (Odocoileus aler PV, white tail deer (Odocoileus virginianus PV and rein deer (Odocoileus hemines) PV contain transforming E-9 gene (Erikson et al., 1994).

The PV genome is contained within the capsid region which consists of the major and minor structural proteins L1 and L2 respectively. The L1 open reading frame (ORF) is the most highly conserved ORF within the PV genome (de Villiers et al., 2004) and represents 80% of the total viral protein (Howley and Lowy, 2001). The L1 protein can self-assemble into virus like particles (VLPs) or in combination with L2, although L2 protein is not required (Hagensee et al., 1993; Day et al., 1998). The L2 protein may enhance packaging (Stauffer et al., 1998) and infectivity (Roden et al., 2000). The early genes E6 and E7 and in some PV types, E5, contain oncogenic properties that can modulate the transformation process (Baker and Howley, 1987). With the exception of E5, which is present only in HPV-1 and BPV-1, all of these genes of different PVs are homologous to one another (Chan, 1997). BPV-3, 4, and 6 constitute the only major exception to the standard gene layout of PVs by lacking the homologue of an E6 gene. In place of E6 their genomes contain ORFs termed E8 which encodes a hydrophobic membrane protein with functional similarities to E5 proteins (Jackson et al., 1991 and Faccini et al., 1996). However, there is no indication that the BPV-4 E8 gene may be homologous to either the E5 gene of genital HPVs, or to the E5 gene of fibropapilloma viruses.

2.2.5 PV Genome Organization and Characterization

Papillomaviruses are notorious for their inability to propagate in in vitro systems. This has hampered their analysis, so that until recently nothing was known of their genetic organization. However, much progress has been made in recent years on the physical organization of their genomes, due to the availability of restriction enzymes and molecular cloning techniques.

Like mammalian cells, PVs have its genetic instructions encoded in double stranded DNA. Unlike mammalian DNA, however, PV DNA is circular in structure and contains instructions for building few proteins and this DNA is held inside as icosahedral 20 sided capsules, between 55 and 60 nanometre in diameter and is made up of a mosaic of 72 roughly star shaped proteins. Viral capsids are composed of two proteins, L1 and L2. L1 is the major capsid protein, which is arranged in 72 pentamers and has the capacity to self-assemble virus like particles (VLPs) and L2 is the DNA binding protein, necessary for viral genome encapsidation (Freitas et al., 2007). Biochemical analysis of the viral capsid has shown two different families of structural protein; a major protein (molecular weight, 5400 Daltons) and a minor protein (molecular weight, 76000 Daltons).
The PV genome is single molecule of double stranded, circular DNA and comprises approximately 8 kb (8000 base pair) lying in a nucleo-histone core. Eight well defined ORFs are encoded which are all transcribed from the same DNA strand within the same orientation. The translated proteins are classified as early (E) and late (L) based on their temporal expression. They include 3 regulatory genes involved in transcription and replication (E1, E2, and E4), 3 oncogenes (E5, E6 and E7) and 2 genes coding for self-assembling proteins that give rise to viral capsid (L1 and L2) (Munger and Howley, 2002). The organization of late genes is simpler than early genes (Garcia et al., 2005). The late gene L1 and L2 slightly overlaps each other in most of the cases (Lancaster and Jenson, 1987). All ORFs are located on one strand, indicating the transcription occur on only one strand. The complete L1 gene or fragment of it is commonly used for detecting PV infections and for typing of PVs. PV systematic have traditionally been inferred from the L1 gene, defining clear cut nucleotide identity thresholds for the delimitation of higher taxonomic units such as species and genera (de Villiers et al., 2004; Bernard, 2005).

Expression of papilloma viral genes takes place through a complex pattern of RNA splicing process in lack of virion transcriptase (Yang et al., 1985; Campo et al., 1994). Transcription is regulated by the differentiation state of the infected cells and is complex, due to presence of multiple promoters, alternate and multiple splice patterns, and differential production of mRNA in different cells (Howley and Lowy, 2001).

A PV genome usually comprise nearly 8 kb and contains 7 major ORFs that code for five early (E) proteins and two late (L) capsid proteins plus an upstream regulatory region (URR) or long coding region (LCR) (Tachezy et al., 2002). The LCR (about 500-1000 nucleotides) contains transcriptional regulatory sequences and the replication origin (Munger and Howley, 2002). The early region of the PV genome encodes the viral regulatory proteins E1 to E7, which are necessary for initial viral DNA replication. The late region encapsulates the genome and encodes the L1 and L2 capsid proteins (Howley and Lowy, 2001). The LCR contains no ORF but does contain the origin of viral DNA replication. Elements present in LCR regulate viral DNA replication as well as transcription (Desaintes and Demeret, 1996, Day et al., 1998).

**2.2.6 Molecular features and histochemistry of papillomatoses**

Papillomaviruses were once classified along with polyoma viruses in the family papova viridae because of superficial similarities in electron microscopic appearance and biological properties. However, sequence and functional analysis now indicates that these two classes of virus are not related. The polyoma-viruses are much smaller and RNAs are transcribed from both DNA strands in a bidirectional fashion.
PVs are epitheliotropic and mucoso-tropic, small, naked, double stranded, covalently closed, circular, super-coiled DNA viruses that replicate in the nucleus of squamous epithelial cells. The virion particles consist of a circular double stranded DNA genome ranging from 7.3-8.0 kb in size, contained in a nucleo-histone core within a spherical capsid, composed of 72 capsomeres. It forms para crystalline arrays in the nucleus of infected cells. The virus particles have a sedimentation coefficient (S20 W) of 300 and 52 to 55 nm in diameter and have a density in caesium chloride of 1.34g/ml (Crawford, 1969). So far, some ten polypeptides have been identified by polyacrylamide gel electrophoresis, with major capsid proteins of 50-63 KD. Both empty and full virus particles are seen by electron microscopy and moreover tubular and other aberrant morphological forms are also common.

Like polyomavirus capsids, the capsomers exist in two states, one capable of making contact with six neighbours, as observed in the 60 hexavalent capsomers and the other with 5 neighbours in the pentavalent capsomers. In the virion their nucleic acid occurs as a cyclic double stranded molecule, complexes with histones, and it is infectious. The capsid of the virion consists of two structural proteins. The major capsid protein (L1) has a molecular weight of approximately 55 kd (Farve et al., 1975; Gissmann and zur Hausen, 1980; Pfister et al., 1977) and represents approximately 80 percent of the total viral protein.L1 is the major capsid protein arranged in 72 petamers and has the capacity to self-assemble in virus like particles (VLPs) (Campo, 2003). A minor protein (L2) has a molecular weight approximately 70 kd and it is the DNA binding protein necessary for viral genome en-capsidation. Both L1 and L2 are virally encoded and in addition, analysis of these proteins in the virion reveals that the viral genome is associated with cellular histones forming a chromatin like complex (Farve et al., 1975; Pfister et al., 1979).

The genomic organization of each of the papilloma virus is remarkably similar. Fine structural analysis by cryo-electron microscopy on three dimensional image recognition techniques have revealed that the viruses consist of 72 pentameric capsomeres arranged on a T = 7 surface lattice (Baker et al., 1991). The coding strand for each of the papilloma viruses contains approximately ten designated transitional ORFs that have been classified as either the early (E) or Late (L) ORFs of the BPV-1 genome are located within the fragment of the BPV-1 genome which is sufficient for inducing cellular transformation, i.e. the 69% sub genomic transforming fragment (Lowy et al., 1980). It is the segment of the viral genome which is expressed in non-productively infected cells and in transformed cells.

The analogous region of the other papillomaviruses genomes is also referred to the early region. The L ORFs are expressed only in productively infected cells (Amtmann and Sauer, 1982).The position , size, and function of many of the ORFs are well conserved among the papilloma viruses that have been sequenced so far (Baker and Howley,1987). The
functions of individual ORFs have also been well characterized. There is a region in the papilloma virus genomes in which there are no ORFs. The region varies slightly in size among the different papilloma virus genomes; in the BPV-1 genome, it is approximately one kilo base in size. This region has been referred to by several terms, including the long control region (LCR), the upstream regulatory region (URR), and the non-coding region (NCR). The organization of all of the PV genomes is remarkably similar.

2.2.7 PV Replication

The life cycle events of PV biomolecules seem to be similarly regulated in both human and non-human PVs (Peh et al., 2002). The PV life cycle is strictly dependent on differentiation of the epithelial tissue (Barksdale and Baker, 1993). PV replication is tightly linked to the growth and differentiation of cells in stratified squamous and mucosal epithelium from their origin in basal layers to their shedding at the epidermal surface of the skin or mucous membranes. Actively dividing basal cells in the stratum germinativum are infected initially and are believed to maintain the virus in a proviral, possibly latent, state throughout cellular differentiation. Virus induced hyperplasia, induced by early gene products, leads to increased basal cell division and delayed maturation of cells in the stratum spinosum and stratum granulosum (Howley, 1996; Howley and Lowy, 2001). These cells become massed into nascent papillomas. PV replication can be divided into three stages (McBride et al., 2000). First the PV virion must bind to a basal keratinocyte and of course may also bind to some other cell types (Muller et al., 1995). During this particular stage, the viral genome is maintained as an episome in the nucleus (McBride et al., 2000). The viral genome is then amplified and viral copy number is increased up to 1000 per haploid cell genome (Lepik et al., 1998). As the basal cells differentiate, the viral DNA is maintained as a stable genome. The viral genome during this second maintenance stage, replicates in synchrony with the host cell chromosome (Gilbert and Cohen, 1987). The earliest PV DNA synthesis is within the fragment containing the PV replication origin and synthesis proceeds in both directions from the replication origin (Melendy et al., 1995). The third replication stage takes place in the terminally differentiated epithelial cells of the papilloma (Howly and Lowy, 2001). And in next layer of stratified epithelium, the stratum granulosum, late viral gene expression, synthesis of capsid proteins, vegetative viral DNA synthesis and assembly of virions occur (McBride et al., 2000). The PV DNA is thought to remain in the basal epithelial cells and to be reactivated when levels of immune system monitoring got declined (Doorbar, 2005).

2.2.8 Non Human PVs

More than 100 types of HPVs have been described while only dozens of types have been confirmed in animal hosts (de Villiers et al., 2004). This is likely a result of a bias in
research efforts rather than a true difference in viral diversity (Chan et al., 1997). In recent years, the identification of a broad range of new and putative new animal PV types has been made with the advent of modern molecular tools (Forsslund et al., 1999; Antonsson and Hansson, 2002).

Warts in animals have been recognized for centuries. Equine papillomas were described as early as in the 9th century AD and the first exceptional transmission of animal papillomas occurred in 1898 (Lancaster and Olson, 1982). Warts in wild cotton tail rabbits were the first animal papillomas thoroughly examined for properties of transmissibility, aetiology and histology. That the warts or papillomas have a viral aetiology was recognized as long ago as 1907, but it was not until 1978 that it was realized that bovine papillomas and papillomas in other species are caused by several different viruses. The infectious origin of bovine warts was initially demonstrated in Brazil (Magelhaes, 1920). Studies on transmission to another species (horse) resulted in the giant papillomas or induction of sarcomas (Olson and Crook, 1951). The activities and characteristics of the papilloma producing agent in cotton tail rabbits classified it as a virus (Shope, 1933). Additional non-human PVs initially characterized include the Bovine Papilloma Virus (BPV) (Lancaster and Olson 1978), equine PV, canine oral PV, deer fibroma virus (Shope et al., 1958), and chaffinch PV (Lina et al., 1973).

Presently, 22 animal PVs have been fully characterized and classified into genera and species based on the L1 ORF sequences (de Villiers et al 2004). As many as 53 putative new animal PV types have been identified by PCR in 7 animal species including chimpanzees, gorillas, spider monkeys, long tailed macaques, domestic cattle, aurochs and European Elk (Antonsson and Hansson, 2002). Amongst the animal PVs, BPV has been identified to have good viral diversity present in cattle and the number of characterized BPVs is higher than the number of characterized viral types for any other animal species (Claus et al., 2009 a,b).

Infection with non-human PVs is generally contained to the skin or mucous membranes of the host species (Lancaster and Olson, 1982), however, canine oral PV can also infect the eyelid, conjunctiva and skin around the nose and mouth (Chambers and Evans, 1959). Some animal PV types such as BPV, Cotton tail rabbit PV and European harvest mice PV have been implicated in cancers (Antosson and Hansson, 2002). Of course BPV has been found to be the most oncogenic among the PV types (Lancaster et al., 1977).

Most PVs are species specific or may infect closely related animals with in the same genus (Sundberg et al., 2000), although BPV1 and BPV2 can induce fibroblastic tumours in a strain of inbred mice (Boiron et al., 1965), hamsters (Cheville, 1966) and rabbits (Breitburd et al., 1981). Recent studies have shown that many domestic and wild species of mammals
and birds can be infected by one or more PVs (Sundberg et al., 2000). New PVs in marine mammals have also been described (Van Bressem et al., 1996; Bossart et al., 2002).

