Indian J. Virol. (October–December 2013) 24(3):332–341
DOI 10.1007/s13337-013-0165-9

REVIEW ARTICLE

Bovine immunodeficiency virus: a lentiviral infection

Sandeep Bhatia · S. S. Patil · R. Sood

Received: 6 February 2013 / Accepted: 16 September 2013 / Published online: 27 September 2013

Abstract The bovine immunodeficiency virus (BIV) is a lentivirus which is known to infect cattle worldwide. Though serological and genomic evidence of BIV in cattle has been found throughout the world, isolation of the virus has been reported only from few places. Very little is known about its impact on animal health status, pathogenesis and mode of transmission. BIV is considered generally non-pathogenic and is not known to cause any serious disease in cattle. BIV is genetically and antigenically related to Jembrana disease virus (JDV), the cause of an acute disease in Bali cattle (*Bos javanicus*) and human immunodeficiency virus, the cause of acquired immunodeficiency syndrome in human. Therefore, it is important to monitor the presence of BIV in cattle to keep vigil over its possible evolution in its natural host to emerge as pathogenic lentivirus like JDV. Differentiation of BIV infection in cattle from the acutely pathogenic JDV is important for diagnosis of the latter. Currently, BIV is considered as a safe model for understanding the complex genome of lentiviruses. Further research on BIV is indeed needed to elucidate its possible role in animal health as well as for insight into the molecular mechanisms adopted by related lentiviruses.

Keywords Bovine immunodeficiency virus · Lentiviruses · Molecular biology

S. Bhatia (✉) · R. Sood
High Security Animal Disease Laboratory (HSADL), Indian Veterinary Research Institute (IVRI), Bhopal, India
e-mail: sandeep.bhatia@hsadl.in

S. S. Patil
Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS), Bengaluru, India

Introduction

The bovine immunodeficiency virus (BIV) belongs to the lentivirus genus of the subfamily *Orthoretrovirinae* under *Retroviridae* family [50]. The members of *Retroviridae* family are characterized by the expression of a unique enzyme reverse transcriptase (RT). The RT enzyme facilitates the transcription of the RNA of the infectious virus to a complimentary DNA copy which is incorporated in the host's cell nucleus as a 'provirus'. The provirus remains latent for many years without doing any harm to the host. In presence of pre-disposing factors such as concurrent infection, stress or age, the provirus may get reactivated into infectious RNA virus and may initiate pathogenesis inside host. The BIV causes a persistent viral infection in cattle and buffalo. Infection with BIV has never been linked to a specific disease or clinically identifiable syndrome, but it has been associated with lymphadenopathy, lymphocytosis, central nervous system lesions, progressive weakness [22, 88], decreased milk yield [60], decreased lymphocytic blastogenic response [59] and bovine paraplegic syndrome [90]. Though enough experimental evidences are available to believe that BIV can cause immune dysfunction in animals making them vulnerable to secondary infections [22, 33, 59], the significance of natural BIV infection to health of cattle has not been clearly established. Interestingly, a closely related bovine lentivirus—Jembrana disease virus (JDV) is known to cause acute disease in Bali cattle and can not be differentiated serologically with currently available immunodiagnostic methods [52].

Presence of BIV infection among cattle and buffaloes in India has been reported on the basis of genomic [73] as well as serological detections [11–14]. Several other countries have reported BIV infection in cattle viz.

Southwest USA [15], Canada [60], Germany [67], Japan [47], Italy [24], Australia [19], Korea [26], Pakistan [62], Brazil [63] and Zambia [64]. The non-pathogenic nature of BIV, despite its close genetic and antigenic similarity with pathogenic lentiviruses like JDV and human immunodeficiency virus (HIV), is an interesting feature which makes this virus a good model for lentiviral research especially for understanding the pathogenesis and evaluating methods for effective treatment and control of pathogenic lentiviruses [34, 36]. This review focuses on the biological and molecular properties of BIV and its role in animal health system.