Animal PVs have been studied both as infectious viral biomolecules of diseases in animals and as models of HPV infection (Campo, 2002). Animal PVs causing most distressing diseases in both farm and companion animals notably the teat papillomatosis in cattle, sarcoïds in horse, donkeys and mules, papillomatosis in camels and canine oral papillomatosis etc. warrant an urgent need for the understanding of the complex mechanism of viral pathogenesis of these problematic infections. Canine oral papillomatosis can be very extensive and persistent and lead to great distress (Chambers and Evans, 1959). Equine sarcoïds are often recurrent and untreatable and lead to loss of valuable animals. Persistent and florid teat papillomatosis in cows can lead to mastitis, prevent the suckling of calves and make milking impossible (Campo, 2002).

2.2.9 Papillomavirus Pathogenesis

Papillomavirus enters infected skin via skin surface abrasions, allowing the virus access to the proliferative cells of the skin (Egawa, 2003), where it promotes cellular proliferation and accelerated epithelial growth (Silverberg, 2004). Infection with PV is primarily found on extremities, face and body. Warts (papillomas) are induced in the skin and mucosal epithelia at specific sites (de Villiers et al., 2004) and differ in their tissue specificity and the associated disease (McMurray et al., 2001). The highly tissue-specific papillomaviruses can be divided into two groups: one group is primarily found in cutaneous epithelia (skin), in which there is thickening of the epidermis, and the other group is predominantly present in mucosal epithelia (Smits et al., 1992), involving the oral pharynx, oesophagus, or genital tract (Howley and Lowy, 2001).

Warts are diagnosed by physical examination and are defined by morphology, location, and host immune response. The three main types of lesions observed are: common warts (rough plaques of skin), mosaic warts (groups of common warts), and flat warts (smooth, flesh-coloured papules). Mucosal warts may appear as single plaques or as a group with a grapelike appearance (Silverberg, 2004).

Like all members of the papillomavirus class, the Delta papillomaviruses infect only the epithelial cells (keratinocytes); however, unlike other PVs they cause proliferation of both keratinocytes and fibroblasts, causing benign fibro-papillomas involving both the epithelium and the underlying dermis. The specificity of types differs: BPV-1 infects para genital areas, including penis, teats and udders, and BPV-2 infects skin, alimentary canal and urinary bladder. The epitheliotropic BPVs or Xi papillomaviruses infect keratinocytes, causing pure papillomas involving only the epithelium and the specificity of the types differs. BPV-3 infects skin, BPV-4 infects upper alimentary tract (Bloch et al., 1995) and BPV-6 infects teats and udder. Epsilon papilloma virus having single member, i.e. BPV-5 infects teats and
udders, and can cause both pure papillomas and fibro-papillomas. Warts caused by the Xi-papillomavirus group have a cauliflower-like appearance and can attain the size of a fist; most common on the head, neck and shoulders and may also occur in other locations (Merck Veterinary Manual: papillomas/warts). Cutaneous fibro-papillomas caused by Delta papillomavirus group have a nodular appearance.

The genital mucosal HPV types are defined as either high risk or low risk based on their involvement with lower genital tract cancers (McMurray et al., 2001). In the low risk types, such as HPV-16 or HPV-18, the virus deregulates checkpoints that normally monitor the fidelity of chromosome replication and segregation, leading to the development of cancer (Galloway, 2003). Ano-genital carcinomas caused by HPV infection include penile (Rubin et al., 2001), vaginal (Daling et al., 2002), vulvar (Trimble et al., 1996), and anal cancers (Krzyzek et al., 1980). The high-risk HPV types are also associated with cervical dysplasia (Kurman et al., 1982), uterine cancer (zur Hausen, 1996) and cervical cancer, one of the most common cancers of women worldwide (Schiffman, 1992, and zur Hausen, 1991). The pathogenesis of HPVs differs for viruses that are considered high risk or low risk group members. In the case of epidermodysplasia verruciformis (EV), the disease behaves like a genetic cancer of viral origin, which may result from an abnormal recessive gene (Jablonska, 1991).

2.2.10 Diagnosis of Papillomavirus infection

Warts are diagnosed by physical examination and are defined by morphology, location and host immune response (Pfiste, 1980). Papilloma virus induced lesions are usually multiple and may be sufficiently characteristic to confirm the diagnosis macroscopically, however there may be simulants of warts, which needs a definitive diagnosis requiring identification of the virus or its cytopathic effects (CPE) on the individual cell i.e. a change known as koilocytic atypia or koilocytosis (Marins and Ferreira, 2011).

Testing for the presence of HPV viral DNA includes method such as highly sensitive polymerase chain reaction (PCR), Southern blots, dot blots, in situ hybridization, and solution hybridization (hybrid capture assay) (Trofatter, 1997). Detection of HPV by PCR is more sensitive than the other methods, enables the detection of a single genome copy per cell for HPV DNA that has integrated (Shamanin et al., 1994) and allows for the detection of a broad spectrum of genital HPVs (Ting and Manos, 1990). The widely used consensus PCR primers, MY 09 a MY 11, are based on sequences obtained from the highly conserved PV L 1 capsid protein gene (Manos et al., 1989). HPV DNA can be detected by method of PCR in fresh or frozen cervical biopsies (Li et al., 1988; Manos et al., 1989), genital wart tissues (Brown et al., 1999), cutaneous wart tissues (Harwood et al., 1999), and in swab samples taken from the top of lesions (Forsslund et al., 2004). Harwood et al., (1999) have described a degenerate
nested PCR that is capable of detecting cutaneous, mucosal, and EV HPV types. Recently a new method of HPV detection using high density DNA microarrays is able to detect single and multiple mucosal HPV infections (Klassen et al., 2003). New tools in molecular biology such as broad range PCR assays and multiply primed rolling-circle amplification as well as the increasing interest in animal PVs have permitted the detection and characterization of several new animal PVs (Munday et al., 2007; Nespeca et al., 2006; Rector et al., 2004, 2005a, b, 2008; Stevens et al., 2008; Tobler et al., 2006, 2008).

2.2.11 Treatment and prophylaxis of Papillomavirus Infection

The host response to papillomavirus infection is a complex process of skin barrier protection, innate immunity, and acquired immunity (Silverberg, 2004). Warts usually will regress over time and after six months of infection, 30% of warts will clear on their own (Messing and Epstein, 1963). Following immune regression, PV DNA persists in a latent state, with only a few cells, if any, capable of supporting the productive cycle that occurs during epithelial cell differentiation (Doorbar, 2005). The best approach for controlling the prevalent HPV associated disease is to prevent HPV infection (McMurray et al., 2001); however, the PV life cycle requires a differentiated stratified epithelium to replicate and this has been difficult to generate in cell culture (McBride et al., 2000). Owing to such difficulty, the development of a capsid directed vaccine was hindered for long time (Biemelt et al., 2003). A recently described raft system has allowed the genetic analysis of the complete viral life cycle of BPV-1. Using a combination of organo-typic raft cultures and xeno-grafts on nude mice, BPV-1 DNA can be amplified and capsid antigens and infectious BPV-1 virus particles can be produced (McBride et al., 2000).

Several animal models of PV infection have shown that neutralizing antibodies can block new infection (Galloway, 2003). Vaccination against PV infections using virus like particles (VLPs based on the L 1 capsid protein or the L 1 plus the L 2 protein is currently being developed (Leder et al., 2001)). Vaccines based on VLPs are desirable because they retain repetitive, highly immunogenic epitopes found on the surface of infectious virions, but lack the potentially harmful PV genomes. Three types of HPV VLP-based vaccines are currently being developed. The first most basic type of vaccine is designed to prevent genital HPVs by inducing virus-neutralizing antibodies against the L 1 major capsid protein. The second type of vaccine is based on chimeric VLPs which incorporate polypeptides of other viral and cellular proteins into the VLPs. Such vaccines induce cell mediated immune responses (CMI) to non-structural viral proteins. The third type of vaccine is unique one that incorporates self-peptides into the outer surface of the VLPs and is designed to induce antibodies against central self-antigens (Schiller and Lowy, 2000).
2.3 Bovine Papillomatosis

Papillomatosis, (common warts) is an infectious skin disease caused by papilloma viruses and associated with a number of hyperplastic and neoplastic lesions of the stroma and epithelium in a wide variety of vertebrate species. Bovine papillomatosis (BP) is a chronic contagious proliferative disease characterized by warts or papilloma that occur mostly in cutaneous form and less frequently in mucosal form as exophytic papilloma (Marins and Ferreira, 2011). Cutaneous fibro-papillomatosis in cattle and buffalo is very common and is characterized by the presence of multiple benign exophytic proliferations of the epidermis and of the underlying derma, which is again associated with infection with a BPV (Silvestree et al., 2009; Somvanshi, 2010; Singh and Somvanshi, 2010; Nagarajan, 2011; Kumar, 2012). It has been described in cattle and other ungulates such as red deer, roe deer, elk, and less frequently in horses, dog, cat, sheep, goat, pig, and bison (Borzacchiello et al., 2003; Goldshmidt and Hendrick, 2002; Bogaert et al., 2005; Litera’k et al., 2006, Brandt et al., 2008, Dhule, 2013). Genital papillomavirus infection has also been described in cows and pigs (Elzein et al., 1991). The incidence of papillomatosis is reported to be much higher in imported and cross-bred cattle than in indigenous cattle. Although the disease is not of much importance, its high infectivity, chronic illness leading to un-thriftiness and the damage of the skin due to generalized warts are matter of concern and economic losses. Extensive warts lead to depreciation in both aesthetic and economic value of the animal due to loss of body condition, hide’s value, increased risk of conditions like mechanical trauma, wounds, haemorrhages, fly blown condition (myiasis), necrotic dermatitis, mastitis, interference in suckling, milking and coitus. Malignant tumours of visceral organs lead to death of animals.

Many times importance of BP is not verified by many farmers and even the veterinarians badly clarified. Much more than an aesthetic issue, bovine papillomatosis has recently grown in importance due to its association with cancer and immunosuppression conditions (Campo, 2002). Even the benign progression demands attention, once hyperplastic lesions may depreciate the pelt in affected animals; when located in the udder, it may lead to secondary infections and lactation problems. In fact, Campo (2006, a, b), described several economic consequences, as cows with teat papillomas cannot be milked, young calves cannot suckle, and often the pedunculated papillomas snap off, the sites become infected and mastitis may ensue with distortion of the milk canals. The disease affects young animals more often and more severely, but may affect cattle of all ages (Nicholls and Stanly, 2000; Olson, 1993; Smith, 1996, Dawlat et al., 1997). Affected cattle are usually less than 2 years old and the tumours regress spontaneously within one year (Campo et al., 1994, Jelinek and Tachezy, 2005; Olson et al., 1992; Smith, 1996). Although the spread of the disease is usually by direct contact, many factors such as contaminated food and equipment, castration, injections,
inheritance, nutritional imbalance, hormonal imbalance, and suppressing the immune system may play roles in the spread of the disease (Campo et al., 1994; Dinc, 1995; Nicolls and Stanley, 2000; Otter and Leonard, 2003).

Infectious papillomatosis (warts) are contagious in animals in which they naturally occur. These lesions may be regarded as either hyperplasia or benign neoplasms since they do not metastasize and kill the host (Lancaster and Olson, 1982). BP is self-regressing in contrast to other PVs affecting canine, equine and human beings. Mucosal form may occasionally persist and provide the focus for malignant transformation to squamous cell carcinoma, under the influence of additional genetic or environmental factors and immuno-suppressents (Campo, 1987; Campo et al., 1992). This has been experimentally demonstrated for cancer of the urinary bladder and upper gastro-intestinal (GI) tract in cattle feeding on bracken fern (Campo, 2006). It is thought to be a multi-step affair (Koller and Olson, 1972; Lancaster and Olson, 1982). Furthermore, BPV infection in cattle could be connected with serious disorders of the metabolism, probably caused by damage of the liver and kidneys with mutagenic, carcinogenic and immunosuppressive cadmium, arsenic and lead, as observed in the serum and tissues of tested animals (Lesnik et al., 1999). Infection of BPV occurred as a result of the viral exposure to single or multiple lesions of the epithelium. In experimental infections the lesions appear after 30 to 59 days and tumours regress in 1 to 14 months (Sundberg, 1987). BPV infection, transformation, and multiplication of basal cells, lead to wart formation, but most warts are benign and do not proliferate indefinitely (Bodron et al., 1964; Brunner and Gilepsie, 1973; Shah and Howley, 1996).