Historical background

BIV was first isolated in 1969 in Lousiana, USA from a Holstein cow with clinical signs of mild persistent lymphocytosis, generalized hyperplasia of lymph nodes, central nervous system lesions, weakness, and emaciation [88]. Histological examination of the tissues from the dead animal revealed a generalized follicular hyperplasia of lymph nodes and perivascular cuffing of the brain. The isolated virus induced formation of syncytia in cell cultures and was structurally similar to maedi-visna virus, hence designated as 'bovine visna-like virus.' Because this bovine lentivirus was not considered as the causative agent of leukemia/lymphosarcoma, its biology went unstudied for nearly a decade and half after its initial discovery until HIV was discovered in 1983 [7]. Twenty years later, it was demonstrated that the bovine R-29 isolate was a lentivirus which was very similar to the human immunodeficiency virus [37–39]. BIV was named on the basis of its morphologic, serologic and genetic features similar to the HIV and simian immunodeficiency viruses (SIV). The other two retroviruses discovered were bovine syncytial virus, a foamy virus or spumavirus; and bovine leukemia virus (BLV), an oncovirus [57, 65]. Generation and characterization of infectious cDNA clones BIV106 and BIV127 derived from R-29 isolate [18, 35] led to extensive studies on molecular biology of BIV. Two additional BIV field strains, termed FL491 and FL112, were isolated which were associated with the development of leukocytosis [84]. However, most pathological, serological and molecular biology information has been obtained from studies with the original BIV R-29 isolate.

Relationship with other lentiviruses

Lentiviruses that share structural, genetic, biological and/or pathological properties include maedi-visna virus (MVV) in sheep, caprine arthritis-encephalitis virus (CAEV) in goats, equine infectious anemia virus (EIAV) in horses, JDV and BIV in cattle, feline immunodeficiency virus (FIV) in cats, SIV and HIV in primates. Lentiviruses, which are not oncogenic, induce slow, chronic and degenerative pathological changes in infected hosts, often associated with the development of immune-mediated lesions [32]. All lentiviruses infect monocyte/macrophage cells. Moreover, FIV, SIV and HIV infect T cells and, consequently, are mainly associated with clinical signs of immunodeficiency in the infected hosts [3, 25, 39, 53, 87]. In contrast to the other retroviruses, lentiviruses may replicate in non-dividing cells. In addition, the lentivirus genome offers a complex structure including several regulatory/accessory genes that encode proteins, some of which are involved in the regulation of virus gene expression. HIV, the causative agent of the human acquired immune deficiency syndrome (AIDS), is the most studied lentivirus. Other lentiviruses, including BIV, may constitute alternative surrogate animal models for certain aspects of HIV research. The serological relationship of BIV with other lentiviruses (HIV-1, EIAV and SIV) has been studied in detail by Battles and co-workers [8]. In this study, it has been shown in Western blot analysis that BIV antiserum and capsid antigen cross-react with corresponding capsid antigen and anti-sera, respectively, for EIAV, SIV, and HIV-1. Amino acid alignment of the predicted sequence of capsid proteins of BIV, SIV, EIAV and HIV-1, revealed a highly conserved domain spanning 10 amino acids (p10). Through immunoprecipitation of HIV-1 p24 and BIV p26, p23 and p10 proteins with antiserum prepared against a 20 amino acid stretch in BIV capsid protein, it was shown that cross-reactivity among capsid proteins of BIV, HIV-1, EIAV and SIV is due to p10.

Genomic structure and function

BIV has the most complex genome organization among the lentiviruses with several regulatory genes involved in the regulation of the gene expression. Molecular cloning and sequencing of the proviruses from BIV-infected cells have been used to develop a complete genetic map of BIV [18, 35]. The deduced genetic complexity of BIV has been substantiated by Northen blotting and cDNA experiments used to characterize viral transcripts [56, 69, 70, 72]. Based on these experiments and the genetic map of BIV, Gonda et al. [41] described the genomic organization of BIV (Fig. 1a). As per their description, the virus particle contains two copies of a single stranded RNA genome (dimer), similar to other retroviruses. The linear genome of BIV contains 8,960 base pairs in the form of proviral DNA which comprises of the obligatory retrovirus structural genes '*gag*', '*pol*' and '*env*', flanked on the 5′ and 3′ ends by a complete copy of a long terminal repeat (LTR). The

LTRs contain the promoters, enhancers and terminators of transcription. BIV also contains the complex lentivirus 'central region' between and overlapping the *pol* and *env* reading frames. The central region of BIV genome contains coding exons of several putative non-structural accessory genes including vif (viral infectivity factor), tat (trans-activator of transcription), rev (regulator of virus expression), vpw, vpy and tmx. The products of the accessory genes 'vif' and 'Tat' as well as those of the structural genes '*gag*' '*pol*' and '*env*' of BIV have some sequence similarity to their counterparts in HIV 1. However, the genomes of BIV and HIV-1 show overall divergence, with the '*gag*' and '*pol*' ORFs having the greatest sequence similarity.

The Tat protein is a non-structural regulatory protein of BIV. Tat gene of BIV encodes for Tat protein that basically enhances the level of viral RNA transcription. Two exons code for Tat protein, first is coded by an exon 5′ of the envelop gene from nucleotides (nt) 5228 to 5536 and the second is encoded by an exon located within the envelop gene from nt 7657 to 7782. In a similar way, JDV-Tat is also coded by two exons i.e., exon Tat-1 and exon Tat-2. The sequences of Tat-2 were completely conserved than that of Tat-1 sequences [77]. JDV-Tat protein binds to BIV-TAR with higher affinity than BIV-Tat peptide itself [21]. A new BIV-Tat236 and long terminal repeats (LTRn) protein expressed by variant of BIV has been reported [29]. The variant BIV-LTRn has three nucleic acid mutations at positions −194, −135 and −114 upon comparison with the wild type BIV. Such LTRn promotes a higher Tat mediated trans-activation. The Tat protein of BIV, after expression in host cells, binds to a viral RNA stem loop structure called trans-activating response element (TAR) which is located at 5′ ends of BIV transcripts. In association with positive transcription elongation factor (P-TEFb), Tat enhances the production of full length viral RNA [56]. It was also reported that BIV-Tat has a role in proapoptotic action of BIV in inducing apoptotic cell death which may be related in causing cytopathic effect in cell culture. BIV-Tat also regulates microtubule dynamics in host cells [95]. Internalization of BIV-Tat in infected cells helps BIV in influencing neighboring cells and makes the cellular environment conducive to viral replication [31]. Functions of BIV-Tat have been exploited for analysis of the TNRC6B protein, a component of the microRNA induced silencing complex in cell multiplication [89]. The knock-down of bovine hexamethylene bisacetamide (HMBA)-

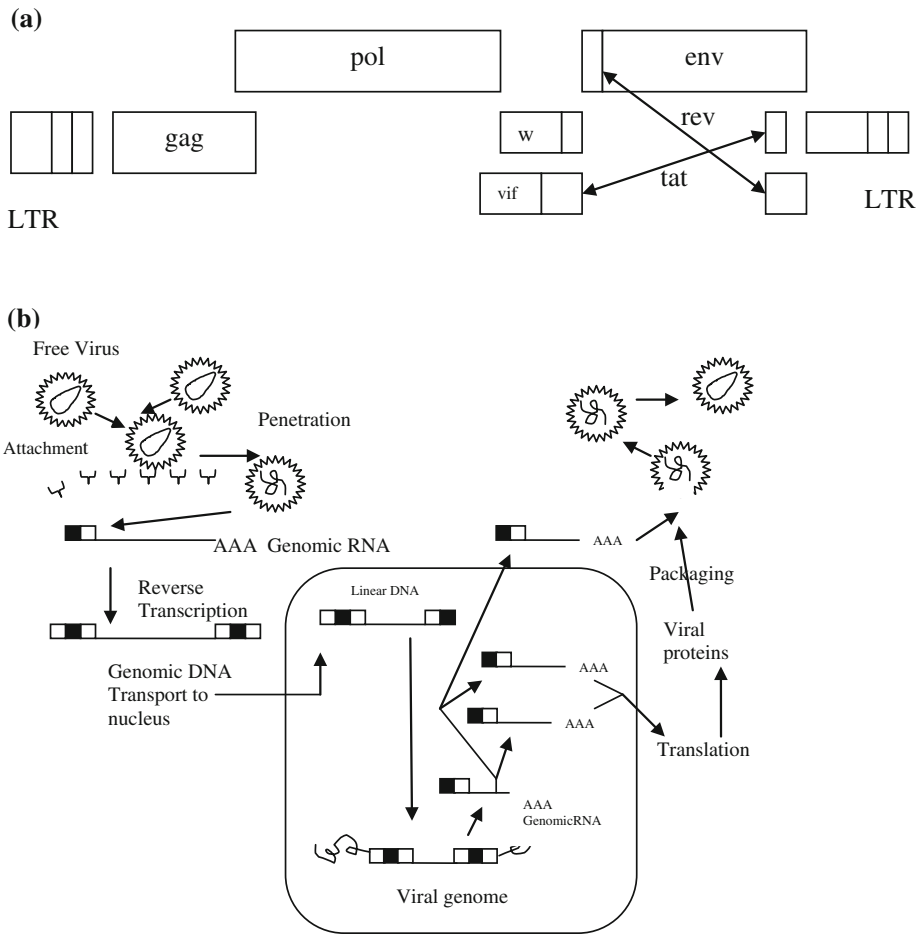

Fig. 1 **a** BIV genome organization **b** BIV infection cycle. (Reconstructed from [40])

induced protein (BHEXIM1) enhances BIV replication and it competes with BIV-Tat by binding to B-cyclin T1 [45]. This information may help in understanding the BIV latent life cycle. The molecular research work on BIV-Tat-TAR is exploited towards either for up regulation or for inhibitory purpose of the HIV-1 TAR [5].