BPV induce both lytic and proliferative changes in cells, most notably in keratinocytes. Lesions arise on the haired and glabrous skin and mucous membrane, all of which are covered by stratified squamous epithelium. It is notable that ruminants are the primary group that develop papillomas and fibro-papillomas (a benign fibroblastic proliferation with overlying acanthosis, hyperkeratosis, and a down growth of rete pegs) depending on the ratio of fibrous connective tissue to hyperplastic epithelium (Yager et al., 1993). BPV 1, BPV-2 and BPV-5 (Bloch et al., 1994, a, b) cause cutaneous fibro-papillomas, while BPV-3, BPV-4 and BPV-6 cause squamous papillomas of the skin and oesophagus (Smith and Campo, 1985; Yager et al., 1993 and Goldshmidt and Hendrick, 2002). Cells undergoing the lytic form of infection shed large numbers of viral particles (Cheville, 1994).

2.3.1 Bovine Papilloma Virus (BPV)

BPVs are a heterogeneous group of epitheliotropic viruses that recognize bovines as its primary host. Thirteen BPV types (BPV-1-13) have been described and classified into four genera: of which Delta-papillomavirus genera includes BPV-1 , 2 and 13 (inducing fibropapilloma in bovine species and sarcoid in equine), Epsilon-papillomavirus that includes
BPV-5 and BPV-8 (causing fibro-papillomas and epithelial papillomas), and Xi-
papillomavirus includes the BPV-3, -4, -6, -9, -10, -11 and -12 types (inducing only the
epithelial papillomas), and an as yet unassigned PV genus (BPV-7 type) (Ogawa et al., 2004;
Bernard et al., 2010; Zhu et al., 2012; Dong et al., 2013).

Infection by BPV occurs as a result of virus exposure to single or multiple lesions of
the epithelium. Papillomaviral infection, transformation and multiplication of basal cells, lead
to wart formation but most warts are benign and do not proliferate indefinitely (Shah and
Howley, 1996).

It was concluded that BPV-1 and -2 comprises the major cutaneous fibropapillomas
(Catroxo et al., 2013). The BPV-1 can induce the lesions in teats and penis (Bloch et al.,
1994, a, b; Campo, 1997, a, b; Jelinek and Tachezy, 2005) while the bovine enzootic
hematuria that affects digestive tract is related to BPV-2 (Wosiacki et al., 2002). Hatama et
al., (2008) and Borzachiello and Roperto (2008) demonstrated that BPV-5, -9 and -10 lead to
the development of fibropapillomas in the udder. The BPV-3 and -11 were isolated from skin
epithelial papillomas of different body parts (Pfister et al., 1979; Hatama et al., 2011). Other
experiments revealed in the epithelia of the digestive tract the presence of BPV-4
(Borzachiello and Roperto, 2008) of teat the presence of BPV-6 and -9 (Jarret et al., 1984;
Hatama et al., 2008) of the tongue the BPV-12 (Zhu et al., 2012) and in ears the BPV-13
(Lunardi et al., 2013).

Bovine papilloma virus affects cows worldwide and is a good model system for HPV.
BPV is perhaps the most extensively studied animal PVs and it has been found invaluable in
the investigation of virus biology, the direct link between virus infection and neoplasia, the
relationship between virus, host and environment, the host immune response to the virus and
in the development of the first anti papilloma virus vaccines. Additionally and equally
importantly, BPV is an infectious agent of diseases in farm animals and therefore of
considerable veterinary and agricultural importance (Campo, 2002 and 2006, a, b). BPV
cause both benign and malignant epithelial and mesenchymal tumours in cows and equids.
They are strictly specific and even in experimental conditions, do not infect any other equids
by BPV type-1 or BPV type 2 (Campo, 2006, a, b).

The BPV has a constant morphology and structure independent of the site or type of
lesion. The detailed structure of the BPV virion has been determined recently and an atomic
model has been generated which shows that the c- terminus of L1 and N- terminus of L2 are
exposed on the surface of the virion and are likely to have a role in infection and
immunogenicity (Day and Schiller, 2006).

BPVs are a heterogeneous group of non-enveloped ds DNA viruses, specific to each
animal species and in some cases specific to epithelial sites in that species. They induce

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papillomatosis of the skin, the genital and paragenital area, the eye, the upper alimentary tract and the urinary bladder manifesting as papilloma or fibropapilloma following a cytopathic mechanism of cell proliferation (Sundberg, 1986). The disease affects young animals more often and more severely, but may affect cattle of all ages (Nicholls and Stanley, 2000; Olson, 1993; Smith, 1996). Seven members of BPV, (BPV-1 to -7) have been isolated, identified and described in detail (Pfister et al., 1979; Campo et al., 1980; Jarrett et al., 1984; Carvalho et al., 2003; Ogawa et al., 2004; Ogawa et al., 2007) and a further 13 types were identified recently (Antonsson and Hansson, 2002; Ogawa et al., 2004). Recently Bovine papilloma virus types up to 10 have been reported and characterized so far (Campo et al., 1980; 1981; Chen et al., 1982; Hatama et al., 2008; Jarrett et al., 1984; Ogawa et al., 2007; Pfister et al., 1979; Tomita et al., 2007). Additional candidate types, including bovine alimentary papillomavirus-11 (BPV-11) have been described (Ogawa et al., 2004). Only three types of BPV (BPV 1, BPV-2 and very recently BPV-10) are reported from India (Leishangthem et al., 2008; Singh et al., 2009; Pangty et al., 2010 and Rai et al., 2010).

BPVS have a specific tropism for squamous epithelial cells. Full viral replication, including vegetative viral DNA synthesis, production of viral capsid proteins and the assembly of virions, occur only in the more terminally differentiated squamous epithelial cells. The wart specific viral genes, which encode the viral capsid proteins, are expressed terminally in the differentiated epithelial cells of the wart. This apparent absolute requirement for terminal differentiation of squamous epithelial cells for the expression of the wart virus specific gene for viral replication is probably the reason that virologist have not yet succeeded in propagating these viruses in tissue culture (Howley and Schlegel 1987).

2.3.2 BPV induced papillomatosis and the distressing diseases

The BPV is responsible for a chronic contagious illness, whose remarkable clinical sign is the hyper-proliferative lesions, known as papillomas, on cutaneous tissue and mucosa (Campo, 2006, a, b). Despite being primarily considered epitheliotropic, BPV DNA has already been isolated from peripheral blood mononuclear cells, milk, urine, seminal fluid and sperm cells of animal’s infection with BPV1, BPV-2 and BPV-4 (Carvalho et al., 2003; Yaguiu et al., 2006; 2008; Roperto et al., 2008; Lindsey et al., 2009).

The fibropapillomas affect both dairy and beef cattle (Paintsil et al., 1998; Veiga, 2000). When papillomas are located in the teats and udders it makes milking difficult and can cause a drop in milk production predisposing to mastitis and spread of the virus through the perineum (Veiga, 2000; Campo, 2002). Jarret (1985) and Borzacchiello and Roperto (2008) reported that wart lesions can induce to loss of reproductive function with consequent collapse and death of the animals. Young animals infected with these viruses and undergoing
stressful confinement system, they become susceptible to secondary infection with significant weight loss and damage to the skin causing economic losses (Fenner, 1987; Veiga, 2000).

Infection by BPV causes cutaneous papillomatosis and benign proliferative lesions that can result in severe injuries and losses in animal production (Jelinek and Tachezy, 2005). The lesions can regress spontaneously over a period of 6-12 months and most of the affected animals are less than 2 years old. However, large warts may bleed, potentially leading to secondary infections, and florid warts of the teat can cause mastitis and interfere with suckling and milking (Campo, 2006). Fibro-papillomas can be troublesome when present in genital area, causing pain and sometimes loss of reproductive functions as well as interfering with calving (Campo, 2006). Chronically immuno-suppressed animals may develop extensive papillomatosis in the upper gastrointestinal tract, which can cause difficulties with eating and breathing (Campo, 2006).

In cattle BPV infection causes cutaneous warts (BPV-1 and BPV-2), papillomatosis and cancer of the upper gastrointestinal (GI) tract (BPV-4), papillomatosis of teats and udder (BPV-1,-5 and -6) and penis (BPV-1) and cancer of the urinary bladder (BPV-1 and BPV-2) (Campo, 1998). Immunosuppression is instrumental in the development of widespread and persistent papillomatosis of the GI tract. If the papillomatosis is not too severe, the animals survive but are great risk of developing squamous cell carcinomas.

Most of the BPVs have been described as causative agent of specific lesions in distinct body sites of bovines. BPV-1 has been found causing teat frond and penile fibro-papillomas and BPV-2, has been described as the agent of common warts and oesophageal fibro-papillomas. BPV-3 and BPV-8 have been found to cause epithelial papillomas of the skin. The teats and udders of cows are subjected to infection by three different types of BPV (Campo, 1998). This disease, especially if caused by BPV-6, is not only a health problem but has also economic consequences, as cows with teat papillomas cannot be milked, young calves cannot suckle, and often the pedunculated papillomas snap off, the site become infected and mastitis may ensue with distortion of the milk canals.

Papillomatosis of the penis interferes with the normal function of bulls and the animals have to be sacrificed. BPV-1 and BPV-2 are the only PVs that can infect a host of a different species. Horses, donkeys, and mules develop sarcomas as a result of BPV infection (Olson and Crook, 1951; Angelos et al. 1991). The tumour is locally invasive, refractory to treatment and can lead to the animal being sacrificed. The tumour is not permissive for virus replication and infection is abortive; the BPV DNA is present in tumour cells in multiple episomal copies and its early genes are transcribed, indicating an active role of the virus in sarcomatous aetiology (Angelos et al., 1991; Nasir and Reid, 1999, Otten et al., 1993; Nasir et al., 2007).
In cattle feeding on bracken fern the BPV-4 induced papillomas of the upper GI tract are at a high risk of progressing to cancer. Bracken fern contains immune-suppressants and chemical carcinogens and its presence in animal feed causes a variety of diseases (Campo 1997a, b). BPV-4 infects mucosa of the upper GI tract, expresses its transforming proteins and induces papillomas (Bloch et al., 1995). The rapidly dividing papilloma cells start invading the underlying derma and full transformation to squamous carcinoma ensues (Campo et al., 1980).

### 2.3.3 Bovine papillomavirus and Co-infection

A diversity of multiple BPV infection has been described in bovine and other mammals (Olone et al., 1968; Olson et al., 1963; Patel et al., 1987; Ogawa et al., 2004; Oliveria et al., 2005; Bogaert et al., 2008; Claus et al., 2009, a, b; van Dyk et al., 2011) suggesting that certain viral types are not restricted as previously thought. Claus et al. (2009, a, b) observed the occurrence of several BPV types in a specific anatomical region; the detection of the same viral type in distinct body sites and determining papillomas with diverse gross aspects; and lesions with similar morphological characteristics caused by distinct papillomavirus.

The occurrence of co-infection with different types of BPV has been reported from India and abroad as well. Co-infection with BPV-1 and BPV-2 was described in India first time by Leishangthem et al. (2008) and later by Pangty et al. (2010).

In Japan, Ogawa et al. (2004) verified the presence of BPV types in the same papillomatous lesions in the Japanese herd. In Brazil, the simultaneous presence of BPV-1 and BPV-2 was detected in the same lesion (Yaguiu et al., 2006, 2008; Lindsey et al., 2009). It is of worth mention that five different combination of multiple BPV infection was recorded in cattle in Brazil (Claus et al., 2009). Co-infection with BPV-1 and BPV-2 was assessed using a multiplex BPV genotyping assay in bovines in Germany (Schmitt et al., 2010) and in Brazil using specific BPV primers (Freitas et al., 2011).

Besides CWT lesions in bovine, the presence of multiple putative new BPV type was also observed in the normal skin (Ogawa et al., 2004). The simultaneous presence of BPV-1 and BPV-2 was demonstrated in bovine tissues other than skin like blood and reproductive cells (Yaguiu et al., 2006, 2008; Dinz et al., 2009; Lindsey et al., 2009). BPV-1 and BPV-2 have also been found co-infecting giraffe (van Dyk et al., 2011), zebra (van Dyk et al., 2009) and horse (Bogaert et al., 2008). However, the occurrence of diverse co-infection by BPV in a single sample suggests a natural competition of different BPV types occurring on the skin (Schmitt et al., 2010). Of course it is not clear if all BPV types found in the same lesion are transcriptionally active and detection of viruses in apparent latency may be a result of immune evasion.
2.3.4 Heterogeneity of BPV:

BPVs are a heterogeneous group of DNA viruses having ubiquitous distribution affecting millions of cattle world-wide and induce papillomatosis of the skin including genital and paragenital area, the eye, the upper GI tract and the urinary bladder. Warts are called bovine papillomatosis or infectious papillomatosis and the most common type of cattle warts are squat, pedunculated, and flat and tags appear as hairless lesions. At least six members of BPV (BPV1, 2, and 5, causing cutaneous fibro-papillomas, while BPV3, BPV4 and BPV 6 cause squamous papillomas of the skin and oesophagus) have been described in detail (Jarrett et al., 1984a, b; Jarrett, 1985), and a further 13 types were identified during 2002 to 2004 by Antonsson and Hansson, 2002; Ogawa et al., 2004, which more than trebles the heterogeneity of BPVs.