BIV infection cycle

BIV infection cycle (Fig. 1b) has been described by Gonda and Oberste [40]. The BIV genome consists of two positive-sense, single-stranded, protein-encapsidated RNA genomes. During the infection cycle, free BIV particles attach to specific cell surface receptors via the envelop glycoprotein of the virus. Subsequently, the viral envelop fuses with the plasma membrane releasing genomic RNA and mature *pol* gene products from the core of the virus into the cytoplasm. The viral RT transcribes the viral RNA into double-stranded DNA which is then transported to the nucleus where it is incorporated into the host genome with the help of integrase (IN) enzyme. The integrated provirus remains transcriptionally silent until appropriate cellular signals activate gene expression from the viral LTR. Cell-mediated expression from the viral LTR is significantly enhanced by the action of the virally coded Tat protein. Splicing of the primary genome-length viral mRNA into sub-genomic messages and transport to the cytoplasm is carried out by the cellular splicing machinery and another virally encoded protein, 'Rev' (regulator of virus expression). Sub-genomic mRNAs are translated on the ribosomes in the cytoplasm of the infected cell. Viral precursors for *gag* (group specific antigen) and *gag-pol* assemble beneath the plasma membrane and incorporate viral genomic RNA during the process of budding. The viral envelop is studded with surface (SU) and transmembrane (TM) glycoprotein. Following release, *gag*-related precursors in the immature particle are cleaved into their functional subunits by the viral protease (PR) as the virus undergoes morphogenesis into a mature infectious particle. The mature particle can begin the infection cycle again by binding to a naïve cell expressing the appropriate receptor for BIV.

Genetic diversity in BIV

Lentiviruses exhibit antigenic variations to evade immune surveillance and thereby replicate fast to produce more number of viruses. Genomic diversity in lentiviruses including BIV is attributed to mutations, recombination events and selective pressure that act on viruses during replication [17, 20, 58, 86]. Lentiviral persistency in host cells is another mechanism to survive for longer period. There are many reports on study of variation and selected stability in certain genomic regions of BIV during its life cycle. A limited genetic variation has been reported during long term persistency in host cells upon comparison of *env* sequences of BIV isolated at 4–5 years post infection [23].

Genetic variation is reported largely in regions of *pol* and *env* sequences [30, 61, 81, 83, 85]. Suarez and co-workers [85] compared nucleotide sequences of RT region of *pol* gene of thirteen BIV isolates which were shown to have up to 10 and 11 % divergence in nucleotide and amino acid homology respectively. There was no size variation in the RT region of all isolates. The sequence variation in the RT gene region was found to be uniform [44, 54]. The conserved regions in the RT domain of *pol* gene have maintained the identity of BIV when it was diverging from HIV-1. The proportion of replacement substitutions remained same in the RT domain and about 85 % of the fixed replacement differences were critical in evolutionary development of the BIV. It has been hypothesized that the existence of BIV *pol* quasispecies is due to presence of non-conservative amino acid changes [30].

Surface (SU) envelope protein is more prone for genetic variation and any change in this protein can alter the cell tropism [28, 74]. Size variation in the SU protein of BIV is a common phenomenon due to recombination events [81, 83]. Size variable viruses do have advantages in antigenicity and multiplicity of BIV [55]. A 5 % sequence divergence was observed upon comparison of SU gene sequences of R-29 and R-29 derived isolates. The sequence variations due to nine amino acid changes were found to be spread throughout the SU gene wherein seven of nine amino acid changes were in the conserved region. The conserved region of the SU gene was recognized as the region larger than 12 aa that had less than 10 % sequence divergence from the consensus. The hypervariable region was defined as the region larger than 12 aa with greater than 30 % sequence divergence from the consensus. Six conserved and hypervariable regions in SU genomic regions have been identified [66, 79]. Five conserved sites were identified in N-glycosylation sites of different BIV isolates. Large size differences of 104 nucleotides in SU gene were reported among BIV isolates being highest in V2, V4 and V6 hypervariable regions. Limited information on variations in other genomic regions of BIV is available. A hybrid Tat236 of Tat protein of BIV was characterized which contained the first 98 amino acids of Tat103 and 3′ end 138 amino acids of *Rev* of BIV variant which had higher trans-activation properties [80].