Based on sequence relatedness, BPV types 1to 10 have been characterized so far (Campo and Coggins, 1982; Campo et al., 1980, 1981; Chen et al., 1982; Hatama et al., 2008; Jarrett et al., 1984; Ogawa et al., 2007; Pfister et al., 1979; Tomita et al., 2007). Additional candidate types, including bovine alimentary papillomavirus-11(BPV-11), have also been described (Ogawa et al., 2004).

BPV infection has been associated with 4 main clinical presentations linked with specific viral types: 1) cutaneous fibro-papillomas (BPV-1 and BPV-2); 2) Cutaneous papillomas (BPV-3) and teat fibropapillomas (BPV-5); and 4) Cutaneous and teat papillomas (BPV-6) (Claus et al., 2007, Ogawa et al., 2004; Wosiacki et al., 2005 & 2006).

2.3.5 BPV Genome

The genomic organization of each of the papilloma virus is remarkably similar. One characteristic of the genomic organization of BPV-1 which is shared with all of other papilloma viruses is that of the ORFs are located on one strand of the viral DNA, indicating that all of the viral genes are located on one strand (Chen et al., 1982).

The circular supercoiled DNA genome of BPV is about 8 kilo-bases (Kb) and consists of three canonical regions : long control region (LCR), formerly called the non-coding region and later the upstream regulatory region containing the cis- regulatory elements necessary for the replication and transcription of the viral DNA, the region containing the early(E) genes encoding non-structural proteins and the region containing the late (L) genes encoding the structural proteins (Chambers et al., 2003; Nassir and Campo, 2008). The BPV genome can be functionally divided into three main domains on the basis of its transforming capability. The three domains are E (early), L (late) genes, and long control region (LCR) (about 500-1000 nucleotides) contains transcriptional regulatory sequences and the replication origin (Danos et al., 1983; Lazo, 1988; Munger and Howley, 2002). There are six early genes,
all of them expressed according to the viral life cycle into the host cell. The early genes are responsible for DNA replication, transcription, control, and cellular transformation (Doorbar 1996; Cresswell et al., 2000). The E-1 protein has helicase activity and plays its role on the viral replication (Lambert, 1991; Wilson et al., 2002). The E2 gene product is responsible for recognition and ligation to the replication origin and furthermore, it has mitotic chromosome binding activity in order to ensure equal distribution of viral episomes among daughter cells (Androphy et al., 1987; Baxter et al., 2005). The E4 gene, completely overlapping E2 gene but in a different reading frame, produces a small protein profusely found in keratinocytes cytoplasm during productive replication (Anderson et al., 1997). Three early proteins are necessary for BPV-mediated carcinogenic process, so, called onco-proteins: E5, E6 and E7 (Nasir and Campo, 2008). E5 is a membrane-associated hydrophobic protein, which plays a role on disrupting cellular growth control. Translation of ORFs E5 of BPV is required for its transforming activity (Di Maio et al., 1986). BPV E6 protein is known to have a multitude of binding partners and activities on the virus lifecycle. The E region represents about 45% of the viral genome and contains eight open reading frames (ORFs) and it is at least 400 bases in size that codes for the proteins associated with genome replication. The L region comprises about 40% of the genome and contains two additional ORFs, L1 and L2, that codes for the viral structural proteins of the virion capsid. The L1 ORF is the most highly conserved ORF within the PV genome (De Villiers et al., 2002) and represents 80% of the total viral protein (Howley and Lowy, 2001). Here L1 is the major capsid protein, which is arranged in 72 pentamers and has capacity to self-assemble in VLPs. L2 is the DNA binding protein necessary to genome encapsidation. LCR is a small region between 5’ end of the E and 3’ end of L region, codes for wart specific late RNA (Lazo, 1988; Reid and Campion, 1988). L1 and L2 are major and minor capsid proteins respectively and the C-terminus of L1 is exposed on the surface of the virion and is likely to have a role in infection and immunogenicity (Modis et al., 2002).

2.3.6 BPV gene products and their functions

It has been stated that the BPV genome has about ten genes viz. the early genes E1 to E8 and the late genes, L1 and L2 respectively. Their functions are as follows: The early genes encode products that are necessary for viral DNA replication and transcription and can subvert cellular proliferation, ultimately leading to cell transformation (Campo, 1997). The BPV late proteins L1 and L2 are expressed into the more differentiated epithelial cells. The former mediates virus interaction with cellular receptors; the latter induces virion assembly by binding to viral DNA (Anderson et al., 1997).

E1: The E1 protein is essential for viral DNA replication and has also been shown to bind a number of cellular DNA polymerase, thus recruiting the cellular DNA replication
initiation machinery to the viral origin of replication (Park et al., 1994). E1 encodes a protein that binds to the viral genome and uses ATP to exert a helicase activity that forces apart the DNA strands, thus preparing the viral genome for replication by cellular DNA replication factors. It is claimed to be the most important regulatory protein that modulate replication (Baker and Howley, 1987; Lambert, 1991).

E2: BPV E2 was the first viral product to be identified as a master transcriptional regulator for viral promoters located primarily in the LCR. It is crucial in the life cycle of BPV. It is both a replication factor for viral DNA and the major viral transcription regulator, and binding to the LCR activates or repress transcription of the viral genes (Hermonat et al., 1998; Jackson et al., 1991). E2 gene product is involved in both control of transcription and viral DNA replication (Yang et al., 1991). The protein has a transactivation domain linked by a relatively unstructured hinge region to a well characterized DNA binding domain. It can activate or repress viral promoters and has critical roles in viral DNA replication (Ham et al., 1991), and targets the E1 protein to the replication origin (Sedman and Stenlund, 1995). E2 also utilizes a cellular protein, Bromodomain-4 (Brd 4) to tether the viral genome to cellular chromosomes (McBride, 2000). This tethering to the cell’s nuclear matrix ensures faithful distribution of viral genomes to each daughter cell after cell division. It is thought that E2 serves as a negative regulator of expression for the oncogenes E6 and E7 in latently HPV infected basal layer keratinocytes. Genetic changes viz. integration of viral genome (DNA) into a host cell chromosome, that inactivate E2 expression tend to increase the expression of the E6 and E7 oncogenes resulting in cellular transformation and further genetic stabilization.

E3: This small gene exists only in few PV types. The gene is not known to be expressed as a protein and does not appear to serve any major function.

E4: It is expressed at low levels during the early phase of viral infection but its expression increases dramatically during the late phase of infection. The E4 protein of many PV types is believed to facilitate virion release into the environment by disrupting intermediate filaments of the keratinocyte cytoskeleton. It is very much abundant in BPV induced lesions where they can make up as much as 30% of total protein. Viral mutants incapable of expressing E4 do not support high level replication of the viral DNA. The precise role of E4 protein is not clear but studies have shown E4 expression coincides with the onset of viral genome amplification (Peh et al., 2002). In cultured cells, E4 interacts with the filamentous network of cyto-keratins, which help virus replication in vivo (Doorbar, 1991).

E5: E5 is the major BPV transforming onco-protein (DiMaio et al., 1986; DiMaio and Mattoon, 2001) that would help in establishing viral infection by promoting both cell proliferation and immune evasion. It is a short, hydrophobic, type 2 transmembrane proteins.
that destabilize the function of many membrane proteins in the infected cells and is expressed in the deep layers of the infected epithelium (Suprynnowicz et al., 2006). It functions as oncogene primarily by activating the cell growth promoting the signalling of platelet derived growth factor receptors. A recently discovered function of BPV5 is the down regulation of the surface expression of MHC -1 protein, which may protect the infected cell from destruction by cytotoxic T cells and or killer T cells (Araibi et al., 2004; Ashrafi et al., 2002). BPV E5 is expressed in both basl and supra-basal transformed epithelial cells with a typical juxta nuclear pattern due its localization in the golgi apparatus (Bohl et al., 2001; Burnett et al., 1992). E5 is a membrane- associated hydrophobic protein and is believed to play key role on disrupting cellular growth control

E6: BPV-E6 is a transcriptional activator and the primary function of it is to inactivate the tumour suppressor protein p53. The E6 protein of BPV type -1 and of the oncogenic HPVs is believed to function through binding cellular targets (Howley and Lowy, 2001) such as binding and degrading the p53 tumour suppressor protein. In Xi papillomavirus, E6 gene is replaced by an E5- like gene, which was initially defined as E8. E7: The E7 protein appears to cooperate with E5 and E6 for cellular transformation, whose production with the two other onco-proteins increases the transformation efficiency (Bohl et al., 2001). In most PV types, the primary function of the E7 protein is to inactivate members of the retinoblastoma protein (pRb) family of tumor suppressor proteins. It is crucial for morphological transformation of cells. The E7 protein of the oncogenic HPVs binds a number of important cellular regulatory proteins such as the pRB (retinoblastoma tumor suppressor protein) and the related poicket proteins p107 and p130 (Dyson etal., 1989). The early genes E6 and E7 and in some PV types, contain oncogenic properties that can modulate the transformation process (Baker and Howley, 1987). Together with E6 and E7 serves to promote cell cycle progression, thus priming the cell for replication of the viral DNA. E7 also participates in immortalization of infected cells by activating cellular telomerase.

E8: it is truly an early protein, produced both before viral DNA replication and in early papillomas. Notably few PV types have the capacity to express a short protein from the E8 gene.

L1: It spontaneously self assembles into pentameric capsomeres. Purified capsomeres can go on to form capsids, which are stabilized by di-sulphide bonds between neighbouring L1 molecules. The L1 capsids assembled in vitro. However, the surface loops of L1 can differ substantially even for different members of PV species. The expression of L1 gene is exclusively restricted to differentiating keratinocytes in the outermost layers of the skin or mucosal surface. The increased expression of L1 is typically correlated with dramatic increase
number of copies of viral genome. C-Terminus of L1 is exposed on the surface of the virion and is likely to have a role in infection and immunogenicity (Modis et al., 2002).

L2: In addition to its direct interaction with a number of cellular proteins during the infectious entry process it provides stability to the virions and facilitate packaging in cooperation with L1 (Hagensee et al., 1993) and en-capсидation of viral DNA, by interaction of charged amino acids with nonspecific DNA sequences. L2 is detected solely as a nuclear Ag (antigen) in the differentiated layers of papilloma and may enhance packaging (Stauffer et al., 1998) and infectivity (Roden et al., 2001). The BPV-4 type codifies the L3 protein with unknown function (Patel et al., 1987, Doorbar and Sterling, 2001).

2.3.7 BPV Diversity

The understanding of PV diversity is very limited. Virus host- divergence is an important evolutionary force which can partially explain the evolution of PVs and their diversity. Thus alternative mechanisms such as virus duplication within the host, recombination, viral sorting, or viral adaptation after a host switch, may contribute to a certain extent to explain the BPV diversification (Gottschling et al., 2011). Gottschling et al., (2007) used a rigorous phylogenetic approach; taking into consideration of the choice of appropriate out groups, as well as the assessment of confidence values of internal nodes. Gottschling et al., (2011) used different statistical approaches to assess topological and branch length congruence, evidencing the importance of alternative mechanisms other than co divergence. Another statistical approach based on entropy was used to assess the evolution of PVs, showing the hot spots in the genome could be used as markers in order to infer PV phylogeny. When it comes to BPV, 12 types have been currently well described and their genomes have been sequenced and about 14 new putative types were also isolated based on these sequencing (Antonsson and Hansson, 2002; Ogawa et al., 2004; Campo, 2006; Ogawa et al., 2007; Tomita et al., 2007; Claus et al., 2008; Hatama et al., 2008; 2011; Zhu et al., 2012).

In phylogenetic analysis, BPVs are found in at least three distantly related lineages. First, BPV-1, BPV-2, BPV-5, and BPV-8 form a paraphyletic group with OvPV-2, which infects a close related host. Other PVs infect Artiodactyla are also close relatives of those BPVs. However, this group is clustered together with equine and canine PVs. Second, BPV-3, BPV-4, BPV-6, BPV-9, BPV-10, BPV-11 and BPV-12 are grouped together with caprine and canine PV (ChPV-1). Therefore, all this diversity found in BPVs that infect one host (Bos taurus or Bos indicas) is a case of evolutionary incongruence between host and PV phylogeny, including that co-divergence alone cannot explain the PV diversity (Gottschling et al., 2007, 2011). For BPVs, at least three lineages probably passed through a prior divergence process preceding the host divergence. This could explain the proximity of BPVs to PVs that infect distantly related hosts. In addition, zoonotic transmission of PVs is rare event but it
occurs in BPVs as they were found in zebras, horses and buffaloes (Silvestre et al., 2009, van Dyk et al., 2009; Bogaert et al 2010). However other evolutionary mechanisms could also be associated with BPV diversification which needs further research with hues samplings.

2.3.8 BPV Life Cycle

Every viral family has a different strategy of replication, and an understanding of viral replication provides a basis for understanding pathogenesis, immunity, chemotherapy, and the role of viruses in cancer. Campo (1987) found that viral replication in basal epithelial cells stimulates hyper-proliferation and hyperplasia with formation of warts and benign papillomas. The early and late events in the replication cycle of the virus were thought to be attachment, penetration, intracellular maturation and budding. The life cycle of the virus is tightly coupled with the differentiation process of the epithelial cell: the virus infects the basal keratinocytes, express part of its genes in the basal and supra basal layers, replicates its genome in differentiating spinous and granular layers, expresses its structural genes and packages its DNA in the squamous layers, and new infectious virus is finally released with the keratinized squamous (Campo, 1994, 1995). BPV has distinct four stages in its life cycle and key life cycle events seem to be similarly regulated in both human and non-human PVs (Peh et al., 2002). Main features of the life cycle is as follows

Infectious entry: PVs are believed to gain access to keratinocyte stem cells through small wounds or micro abrasions, in the skin or mucosal surface. Interactions between L1 and sulphated sugars on the cell surface promote initial attachment of the virus (Joyce, 1999 and Giroglou, 2001). Following attachment, the virion penetrates the host cell and is partially uncoated to expose the viral genome or is internalized from the cell surface and transported to membrane enclosed vesicles called endosomes (Selinka et al., 2002 and Day et al., 2003) where the minor capsid protein L2 disrupts the membrane of the endosome, allowing the viral genome to escape and traffic, along with L2, to the cell nucleus (Kampar et al., 2006 and Day et al., 2004).

2.3.9 Maintenance and latency

After successful infection of a keratinocyte certain early viral genes are transcribed into RNA which may then be processed by splicing. The virion expresses very low levels of the early viral proteins E1 and E2, which are responsible for replicating and maintaining the viral genome as a circular episome. The viral oncogenes E6 and E7, which promote cell growth by inactivating the tumour suppressor proteins p53 and pRb, respectively, may also be expressed at very low levels. Keratinocyte stem cells in the epithelial basement layer can maintain papillomavirus genomes in a dormant or latent state for decades (Doorbar, 2005).
Latent papilloma virus infection in cattle has also been reported (Campo et al., 1994a). Persistant papillomatosis associated with immunodeficiency has been reported (Duncan et al., 1975).

2.3.10 Production of progeny virus

The expression of the viral late genes, L1 and L2, is exclusively restricted to differentiating keratinocytes in the outer most layers of the skin or mucosal surface. The increased expression of L1 and L2 is typically correlated with a dramatic increase in the number of copies of the viral genome. Since the outer layers of stratified squamous epithelium are subject to relatively limited surveillance by cells of the immune system, it is thought that this restriction of viral late gene expression represents a form of immune evasion.

2.3.11 Assembly of progeny virus particles

New infectious progeny virus particles are assembled in the cell nucleus. The surface layers of the stratified squamous epithelia are shed into the environment through process of spontaneous cellular disruption known as desquamation. Papilloma viruses have evolved to exploit this normal process as a mechanism for releasing virions into the environment. Other kinds of non-enveloped animal viruses utilize an active lytic process to kill the host cell, allowing the release of progeny virus particles. Often this lytic process is associated with inflammation, which might trigger immune attack against the virus. PVs exploit desquamation as a stealthy, non-inflammatory release mechanism. Cells undergoing the lytic form of infection shed large number of virions or virus particles (Cheville, 1994).

2.3.12 Biological properties of BPV

BPV is the long sought viral cause of cutaneous neoplasia of cattle and buffaloes worldwide and it is an aetiological agent associated with several forms of cutaneous and mucosal papillomas (Campo, 2002). BPV induces hyper proliferation of epithelial cells of the skin or mucosa, and certain types can also infect fibroblasts. They are very diverse or heterogeneous in genomic properties and individual types are associated with specific lesions. BPVs have a specific tropism for squamous epithelial cells. Full viral replication, including synthesis of its genome, capsid proteins and assembly of virions, occur strictly in the more terminally differentiated squamous epithelial cells. The wart specific late viral genes, which encode the viral capsid proteins, are expressed only in the terminally differentiated epithelial cells of the wart. This apparent absolute requirement for terminal differentiation of squamous epithelial cells for the expression of the wart virus specific genes for virus replication is probably the reason that papilloma virologist have not yet succeeded in propagating these viruses in tissue culture (Howley and Schlegel, 1987).
Despite the fact that to date there is no tissue culture system for the \textit{in vitro} propagation of the papilloma viruses, BPV have been shown to have reproducible effects in tissue culture. Black et al., (1936) observed cytopathic changes in cultures of bovine conjunctival cells after infection with bovine papilloma extracts. These changes included altered morphology, piling up and an increase in acidity of the medium. Subsequently, cell free extracts of bovine papillomas were shown to induce morphological transformation of primary embryonic bovine skin cells, mouse embryo cell cultures (Boiron et al., 1965), and hamster embryo cells (Geraldes, 1969, Meischke, 1979). Boiron et al., (1965), first reported transformation of foetal bovine skin cells with DNA obtained from virus isolated from a pool of bovine papillomas. Dvoretzky et al., (1980) reported an invitro focus assay for the quantitation of BPV-1 and BPV-2. Chan et al., (1997) reported that the super group of fibro-papillomaviruses (BPV-1 and BPV-2) is the only one with members that also infect mesenchymal cells and fibroblast cell cultures. Neither extensive molecular virological research on BPV-1 gene functions, nor sequence analyses have pointed so far to the origin of this unusual biology.

Neoplastic progression is a dead end process for BPV, as the transformed cell is no longer permissive for virion maturation; the viral genetic material is either incorporated into the cellular one (Schwarz and Bhandoola, 2006), maintained as an extra chromosomal element, which replicates in synchrony with the cell cycle (Botchan et al., 1986), or may even be lost by the transformed cells (Campo et al., 1985). BPVs fall into two groups (Jarrett et al., 1984); subgroup, comprising the fibro-papillomaviruses BPV-1, -2 and -5, and subgroup B, comprising the epitheliotrophic papilloma viruses BPV-3, -4 and -6. Infection by fibro-papilloma viruses leads to an initial transformation of the sub-epithelial fibroblasts followed by epithelial plexiform acanthosis and then papillomatosis (Jarrett, 1985), while infection by epitheliotrophic papillomaviruses induces epithelial papillomas without fibroblast involvement. BPV E5 induces cell transformation of cultured rodent fibroblast and keratinocytes by binding to and activating the platelet derived growth factor (PDGF) beta receptor. Furthermore E5 induces receptor activation by forming a stable complex with it, inducing its dimerization and phosphorylation. This mechanism has been demonstrated in transformed cells and in naturally occurring epithelial and vascular bladder cancer in cattle (Borzacchiello et al., 2007).

The characteristics of the BPV have made it an attractive choice for eukaryotic expression system. These include the episomal nature of the genome in transformed cells and the fact that these cells remain viable over many years (Stephens and Hentschel, 1987). PVs will transform fibroblasts in culture and in vivo. However, viable viruses are only produced terminally differentiated papillomas and cannot be cultured \textit{in vitro}. 

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Antonio Carlos de Frietas et al., (2003) have detected BPV-1 DNA sequences in peripheral blood, warts and plasma samples of bovine affected by cutaneous papillomatosis. BPV-1 has also been detected in blood, placenta and amniotic fluid obtained from a bovine and her calf thus showing the evidence of vertical transmission of BPV-1.

2.3.13 Mode of Transmission of BPV

Little is known about the mode of transmission of the disease between animals. It is known that the confined populations are more vulnerable because virus dissemination could occur by direct animal to animal contact or indirect contact through contaminated objects (Hama et al., 1998; Nasir and Campo, 2008). Besides the established skin to skin path way, another mode of transmission has been claimed like transmission by arthropod vector such as ticks that act as mechanical transmitters (Dalmat 1958; Somvanshi et al, 1986) and also by vertical transmission (Carvalho, 2003; Freitas et al., 2003; Finlay et al., 2009; Yaguiu et al., 2006; Roperto et al., 2008). Preliminary studies indicate the presence of these viruses in the blood of cattle suggesting that this latent site may exert a role in the transmission of the disease (Diniz et al., 2009). Spreading of BPV through non-epithelial tissues and fluids has also been claimed as a possible mode of transmission of BPV (Stocco dos Santos et al., 1998; Freitas et al., 2007). This mode of transmission has been justified by the detection of BPV in different tissues and cells, including reproductive sites as oocytes, ovary, uterus, cumulus cells, and uterine lavage (Carvalho, 2003; Freitas et al., 2003; Yaguiu et al., 2006; Lindsey et al., 2009). The vertical transmission of the disease has been suggested and this mode of transmission has gained tremendous impetus in studying the biology of BPV biomolecules (Stocco dos Santos et al., 1998; Freitas et al., 2003; Yaguiu et al., 2008). It has been observed that HPV infected women could transmit the infection to the foetus by trans-placental mechanisms. The mechanism behind the transmission of BPV to or nonspecific hosts like horse, mules, donkey, giraffe, stable antelope, buffalo etc. is not clear. Recent findings of BPV DNA in epidermis and formation of L1 capsomers of sarcoid in horse and active BPV in healthy skin of equine (Bogaert et al., 2008; 2010a, 2010b; Brandt et al., 2011) could help in explaining the occurrence of equine sarcoid in animals kept far away from any bovine virus source, especially when accommodated in close contact with other affected equids (Brandt et al., 2011).

However, it is believed that flies can be a vector for BPV and transmit the virus between bovine and horses (Nasir and Campo, 2008; Finlay et al., 2009). BPV infection may be transmitted via routine management practices by way of tattooing, contact with contaminated halters and farm equipment, or passed into existing wounds from contaminated pasture. More research drive is necessary to explore all of possible mode of transmission (Chambers et al., 2003).The heavy traffic of animals between different regions could contribute to the spread of this agent.
2.3.14 Dissemination and cross species infection of BPV

Present day research should be directed to investigate the dissemination of BPVs to other mammals that infecting or co-infecting these animals due to its plasticity. For long time, PVs had been described as species specific (Campo, 2006, a, b). PVs appear to be widespread and have been found in large number of vertebrate species and are assumed to have co-evolved with their hosts (Bernard, 1994; Antonsson and McMillan, 2006). A series of PVs could infect a variety of phylogenetically distant hosts (Bravo et al., 2010).

BPV can infect cattle but also infect close relatives of cattle such as buffaloes (Silvestre et al., 2009; pangty et al 2010) and giraffe (van Dyk et al., 2011) causing fibropapillomas and bladder lesions (Pathania et al., 2011). BPVs have also been found naturally infecting more distantly related species, such as tapirs (Kidney and Berrocal, 2008), horses (Bogaert et al., 2008), sable antelope (van Dyk et al., 2011) and Zebras living either in zoos or in the wild (van Dyk et al., 2009) causing sarcoïds and fibro-sarcomas when inoculated into rodents (Robl and Olson, 1968). Recently a variant of BPV -8 has been detected to induce papillomas in Bison (Literak et al., 2006). Presence of L1 capsomers in epidermis of equine with sarcoïd is suggestive of productive infection by BPV (Brandt et al., 2011). The ability of BPV-1 infection in related hosts can be a result of human domestication of cattle and horses or a phenotypic acquisition driven by vector mediated inters species transmission (Finlay et al., 2009; Gottschling et al., 2011).

2.3.15 Classification of BPV

BPVs originally fall into two groups (Jarrett et al., 1984); subgroup-A, comprising the fibro-papilloma-viruses namely BPV-1,2 and 5) , i.e. viruses that infect both the epithelium and the underlying derma , giving rise to fibro-papillomas and subgroup-B, defined as truly epitheliotropic BPVs comprised BPV-3,4 and 6, inducing true papillomas. This classification was originally based on the BPV genome structure and recognized pathology induced by them.

Based on host response, BPVs can be subdivided into further three groups as to tissue tropism and the histopathology of the lesions. One group is composed of BPV-3 and BPV-5 induces neoplasia of cutaneous stratified epithelium; BPV-4 constitutes a second group that primarily induces hyperplasia of either normal non stratified squamous epithelium or metaplastic squamous epithelium. The third group includes BPV-1 and BPV-2, which induce cutaneous papilloma and underlying fibroma of connective tissue. In all histological types, intact virus can be demonstrated only in outer layers of keratinizing cells of the epithelium (Cheville, 1994; Tajima et al., 1968).
BPVs have recently been re-classified (de Villiers et al., 2004) following the Greek letter nomenclature for other virus families. According to the new nomenclature, the epitheliotropic BPVs 3, 4 and 6 are defined as xi-papillomaviruses and BPVs 1 and 2 as delta-papillomaviruses which are associated with fibropapillomas; and epsilon-papillomavirus comprising BPV-5, whose seems to share similarities with the preceding two BPV groups, (Bloch and Breen, 1997) but BPV-5 appears to have dual pathology and causes both fibropapillomas and epithelial papillomas (Bloch et al., 1994a). These two observations have led to the re-classification of BPV-5 as the only member of the epsilon- papillomavirus genus (de Villiers et al., 2004). Two additional BPV were characterized: one was designated BPV-7 and classified as a member of a new papilloma virus genus, and other was designated BPV-8 and classified as a member of the Epsilon- papillomavirus genus (Borzacchiello and Roperto 2008). Recently, Hatama et al., (2008) identified two new BPV types belonging to the genus Xi- papillomavirus, designated BPV-9 and BPV-10.