Pathogenesis of BIV

Like other lentiviruses, BIV infect cells of the immune system, primarily monocytes/macrophages and lymphocytes

in vivo [39]. The in vitro tropism of BIV is quite broad. BIV replicates in fibroblast like cells and is cytopathic in most, causing syncytia and cell death [41]. Productive infections have been established in primary cultures of embryonic bovine spleen, brain, lung, choroid plexus, testes, thymus, kidney, and synovial membrane [38]. In addition, established canine thymus (Cf2Th), embryonic rabbit epithelium (EREp) and various other bovine cell lines have been used for infecting the virus [36, 40, 42]. Only Cf2Th cell line is known to sustain a long-term productive infection [16, 36, 42]. In peripheral blood mononuclear cells (PBMC) collected from naturally infected animals, BIV has been shown to infect and transcribe its genome in different subsets of cells—CD3+, CD4+, CD8+ and γδ-T cells, B cells and monocytes [93, 94].

BIV is pathologically more related to lentiviruses that cause chronic inflammatory diseases (CAEV and EIAV) as compared to those that cause severe immunodeficiency (HIV, FIV and SIV). However, unlike CAEV and EIAV which are known to cause clinically identifiable disease in the susceptible hosts, it has not been clearly established whether natural infection of BIV causes any significant effect on health of cattle even though experimental evidences suggest that BIV can cause immune dysfunction and can predispose animals to secondary infections. Earliest evidence of BIV infection being cause of immunodeficiency in cattle comes from a long term study (more than 7 years) at Louisiana State University dairy herd which had high seroprevalence of BIV [78] and had high incidence of common diseases that reduced the economic viability of the dairy. The herd had a high percentage of cows with encephalitis associated with depression and stupor, alteration of the immune system associated with secondary bacterial infections, and chronic inflammatory lesions of the feet and legs. Histological examination of brain tissue from BIV-infected animals in this herd revealed a non-suppurative perivascular cuffing indicative of viral meningo-encephalitis. These central nervous system lesions were similar to those described earlier by Van Der Maaten et al. [88] and could not be attributed to other viral infections. Later on, studies were conducted by various groups to elucidate the pathogenesis of BIV in cattle. In several studies, experimental infection of calves with BIV R-29 isolates has demonstrated a transient lymphocytosis and lymphadenopathy without any overt clinical signs [22, 71, 84, 88]. Investigations on immune dysfunction have been carried out in animals experimentally infected with BIV. Onuma and co-workers [71] have shown that BIV infection of cattle reduces the responsiveness of various important monocyte functions without a change in CD4/CD8 ratios. Other studies examining the effect of BIV infection on immune function have demonstrated either mild or no immunosuppression on the basis of lymphocyte blastogenesis tests, neutrophil function tests, mononuclear subset analysis, and histopathological changes [22, 33, 59]. In another important study, Zhang and co-workers [96] observed a decrease in CD4/CD8 ratio and an overall increased lymphocyte proliferation 2–6 weeks post-infection in calves inoculated with BIV, suggesting a possible immune dysfunction in BIV-infected calves. The antibody response to BHV-1 and bovine viral diarrhea vaccine was significantly lower in BIV-infected calves than in uninfected controls.

Immune response to BIV in cattle

Humoral immune response

A number of studies have characterized the humoral immune response to BIV in naturally as well as experimentally infected cattle. In one such significant study, virus-specific antibodies could be detected in calves inoculated with BIV R-29 strain as early as 2 weeks post-inoculation (PI) and remained for about 2–2.5 years PI [92]. As detected by western blot, they have shown that first serum antibody response was against the p26 protein followed by gp110 (SU or surface part of envelop glycoprotein), p55 (*gag-pol* precursor polyprotein), gp42 (TM or transmembrane part of envelop glycoprotein), p18 (MA or matrix part of *gag*) and p13 (NC or nucleocapsid part of *gag*). Results from this study and other studies have shown that p26 is the most immunodominant protein of BIV and in experimentally infected calves antibodies to p26 can be detected as early as 2 weeks PI and can last for 2 years. However, the antibodies to p26 were observed decreasing after 1.5 years after experimental infection of animals by BIV. In contrast, antibodies to the *env*-encoded TM protein appeared later than p26 protein and persisted for more than 3.5 or 4 years in animals exposed to BIV [1, 2, 51]. It has also been shown that immune sera from animals infected with other bovine viruses like BVDV and BLV do not react with BIV p26 antigen in Western blot indicating the specificity of p26 for anti-BIV antibodies [99]. Due to specificity of p26, most of the serological tests for BIV like Western blotting, indirect fluorescent antibody technique (IFAT) and indirect ELISA have used this protein as antigen for detection of antibodies in cattle.