The new BPV types were found in teat papillomas and in healthy teat skin but their pathology and whether they are delta, xi or epsilon- papillomaviruses are not yet known. BPVs 1, 3 and 6 were also found in healthy teat skin, which strongly suggests latent or subclinical infection.

Thirteen additional novel BPVs have been reported recently (Ogawa et al., 2004). In addition to lack of immunological cross reactivity, these viruses are readily distinguished by characteristic RE cleavage patterns of their genomes and degree of polynucleotide sequence homology. It is clear that this virus needs further investigation. It is likely that further investigations would reveal the existence of even more BPVs and it would be very much interesting to establish whether their pathology follows Delta-, Xi- or Epsilon- PVs.

BPV-2 has been detected in reproductive tract tissues, fluids and oocytes from slaughtered bovine females not afflicted by 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). It is likely that BPV-2 can persist and be maintained in an active status in the bloodstream, in particular in the lymphocytes, as a reservoir of viral infection that, in the presence of co-carcinogens, may cause the development of urinary bladder tumours (Roperto et al., 2008). Recently, on the basis of complete genome sequence and phylogenetic analysis two new types BPV-7 and BPV-8 in European bison have been reported in Japan (Ogawa et al., 2007, Tomita et al., 2007), respectively. BPV-7 genome is consisted of 7412bp and encoded by five early (E1, E2, E4, E6 and E7) two late (L1 and L2) genes, but did not encode the E5 gene, whereas BPV-8genome is consisted of 7791bp and had 70% similarity with total genome of BPV-5 but having additional E5 ORF.
2.3.16 Incidence of Bovine papillomatosis in global perspective

BPVs are widely distributed world-wide with widespread occurrence of papillomatosis in cattle throughout the globe. Cases have been reported in the form of bovine papillomatosis in cattle (Bos taurus) in Europe, America, Asia, and Oceania (Freitas et al., 2011). Warts occur quite commonly on both dairy and beef cattle in Egypt (Dawlat et al., 1997) and in Australia (Fitzpatrick 1998). Numerous cases of bovine papillomatosis had occurred in Croatia over past few years and an incidence of severe bovine papillomatosis with special reference to efficacy of treatment using autogenous vaccine and paraimmunity inducer has been reported (Turk et al., 2005). In Japan, the presence and incidence of BPV infections had not been well documented. BPV-6 has been reported to be specifically associated with the frond epithelial type of teat papilloma and it is thought to be a common type prevalent in the cattle herds in Japan (Ogawa et al., 2004). Prevalence and types of BPV infections seen on teats of selected heifers in Hokkaido, Japan, were reported by Maeda et al., (2007). Subsequently an epidemic of benign teat papillomatosis in cattle affecting 80% cattle at a local farm in Hokkaido, the Northern island of Japan, caused by BPV-6 and unclassified BPVs were reported and characterized by PCR and DNA sequencing of the amplified products (Maeda et al., 2007).

BPV infections are common in cattle, with around 50% of cattle being estimated to bear BPV-induced warts in the UK (Campo, 1995). Genital BPV infection was observed and reported for the first time in the Al-Ahsa region of Saudi Arabia and the Gulf region involving domestic cattle, one female and two male 2-4 year old crossbred cattle causing fibropapillomas (warts) limited to the prepuce and vulva (Elzein et al., 1991). Methods for the detection and prevention of this disease are needed to prevent widespread dissemination as Saudi Arabia begins to expand its cattle production. Cutaneous, peri-vulvar, and vulvar fibropapilloma associated with BPV-1 infection in the water buffaloes (Bubalus bubalis) was reported first time from Italy as another example of cross-species infection by BPV-1 (Silvestre et al., 2009). Incidence of Spontaneous papillomatosis in bovine heifers from South Western Bohemia was reported with special reference to histological, ultra-structural aspects of papillomatosis together with the presence and distribution of virus demonstrated by PCR, immunohistochemistry and in situ hybridization (Jelinek and Tachezy 2005). BPV-1,-6,-8 and -10 were detected in bovine warts from a German cowshed (Schmitt et al., 2010).

Cutaneous papillomatosis in a European bison shipped from a European zoo to Bukovské Vrchy Hills, Slovakia ,was confirmed histologically and by negative stain transmission electron microscopic (TEM) examination and virus typing based on PCR and subsequent DNA sequence analysis (Literak et al., 2006).
An outbreak of severe bovine papillomatosis that occurred during 2000, in Northwest Croatia, was first time detected based on clinical signs, histopathological findings and TEM. The said outbreak was reported by Turk et al., (2005). BPV-1, -6, 8 and -10 were found in bovine warts from a German cow shed (Schmitt et al., 2010). In Brazil, cutaneous papillomatosis is widespread among beef and dairy cattle, and studies have been focused on the identification of BPV types. The occurrence of considerable viral diversity in BPV infections throughout Brazilian cattle herds has been reported (Claus et al., 2007, 2008, & 2009) and amongst them BPV-1 and BPV-6 have been identified as the main types involved in cutaneous warts, whereas BPV-2 has been associated with enzootic haematuria (Wosiacki et al., 2005; Souto et al., 2006). BPV-1, -2, -6 and -8 in the skin warts of cattle from Southern Brazil have been identified by molecular tools (Silva et al., 2010). Studies in North-eastern Brazil also have revealed the presence of ten different types of BPV in spontaneous cases of BP, with the exception of BPV-7 (Carvalho et al., 2003).

As considered before, BPV is also associated with cancer. Field cases of urinary bladder cancer in cattle associated with BPV-1 and BPV-2 infections were reported in continental Europe, Azores Islands, some regions of Kenya, Brazil, New Zealand, India and China (Borzacchiello and Roperto, 2008). Bovine papillomatosis is also a common viral infection in Brazil that is caused by a BPV and in situ hybridization and detection of BPV DNA sequences in the reproductive tissues in Bostaurus taurus has recently been made (Yaguiu et al., 2006). The detection of BPV in these tissues strongly suggests that these sequences could be an important alternative of viral transmission that could contribute to the widespread incidence of bovine papillomatosis and complex pathology. BPV-2 involvement in the chronic haematuria aetiology has opened the perspective of the development of new strategies for the control of this disease that is the major cause of economic losses in beef herds from many geographical regions (Wosiacki et al., 2006). Most recently wide spread occurrence of cattle warts in 48 different cattle herds in three different states of Brazil has been analysed and molecular detection of the largest number of BPV types associated with warts in Brazil have been made (Silva et al., 2010).

Bovine cutaneous warts were reported from India and identified as BPV types 1 and 2 (Leishangthem et al., 2008; Singh et al., 2009; Pangty et al., 2010; Pathania, 2010; Nagarajan, 2011, Kumar, 2012). Recently Rai et al., (2011) identified BPV-10 in teat warts from cattle at a dairy farm in India. Papillomatosis is not uncommon in Pakistan. Shakoor et al., (2012) reported two cases of teat papillomatosis in crossbred cattle and studied effects of Thuja occidentalis as an alternative remedy in the treatment of papillomatosis in cattle. Incidence of clinical papillomatosis characterized by multiple cutaneous tumours in the linea alba and on teats in Ayrshire and Czech breed heifers in a herd of South-Western Bohemia, in Czech
Republic, was reported together with the presence and distribution of the virus demonstrated by PCR, immunohistochemistry and in situ hybridization (Jelinek and Tachezy, 2005). In Scotland, 36% of cattle introduced to a slaughterhouse had suffered from papillomas on their teats and so did 48% of cows at the same slaughterhouse (Hatama-2011).

2.3.17 Incidence of BP

Incidence of BP was higher among the adult cattle, followed by heifers and calves (Jubb and Kennedy, 1970). But in contrary Abu-Samra et al., (1982) reported that the disease occurred in animals of age group between 8 to 15 months and no adult cattle were found affected. Higher incidence of BP was noticed among adult cattle followed by heifers and calves as reported by William et al., (1992) and as regards distribution of tumours is concerned higher degree of affection was noticed in udder and naval flap (46.51%) followed by head and neck (18.06%), limbs (11.63%), trunk (11.63%) and mixed (11.63%). This was in contrary to Abu-Samra et al., (1982) who observed more warts distribution on head, neck, dewlap, trunk and back.

2.3.18 Status of BP in India

Bovine cutaneous papillomatosis or warts are occasionally seen in cattle and buffaloes and in India; this ailment is not uncommon too. Various workers have reported incidences of BP in India from time to time. Nair and Sastry (1955) and Thilakarajan (1980) observed 6.7 % (126 cases) and 6.8 % (44 cases) incidence of bovine papillomas in Madras state during 1940-51 and 1952-62, respectively. Charan (1982) had reported squamous cell carcinoma in reticulum and rumen of Haryana bullocks in UP. Paul Gupta et al. (1984) reported an outbreak of BP in dairy cattle in Haryana. Rao and Nayak (1985) had recorded a case of oral papilloma and five cases of cutaneous fibroma in bovines in Orissa. Transmissible fibropapilloma in cattle was also reported (Chaudhary et al., 1986). Simultaneous occurrence of cutaneous warts and enzootic bovine haematuria (EBH) in same herd of hill cattle was reported from hilly temperate region of Mukteswar (Somvanshi et al., 1986a). Five clinical cases of BP were investigated in 1-3.5 year old Frisian x Haryana and Jersey x Afghan x Hill crossbred heifers maintained at IVRI dairy farm, where the heifers developed multiple, hard, typical pedunculated, keratinized or non-keratinized horny warts of varying size and shape on teats and udder, belly, head, neck, back and legs. Histopathologically, all cases were fibropapilloma. Clinico-pathological characterization of bovine cutaneous warts and assessment of delayed cutaneous hypersensitivity and humoral immune response in spontaneous cases of BP was also carried out by Somvanshi et al., (1988b). Gupta et al., (1989) had observed a case of extensive BP in 2.5 years old cross bred HF heifer in Ludhiana (Punjab) where the animal had developed wart lesions on the skin of both sides of shoulders, neck, dewlap, ears and thigh region of the left hind leg. Singh and

Debasis Jana and Mousumi Jana (2009) reported spontaneous cases of BP in indigenous buffalo calves and heifer from an organized dairy farm in West Bengal. Subsequently Jana and Ghosh (2010) reported a spontaneous case of fibro papilloma in a male buffalo calf and its successful surgical management. Latter persistent bovine cutaneous papillomatosis recorded in an indigenous heifer was reported with special emphasis to its surgical management by the same workers (Jana and Ghosh, 2010). Hegde (2011) reported a severe form of cutaneous papillomatosis in a non-descript cow from Salakini (Karnataka) and its successful by auto-hemotherapy.

Leishangthem (2006) reported natural cases of BP in cattle and Murrah buffalo at organized dairies in Bareilly and Mathura. BPV-2 was demonstrated by TEM and PCR method and confirmed by nucleotide sequencing. Fifty three cases of CWs were recorded in cattle in UP and BPV-1 and BPV-2 was identified as the causal virus bio molecule (Singh et al., 2009). A total of 34 cases of teat warts (33 in cows and 1 in buffalo) were recorded in Nainital and Bareilly district of Uttarakhand and UP, India (Singh and Somvanshi, 2010). Pangty (2009), recorded 16 cases of teat warts (TWT), Pathania (2010), recorded 7 and Kumar (2012) 11 cases of TWT, respectively from station dairy, Mukteswar and field areas in Pithoragarh and Nainital districts. Umadevi and Umakanthan (2013) reported 18 cases of cutaneous papillomatosis in cattle bearing cutaneous warts on various parts of the body, with or without udder or teat involvement from Tamilnadu with successful combined drug therapy for the treatment of papillomatosis in cattle.

2.3.19 Clinical signs and pathology

BPV induced papillomatosis is an infectious disease whose remarkable clinical sign is the hyper proliferative lesions, known as papillomas and or fibropapillomas, on cutaneous tissue and mucosa. Although papilloma or warts appear as epidermal proliferation that have a
keratotic surface resembling a cauliflower (verruca vulgaris), some BPV (BPV-1 and BPV-2) involve dermal fibroblasts and keratinocytes and appear as papulonodule with warty surface. Such fibropapillomas may involve the venereal region where they can cause pain, disfigurement, infection of the penis of young bulls and dystocia when the vaginal mucosa of heifer is affected. Clinically, the typical bovine fibro-papilloma appears as a rough, cauliflower like mass of varying size and irregular shape, elevated above the skin surface and attached by either a narrow stalk or a broad base. The lesions appear as multiple, closely spaced bumps or elevations of the skin, which are round and smooth but soon become rough and horny. The lesions initially grow very slowly, followed by a rapid growth and eventually become larger, horny, pendulant and occasionally appear as cauliflower in shape. The more classical papilloma with only a modest connective tissue support is caused by BPV-3 (skin) and BPV-5 (teat). These are found to be smaller and less pedunculated (Lancaster and Olson, 1982). Lesions over the udder and naval flap were papillomatous but on ears such lesions were of digitative type. Rice grain papilloma and sessile fibroma were most common and seen on neck, trunk, limb and udder. Jarrett et al. (1984) classified the lesions into round, flat and rice grain type with specific etiological virus subgroups (BPV-1, 2, 3, 4, 5, and 6). Lindholm et al., (1984) stated that rice grain papilloma were the intermediary stage in the development of papilloma i.e. fibroma, plaque or papilloma.

Warts or papillomas develop after the introduction of infective virus biomolecule through the cuts, abrasions or micro trauma of the skin. BPV induces the formation of papillomas or warts by targeting the keratinocyte, accompanying the steady maturation of the cell to the surface (Sundberg, 1990; Campo, 2003; Stanley 2006). Infection of epithelial cells results in hyperplasia with subsequent degeneration and hyperkeratinisation. These benign changes occur usually 4 to 6 weeks after exposure. Warts development usually follows the following three stages as stated below:

Stage-1: Slight raised plaques, starting at about 4 weeks after exposure.

Stage-2: Characterised by cytopathology, virus replication and crystalline aggregates of virions in lesions, starting at about 8 weeks.

Stage-3: Characterized by fibrotic, pedunculated bases and rough lobate, or fungiform surfaces, starting after about 12 weeks.

Numerous descriptions of epithelial growths in the skin of many species of mammals have been reported (Karstad and Kaminjolo 1978; Uzal et al., 2000; Sundberg et al., 2000; 2001; Schulmann et al., 2001; 2003; Literak et al., 2006; Silvestre et al., 2009). Conspicuous amount of fibrous connective tissue at the base which forms core upon which the neoplastic epithelial cells are massed, and are commonly associated with the infection with BPV.
2.3.20 Histopathology

Histologically the warts are mostly characterized by fibro-papillomatosis with acanthosis, hyperkeratosis and down-growth of rete ridges. The virus appears to infect the basal cells of the epithelium, causing hyperplasia with hydropic ballooning of their cytoplasms, large eosinophilic keratohyaline granules and vesicular nuclei. Some cells degenerated, while others were stimulated to excessive growth and formation of warts. Microscopically, papillomatous lesions (warts or papillomas) are characterized by hyperplasia of cells in the spinous layer (acanthosis). These cells show an increase in the size and number of desmosomes and tono-fibrils whereas the other epithelial cells show degenerative changes with loss of tono-fibrils, detachment of desmosomes, focal nuclear atypia and cytoplasmic vacuolization. Towards the upper layers of the epithelium these changes are most prominent. In the granular layer, nuclear degenerations, margination and condensation of chromatin are evident. Epidermis is affected by loss of nuclear polarity and hyper chromatic nuclei are visible in the basal cell layer. Some koilocytes i.e. cells with shrunken; irregular nuclei surrounded by clear spaces are present.

2.3.21 Transmission electron microscopy (TEM)

Transmission electron microscopic (TEM) analysis reveals the presence of virions in abundance arranged in the form of crystalline array in nuclei of degenerated cells in the keratinizing layer (Lancaster and Olson, 1982). Araibi et al., (2004) emphasized that koilocytes are typical of papillomavirus infection where the vacuolated cells become enlarged with condensed nuclei. Small intra-nuclear aggregates of virus particles in epidermocytes, damage to desmosomes and disorganization of cytokeratin filaments in many epidermocytes and aggregates of virus particles in fibroblasts in the dermis were revealed under TEM studies (Jelinek and Tachezy, 2005). Electron microscopic examination of cutaneous warts (CWTs) also revealed presence of BPV like virions on negative staining (Brener and Horne, 1959; Catroxo et al., 2005; Turk et al., 2005). In squamous papillomas of viral origin, presence of virus is generally detectable using immunological methods or by TEM. In contrast, when the papilloma has undergone malignant transformation to carcinoma, the structural integrity of the virus is lost and virus is lost and virus or viral structural antigen is not present. This phenomenon of virus masking is intriguing and may be related to the process of differentiation of epithelial cells since virions are almost always confined to mature cells in the outer keratinizing epithelium of a papilloma (Catroxo et al., 2005).

2.3.22 Immunopathology of BPV

Cattle warts are BPV induced chronic condition maintained in the animal’s body usually in a quiescent state. Normally the BPV induced papillomas regress as a result of a cell
mediated immunity (CMI) or immune response (Knowles et al., 1996) but some animals are unable to reject the infection and succumb to widespread cutaneous or mucosal involvement. There is a significant relationship between the development of warts and immunity. The disease mostly occurs in animals which are immunocompromised (Lutzner 1985). Mature viruses and their hosts remain in a delicately balanced relationship. Viruses must be able to overcome the host immune response to replicate them to produce infectious progeny. Nevertheless, despite the viral evasion of immune surveillance, eventually the host mounts an effective immune response and virus and virus infected cells are eliminated.

Host immune response to BPV is surprisingly poor (Campo, 1998). Animals could carry massive tumours, actively could produce hues quantities of infective virions, but cattle do not respond easily to BPV antigens during the course of infection and anti-BPV antibodies are seldom detected. The failure of the immune system to recognize either incoming virus or progeny virus is because of the fact that the virus life cycle is restricted to the epithelium and therefore is not in contact with the immune system (O’Brien and Campo, 2002; Tindle, 2002). This interpretation is supported by the fact that the naturally infected animals bearing warts or papillomatous lesions do have high titres of natural anti-BPV antibodies, and good antibody responses can be obtained after intramuscular inoculation with purified virus or viral proteins, confirming that only when the papilloma is damaged, or threshold of unknown nature is reached via immunization, viral antigens come in contact with immune cells (Campo, 1988). Weak T and B-cell response to capsid proteins or to the transforming protein E7 can be observed in some animals at later stages of infection and appear to be associated with papilloma rejection (O’Brien and Campo, 2002).

The rejection of BPV-4 papillomas has been claimed to be associated with the accumulation of hues masses of activated lymphocytes in the derma underlying papilloma. In these clusters CD4+ lymphocytes are the predominant sub type, followed by gama, delta T cells and CD8+ lymphocytes that predominate in the basal layer and among in the keratinocytes (Knowles et al., 1996; Campo, 1998). However, the contribution of the individual lymphocyte subtypes to papilloma regression yet to be established.

The poor immune response to BPV infection is likely to be the main reasons for the persistence of infection; even in immune-competent hosts, the papillomas persist for many months before regression takes place. In addition to the passive immune escape as a result of the virus lifecycle being confined to the epithelium, papillomaviruses have evolved ways of hiding from the host immune system (O’Brien and Campo, 2002). Among these there is down regulation of MHC-1 by BPV E5 (Ashrafi et al., 2002; Marchetti et al., 2002) that helps the establishment of a successful infection not only through cell transformation but also by down regulating MHC-1, thus allowing the infected cells to evade host immune surveillance. Down
regulation of MCH 1 by BPV E5 takes place at multiple levels: transcription of MHC-1 peptide is degraded (Ashrafi et al., 2002) and the MHC-1 complex is sequestered in the Golgi cisternae and is prevented from reaching the cell surface (Marchetti et al., 2002). Retention of MHC 1 in Golgi cisterne is due, at least in part, to the E5 induced alkalinisation of the Golgi apparatus as a similar reduction of surface MHC-1 is observed in cell treated with ionophores that prevent the acidification of endo-membranes (Marchetti et al., 2002). Notably, BPV E5 down regulation of MHC-1 is observed not only in cultured cells but also in bovine papillomas (Araibi et al., 2004). MHC-1 plays a key role in immune surveillance, as it is responsible for the presentation of antigenic peptides to effector T cells. Once the heavy chain of MHC-1 associates with beta-2 microglobulin and peptides, the complex is transported from the endoplasmic reticulum through Golgi apparatus to the plasma membrane for recognition by T-cells (Cresswell, 2000). The importance of MHC in susceptibility to or protection from PVs infection and associated neoplastic diseases is supported by data from animal model as well as from clinical studies.

In most of PV infections, regression of lesions occurs following activation of the host immune response. However, several immune evasion mechanisms may contribute to persistence and progression of PV-associated diseases. Dendritic cells (DCs) induce effective immune responses after vaccination, but might also induce immune modulation during infection. Natural regression of warts is accompanied by marked infiltration of lymphocytes.

2.3.23 Immunity

Host immune response to PV infection is not well understood. In general, the young stock acquires the infection and lesions persist for variable periods after which the warts regress. The host is left immune to reinfection with the same virus type. Information available on host immune responses to BPV infection is generally limited to virus from fibro-papillomas (BPV-1 and -2). There is a wide range of susceptibility of calves to primary BPV infection, and not all calves develop mature fibro-papillomas. Tumours may regress spontaneously during any stage of development (Cheville and Olson, 1964; Lee and Olson, 1969). Calves respond to BPV infection by generating precipitating antibodies which can be detected at about 6 weeks and persist for at least 26 weeks after infection (Lee and Olson, 1969). Precipitin response of cattle to commercial wart vaccine was studied and response was promising (Barthold et al., 1976).

Multiple bovine warts usually regress on an individual animal simultaneously, but this occurs later than the development of resistance to reinfection with BPV (Lee and Olson, 1968). Regressing fibromas are infiltrated with mononuclear leukocytes, mainly lymphocytes; this occurs generally in perivascular areas but also as a diffuse scattering throughout the tumour. The intensity of infiltration is proportional to the rate of fibroma regression. The
presence of precipitating antibodies does not protect against reinfection unless the warts have undergone resolution. However, a small percentage of animals whose fibromas have regressed are still susceptible to reinfection, but the resultant lesions undergo early regression. The ability of the immune system to reject a fibroma without the concurrent rejection of fibro-papillomas presents a paradox since autologous fibro-papilloma cells cannot be transplanted (Lancaster and Olson, 1982).

Natural regression of papillomavirus induced tumours is accompanied by marked infiltration of lymphocytes. In regressing BPV-4 induced papillomas, there were marked differences in the types of lymphocytes that populated different regions of the lesions. The predominant subtype of T-lymphocyte in such papilloma was by far the CD4+ cells those were present in the sub-epithelial dermis, where they were found to form clusters. Some CD4+ cells were also located inter-digitating with the cells of the basal and supra-basal layers, but in these regions, CD8- and WCl+ lymphocytes were more prominent (Knowles et al., 1996).

Humoral immune response (HMI) studied in regressing and recovered animals by simple agar gel precipitation test (AGPT) revealed negative for precipitins in papilloma negative, progressing cases and in contact animals. The antibodies to BPV antigen prepared from the papillomatous tissue were detected in different by AGPT. The CMI response measured by 2,4 dinitro chloro-benzene (DNCB) and tuberculin tests showed less severe delayed hypersensitivity (DTH) reaction on the skin and skin thickness in wart bearing animals as compared to controls (Somvanshi et al., 1988). However it is suggested that CMI is probably effective in suppression or regression of the lesions. Regression of anogenital warts is accompanied histologically by a CD4+ T cells dominated Th response. Animal model (COP) supports this and provides evidence that the response is modulated by CD4+ T cell dependent mechanism (Coleman et al., 1994; Nicholls and Stanley, 2000; Stanley, 2008). The vaccinated calves produce high titre antibodies directed to three immune-dominant B-cell epitopes (Chandrachud et al., 1994) and developed a strong cellular immune response to two T-cell epitopes (Mc Garvie et al., 1995). Warts regression or rejection involves cell-mediated immune response, with infiltration of the site by large number of lymphocytes and macrophages (Campo 2006).


2.4 Molecular diagnosis of BPV

The DNA of Different types of BPV can be amplified selectively by a series of reactions that could lead to an exponential and reproducible increase in viral sequences
present in the biological specimen of interest, called Polymerase chain Reaction (PCR). Analysis of the amplified products (amplicons) is usually performed by Southern Blotting, restriction fragment length polymorphism and above all direct DNA sequencing. The sensitivity and specificity of PCR–based methods vary, depending mainly on the primer set, size of the PCR product, the reaction conditions efficacy of the DNA polymerase used in the reaction, the ability to detect multiple types and the availability of a type specific assay. The DNA of different types of BPV involved in bovine papillomatosis have been detected by PCR based analysis of warts (papillomata), various tissues and tissue fluids coupled with confirmation further by nucleotide sequencing of PCR amplicons or by dot blotting (Santos et al., 1998, Antonio Carlos de Frietas et al., 2003, Borzacheillo et al., 2003, Cludemir de Carvalho et al., 2003; Tsirimonaki et al., 2003, Ogawa et al., 2004; and Wosiacki et al., 2005).