Cellular immune response

The cell-mediated immunity to BIV has not been studied in great detail. However, there are a number of studies on effect of BIV infection (natural or experimental) on T cell responses to mitogens and other viral agents. A significant increase in specific lymphocyte proliferation to BIV

antigen (*gag*) was demonstrated in BIV-infected calves from 2 to 6 weeks post-infection (PI) while there was no increase in lymphocyte proliferation to mitogens and to BHV-1 and BVDV viral antigens. The CD4/CD8 ratio also declined during 2–7 weeks PI indicating a possible immune dysfunction in BIV infected calves [96]. The reduction of in vitro lymphoproliferative responses to specific antigens or to mitogens (phytohemagglutinin, concanavalin A (Con A) and pokeweed mitogen) was demonstrated with mononuclear cells isolated from cattle [59] or sheep [47] experimentally exposed to BIV.

Diagnosis

Virus isolation

Virus isolation is considered as the 'gold standard' laboratory test for diagnosis of many viral pathogens. Isolation of BIV, especially from naturally infected animals, has been difficult and there have been only four successful isolations till date. The first reported isolation of BIV was from a cow with persistent lymphocytosis and was named as R-29 [88]. The other three were BIVCR1 from Costa Rica [46] and FL491 and FL112 from Florida, USA [84]. All the four isolates used co-cultivation techniques using PBMC from infected animal with either fetal bovine spleen cells, fetal bovine lung cells or embryonic rabbit embryonic cells [84]. Due to the difficulties in isolation, molecular and serological methods have been adopted routinely for diagnosis of BIV.

Molecular diagnosis

Diagnosis of BIV by PCR is considered a reliable method to detect infected cattle [82]. Sensitive PCR diagnostic methods have been developed for the detection of proviral BIV DNA in mononuclear cells [68, 85, 97]. A nested PCR targeting two separate regions of *pol* and *env* was developed and was found to have a greater sensitivity than serology and virus isolation [85]. However, it has been acknowledged by authors that there is no gold standard for BIV testing at present [83]. They reported that their *env* primers had the broadest specificity of all those tested, making them the most appropriate for the testing of field samples. In another study, the *pol* primers were found to be 30- to 100-fold more sensitive than the *env* primers and proved to be the most useful for BIV detection in the experimentally infected bulls [43]. In India, primers specific to *gag* region of BIV genome were used to detect BIV in blood samples and milk samples. Though, the sensitivity and specificity of this PCR was not estimated, all the positive samples (10) were found to be specific to BIV by semi-nested PCR and restriction analysis [73].

Serological diagnosis

For serological studies, the R-29 isolate of BIV was used most widely as a source of antigen before the availability of recombinant BIV antigens till 1999 [4, 15, 27, 48, 49, 92]. It was speculated that the exclusive use of BIV R-29 antigen in serological screening may not detect the seropositivity in animals infected with serologically distinct BIV-variants [8, 35]. Use of recombinant viral proteins in place of native viral proteins facilitated to study sero-epidemiology of BIV infection [2, 10, 98]. Indirect ELISA based on recombinant capsid protein [98] or baculovirus-expressed transmembrane protein [1] have been in use for sero-diagnosis. The '*gag*' gene of BIV has been cloned into a baculovirus expression system [76] and bacterial systems as fusion protein [6, 10, 98]. The bacterial part of the fusion protein in the TrPE system accounted for 50 % of the total, which created problem in ELISA [98]. The recombinant capsid protein has been used to perform Western blot and indirect ELISA for detection of serum antibodies against BIV [98]. Since then, many workers have used recombinant capsid protein of BIV as antigen for serological detection of BIV infection.

In India, a recombinant capsid (p26) protein based indirect ELISA was standardized to test sera of cattle and buffalo for carrying out sero-surveillance of BIV in India [11]. Production of monoclonal antibodies (MAbs) and recombinant antibodies against capsid protein has been reported from India [13, 14]. Elsewhere in the World, MAbs to BIV *gag* proteins have been developed and were found to react specifically with the BIV p26 and recombinant capsid protein in Western immunoblot assay and were found useful in detecting BIV replication in cell culture [91]. Competitive binding assays, using anti-capsid MAbs, demonstrated the existence of at least 3 distinct antigenic determinants on the capsid protein and one of the MAbs differentiated the capsid protein of JDV and BIV indicating that at least one epitope is unique in BIV capsid from JDV capsid [99].