To date, BPV DNA is detected by a variety of polymerase chain reaction based techniques. These PCRs are based frequently on the detection of one or two BPV types using type specific primers. A large number of PCR primers are used for the detection of papilloma viruses. Almost all the primers have been designed from HPVs and are also used for the detection of other animal PVs including BPV (Astori et al., 1998; Antonsson and Hansson, 2002; Ogawa et al., 2004; 2007). The consensus primers FAP59/FAP64 and MY09/MY11 are the most widely used primers for papilloma virus detection in bovine and other animals (Manos et al., 1989; Forslund et al., 1999; Ogawa et al., 2007). However, genotyping is performed either by real-time detection (Bogaert et al., 2007; Yuan et al., 2007), or by sequence analysis (Brandt et al., 2008) or restriction fragment length polymorphism (RFLP) analyses (Carr et al., 2001) of the generated PCR fragments. In addition, consensus primers capable of identifying potentially more than two BPV types have been reported (Borzacchiello et al., 2003). Moreover, PCR systems, designed originally for the detection of HPV, have been used to genotype different BPV types (Antonsson and Hansson, 2002; Ogawa et al., 2004). However, due to sequence diversity between HPVs and BPVs, the analytical sensitivity of these tests is definitely compromised. Furthermore, these latter methods again rely on sequencing of PCR products which is a poorly sensitive, time- and cost-insensitive procedure and thus not suitable for use in large epidemiological studies. Development of a novel multiplex Luminex based BPV genotyping assay has been reported that could detect sensitively and specifically BPV types 1-11.

Santos et al., (1998) studied chromosomal aberrations in three groups of bovines (donors, recipients and controls) and a fourth group (progeny) resulted from mating of some of the recipients. BPV2 DNA was detected in peripheral blood samples used for inoculation, as well as in the calves by Southern Blot and PCR analysis. A significant increase in the rate of chromosomal aberration was also evident.
BPV-2 DNA 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’s (Carvalho et al., 2003). Frietas et al., (2003) detected BPV-1 DNA sequences in peripheral blood samples, warts and plasma samples of spontaneous cases of cutaneous papillomatosis in female bovines and its follower, thus showing the evidence of vertical transmission. BPV-1 DNA could also be detected by in situ hybridization methods (Jelinek and Tachezy, 2005). Wosiacki et al. (2005) employed semi-nested PCR for detection of BPV-2 in urinary bladder tumour and blood from EBH affected cattle. A metagenomic method for identifying viruses, i.e. a recently developed molecular diagnostic tool- DNase SISPA, has been applied as a sensitive tool for diagnosing BPV types (Allander et al., 2001). A sequence dependent amplification like DNASE SISPA for characterizing viruses was employed for the detection and identification of BPVs from papillomatous tissues (Rai et al., 2010).

Leishangthem (2006) applied PCR, cloning and sequencing techniques for diagnosis of BPV-1 and BPV-2 in cattle and buffaloes in UP, India. Singh (2007) demonstrated BPV-2 DNA in cattle and buffalo cutaneous warts infected cattle and buffaloes. BPV-1, 2 and their mixed infections were diagnosed in cattle and buffaloes by employing PCR, (Pangty, 2009). Pangty et al., (2010) used Q PCR for molecular diagnosis and quantification of BPV-1 in natural cases of BP in certain organised dairy herds, in India.

Pathania (2010) standardized Q-PCR for molecular diagnosis and quantification of BPV-2 in urinary bladder tumour of Enzootic Bovine Haematia (EBH) cases. Rai et al., (2010) described that DNase-SISPA, a metagenomic method for identifying viruses, could identify BPV type 10 in bovine teat papillomatosis. The sequence comparison between consensus primers and BPV -10 sequences revealed many differences between consensus primers and BPV-10 sequences. The authors have opined that DNase- SISPA could be used as an alternate method for papilloma virus diagnosis, in cases where PCR failed to identify PVs.

2.5 Treatment and or therapeutic management of BP

Infectious bovine papillomatosis are generally regarded as a self-limiting disease, although the duration of warts varies considerably. A number of drugs and different therapeutic trials have been made to treat papilloma with variable results (antimony preparations- Rajguru et al., 1988; Wadhwa et al., 1992; Khasatiya et al., 2008; autogenous vaccine- Pearson et al., 1958; Theiler and Madewell, 1979; Prasad et al., 1980; Gupta et al., 1984; Wadhwa et al., 1995; Inayat etal., 1999; Lesnik et al., 1999; Vahid and Jayakumar, 1999; Venugopalan, 2000; Turk et al., 2005; Shelar et al., 2007; Sreeparvathy et al., 2011; Hamad et al., 2012; homeopathy-Soni and Parekh, 1977; Rai et al., 1991; Dighe, 1992;
Maclead, 1992; Prakash, 1993; Veena, 2001, Veena and Ravi Kumar, 2002; 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, Ramrishna and Sundaravinayakim, 2013; Ranjan et al., 2013). In most cases lesions disappear as immunity develops. Hence immunomodulation could be an approach of therapeutic management. Persistent warts and very big sized fibropapillomas usually require surgical excision. Cauterization, cryotherapy or excision is usually recommended to get rid of the warts (Valentine, 2004). Cauterization is the burning of the skin growth, used in conjunction with electric current and this mode of treatment is called electro-surgery. This process may be simple but needs adequate skill. Cryotherapy involves freezing of the warts so that those can be removed easily. Freezing of the warts, by applying liquid nitrogen and removal of the warts with forceps or metallic tweezers can also be tried. The risk of skin burns and subsequent wounds do exist in such treatment method. Stedham (1984) attempted cryosurgical approach for treating papillomas and that has been found suitable as also adopted by Olson (1993) and Smith (1996). Several treatment options like Antimony preparations (Dileep kumar and Ansari, 2012, for treating bubaline cutaneous papillomatosis), homeopathic drugs (Umadevi and Umakanthan, 2013, for treating bovine cutaneous papillomatosis), autogenous vaccines (Rao et al., 2000), for treating cattle cutaneous papillomatosis), and ivermectin (Borku et al., 2007, for treating bovine cutaneous papillomatosis) have been tried with varying degree of success. Champawat et al., (1986) reported an outbreak of papilloma in buffalo calves and successfully cured the affected animals with autogenous vaccine. Vadalia et al., (2013) successfully treated fifteen BP affected animals with autogenous vaccine prepared in 70% alcohol. Pattar and Priyanka (2013) adopted autogenous vaccination and immunomodulation for the management of cutaneous papillomatosis in a crossbred cow.

A variety of treatments have been used to treat bovine papilloma (cattle warts/papillomata) without agreement on treatment efficacy (Cimtay et.al., 1994; Hemmatzadeh et al., 2003). In human medicine, the homeopathic doctors, usually used some homeopathic preparations like Thuja, causticum, antin crud etc. along with sulphur for the tumours (Dighe, 1992; Prakash, 1993; Veena, 2001) and claimed complete recovery of the disease. Homeopathic treatment takes some time to exert its effect but the results can be observed (Shakoor et al., 2012). Somvanshi and Sharma (1986) stated that there was no effective allopathic veterinary medicine available for warts management and or its effective therapeutics. Kumar et al., (1987) had successfully used Thuja to treat bovine papillomatosis.
Hossain et al., (2005) attempted homeopathic remedy of warts in cattle. Thuja and antim crud with concurrent use of sulphur were effective in curing bovine cutaneous papillomatosis with a rate of 66.66%. Shakoor et al. (2012) studied the effects of *Thuja occidentalis* as an alternative remedy in the treatment of papillomatosis in cattle.

Wadhwa et al., (1995) used antimony preparations for treating bovine cutaneous warts with varying degree of success. Kavithaa et al., (2014) studied three different regimen of treatment for treating papillomatosis in Jersey cows and got varying results, where auto-haemotherapy was found to be most effective with a cure 92% followed by anthiomaline (81%), oral administration of Thuja extract (70%) and topical application of Thuja ointment. Cihan et al (2004) studied the effects of levamisole on clinical cure of bovine cutaneous papillomatosis and observed 100% clinical recovery of cattle in Kars-Turkey. Borku et al., (2007) found Ivermectin as an effective agent for the treatment of bovine cutaneous papillomatosis. Veiga et al., (2000) evaluated a chemical treatment for cutaneous bovine papillomatosis with daily application of the chemical product (54% calcium carbonate and 11% formaldehyde in an oil base) on the lesions.

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). There seems paucity of reports available on use of auto-hemotherapy in veterinary practice. For treating bovine cutaneous papillomatosis has been gaining importance. Autohemotherapy was reported to cause complete cure of papillomatosis in cattle (Pattanayak, 2004; Chelapati Rao, 2010; Kumar, 2011) and in buffalo (Jana and Jana, 2009). Hali et al., 2003) treated bovine papilloma with a combination of auto-hemotherapy and autogenous vaccine within a period of 1.5-2 months.

The observation that the immune response to BPV infection is poor; a prompt and prolonged immune response is elicited when animals are immunized with BPV proteins has led to the successful development of anti- BPV vaccines. Prophylactic vaccination was achieved with BPV -1virions and virus neutralizing antibodies were elicited by vaccination with BPV-1 L1 protein (Campo, 1997a). Autogenous vaccination is effective in cutaneous papillomatosis (Rao et al., 2000, Inayat et al., 1999, Wadhwa et al., 1995).

Autogenous wart vaccine used for therapeutic purpose gave good results and cured the clinical cases of BP. Commercial vaccines are available and if used as directed; they may help prevention of warts in cattle. Autogenous vaccines are usually prepared from chemically treated warts taken from the infected animals in the same herd. In fact, autogenous vaccine is more apt to have the strain or type of PV causing the wart problem in a herd than some of the commercial vaccines. Commercial wart vaccines have been tried with favourable results in horses and cattle. Foss (1959) obtained better results by injecting autogenous wart vaccine
than by using commercial vaccine. It has been reported that the efficacy of wart vaccine is uncertain when lesions are extensive and the conditions are of long duration. The growths, however, showed tendency to crack and they become slightly greyish in colour after the fourth injection.

Chandran (1961) made a trial on wart affected Sindhi calves with the use of autogenous vaccine, in the clinically affected Sindhi herd stationed at the District Livestock Farm, Trichur and obtained favourable response to the vaccine therapy. Singh and Christopher (1976) reported a clinical case of warts in a buffalo and its clinical cure by use of formalinised autogenous vaccine therapy. Ghaffer et al., (1979) obtained 84% recovery rate in naturally infected cases of 120 Friesian and 4 buffalo calves by using autogenous vaccines prepared from infective wart tissues in buffered glycerol with penicillin and streptomycin or in bovine serum and saline merthiolate. Subcutaneous injection of either vaccine inoculated 17 days apart for two sorts gave favourable response but merthiolate gave better results.

Commercial vaccines for cattle rarely seem to effectively promote regression of existing warts or to prevent malignant progression, 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). Autogenous wart vaccine has been successfully used both in sessile and pedunculated warts in cattle (Theilen and Madewell, 1987; Blood and Radostits, 1989). It activates the immune system of the body and hastens the clinical cure and prevents further occurrence of the disease. A formalinized suspension of bovine warts with inactivated virus provides a vaccine for effective treatment and prophylaxis of bovine papillomatosis (Lesnik et al., 1999; Suveges and Schmidt, 2003). Intra-lesional immune-therapy has also been reported (Hall et al., 1994). Sreeparvathy et al., (2011) claimed autogenous vaccination as an effective treatment method for bovine papillomatosis. Vadalia et al., (2013) stated autogenous vaccination for successful treatment method for infectious bovine papillomatosis. Makay (1990) used herd specific inactivated formalinized vaccine and administered on neck region subcutaneously. He observed regression of warts after four to six weeks post inoculation and clinical cure by 14-16 weeks. Ndorathi and Mbuthia (1994) observed complete regression in animals after 2-4 months of inoculation, only in those from which vaccine was prepared, using individual bovine specific and species specific autogenous vaccine. Suveges and Schmidt (2003) used formalin treated sterile homogenizatum for the treatment and prevention of BP made from cutaneous warts excised from the infected animals. Treatment with vaccine showed 93.5% efficiency, 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). Autogenous vaccine inactivated in 70% alcohol has also been tried for successful treatment of CCWT (Vadalia et

Successful treatment of papillomatosis has been a great challenge for field Veterinarians and practitioners. Although different methods have been used to treat bovine papillomae, effective medicines for wart are not truly determined. The aim of this study was treatment of papillomatosis by different methods and different therapeutic agents and to determine better therapeutic options.