Sero-epidemiology of BIV

Sero-epidemiological data on BIV suggest that the presence of BIV infection is worldwide. Due to lack of BIV isolates and difficulties in producing large amount of BIV antigen, only few countries could under take serological screening of BIV till late 1990s. Later, development of recombinant BIV antigen facilitated the seroprevalence studies worldwide. Expression of BIV capsid p26 protein in bacterial system and use of recombinant p26 protein in Western blot provided an easier way to study sero-epidemiology of BIV infection [10]. After that, many European

and Asian countries took up seroprevalence studies on BIV infection. Some of the important seroprevalence studies are described below.

Serologic screening of randomly selected cattle sera using R-29 as a source of antigen has shown a non-uniform distribution in the USA [4, 15, 27, 92]. From southern and south western part of USA approximately 4 % of cattle sera were positive [13] while cattle sera from eastern or north eastern part of USA were rarely positive [48]. A chemiluminiscence Western blot assay detected anti-BIV antibodies in sera of 5.5 % of 928 adult cows from Ontario [60]. In Germany, serum samples from 6.6 % of 380 cattle were positive for presence of anti-BIV antibodies by cell-ELISA and immunofluorescence assay [67]. In France, a recombinant 53 kDa BIV R-29 antigen was used which gave weaker reaction with French sera than with positives from Louisiana indicating the occurrence of distinct French and Louisiana BIV variant [75]. In Hokkaido, Japan, the seroprevalence of BIV up to 7.5 % in 120 cattle with relatively higher prevalence of BLV (Bovine Leukemia Virus) has been reported [47]. Apart from these reports, serological evidence of BIV infection has been reported from Italy [24], Australia [19], Korea [26], Brazil [63], Zambia [64] and Pakistan [62].

Status of BIV infection in India

The status of BIV infection in Indian cattle was not known till the year 2000. The first evidence of BIV infection in cattle in India was reported in the year 2003 through detection of BIV through PCR [73]. The nucleotide sequence of three PCR amplicons (p26 region) matched with the reference strain (R-29) with 96–97 % homology. Later on, with development of recombinant capsid based ELISAs, seroprevalence studies were taken up to test large number of animals [11–14]. Out of the 672 animals tested by capsid based indirect ELISA, 162 were positive and 510 were negative, giving an overall prevalence of 24 % in India [11]. In a subsequent study in India, a MAb based competitive inhibition ELISA showed much higher agreement (concordance—95.4 %) than the indirect ELISA (concordance- 77.8 %) with western blot [13]. In a further study, a recombinant antibody as ScFv (Single chain Fragment variable) protein molecule was generated against the recombinant capsid protein of BIV and was shown to react specifically with the antigen. The anti-capsid recombinant antibody was used in a competitive inhibition ELISA for detection of BIV antibodies and was found to be more sensitive than the MAb-based ELISA [14]. Looking at the international scenario and worldwide presence of BIV in cattle, it is no surprise that cattle in India are also carrying this infection. Though intermittent monitoring of BIV infection in cattle may be carried out to check any possible changes in the virus, continuous surveillance may not be required as BIV is not considered a serious threat to cattle health.

Conclusions

At present research on BIV is being carried out in two directions. First is the molecular biology study of the virus to unravel the role of various viral genes in regulation of its replication. Secondly, BIV infection in cattle is being studied for its possible role in causing immune dysfunction. Except few studies on effect of BIV infection on immune system, not much experimental work has been carried out in this field probably because of less number of virus isolates available in the world.

The basic studies on BIV are aimed at identifying the parts of the genome of BIV which differ from its more pathogenic but closely related lentiviruses such as HIV. Since there are no safety issues with BIV, it is also a good model for studying lentiviruses. Lentiviruses have the potential to be utilized in gene therapy as they can infect non-dividing cells. Thus far, the lentiviruses used have been primate viruses that may possess the potential to cause disease in humans. As a non-primate virus, BIV does not have this potential and so may represent a safer candidate for gene therapy. BIV has been found to transduce a variety of cells from a variety of organisms [9]. Thus BIV has the potential to be used as research tool and a good and safe model to study lentiviruses. While its role in cattle health does not seem to be serious, its presence in this host cannot be ignored and needs to be observed.

References

1. Abed Y, Archambault D. A viral transmembrane recombinant protein-based enzyme-linked immunosorbent assay for the detection of bovine immunodeficiency virus infection. J Virol Methods. 2000;85:109–16.
2. Abed Y, St-Laurent G, Zhang H, Jacobs RM, Archambault D. Development of a Western blot assay for detection of bovine immunodeficiency-like virus using capsid and transmembrane envelope proteins expressed from recombinant baculovirus. Clin Diagn Lab Immunol. 1999;6:168–72.
3. Agnarsdóttir G, Thorsteinsdottir H, Oskarsson T, Haflidadottir B, Andresson OS, Andresdottir V. The long terminal repeat is a determinant of cell tropism of maedi-visna virus. J Gen Virol. 2000;81:1901–5.
4. Ambroski GF, Lo JL, Seger CL. Serological detection of multiple retroviral infection in cattle bovine leukemia virus bovine syncytial virus and bovine visna virus. Vet Microbiol. 1989;20:247–53.
5. Athanassiou Z, Patora K, Dias RL, Moehle K, Robinson JA, Varani G. Structure-guided peptidomimetic design leads to nanomolar beta-hairpin inhibitors of the Tat-TAR interaction of bovine immunodeficiency virus. Biochemistry. 2007;46(3):741–51.

6. Atkinson B, Liu ZQ, Wood C. Use of bacterial *trpE* fusion vectors to express and characterize the bovine immunodeficiency like virus core protein. J Virol Methods. 1992;36:35–9.
7. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamarand S, Gruest J, Dauguet C, Axler-Blin C, Vezinand-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 1983;220:868–71.
8. Battles JK, Hu MY, Rasmussen L, Tobin GJ, Gonda MA. Immunological characterization of the *gag* gene products of bovine immunodeficiency virus. J Virol. 1992;66:6868–77.
9. Berkowitz R, Ilves H, Lin WY, Eckert K, Coward A, Tamaki S, Veres G, Plavec I. Construction and molecular analysis of gene transfer systems derived from bovine immunodeficiency virus. J Virol. 2001;75(7):3371–82.
10. Betemps D, Mallet F, Cheynet V, Baron T. Over expression and purification of an immunologically reactive His BIV capsid fusion protein. Protein Expr Purif. 1999;15(3):258–64.
11. Bhatia S, Bhatia AK, Sood R, Pattnaik B, Pradhan HK. Serological evidence of bovine immunodeficiency virus infection in cattle and buffalo through use of recombinant capsid (P26) protein based immunoassay. J Immunol Immunopathol. 2006;8(2):128–9.
12. Bhatia S, Patil SS, Sood R, Dubey R, Bhatia AK, Pattnaik B, Pradhan HK. Prokaryotic expression of a 750-bp capsid region of Bovine Immunodeficiency Virus *gag* gene and development of a recombinant capsid (p26) protein based immunoassay for seroprevalence studies. Indian J Biotechnol. 2008;7(1):50–5.
13. Bhatia S, Sood R, Bhatia AK, Pattnaik B, Pradhan HK. Development of a capsid based competitive inhibition enzyme linked immunosorbent assay for detection of bovine immunodeficiency virus antibodies in cattle and buffalo serum. J Virol Methods. 2008;148:218–25.
14. Bhatia S, Gangil R, Gupta DS, Sood R, Pradhan HK, Dubey SC. Single chain fragment variable antibody against the capsid protein of bovine immunodeficiency virus and its use in ELISA. J Virol Methods. 2010;167:68–73.
15. Black JW. Bluetongue and bovine retrovirus committee report. In: Proceedings of the 93rd Annual Meeting of the US. Animal Health Association, pp. 150–152. Carter Printing Co., Richmond, VA. 1990.
16. Bouillant AM, Ruckerbauer GM, Nielsen KH. Replication of bovine immunodeficiency-like virus in diploid and aneuploid cells permanent latent and virus productive infections in vitro. Res Virol. 1989;140:511–29.
17. Boyer PL, Ferris AL, Hughes SH. Mutational analysis of the fingers domain of human immunodeficiency virus type 1 reverse transcriptase. J Virol. 1992;66:7533–7.
18. Braun MJ, Lahn S, Boyd AL, Kost TA, Nagashima K, Gonda MA. Molecular cloning of biologically active proviruses of bovine immunodeficiency-like virus. Virology. 1988;167:515–23.
19. Burkala EJ, Ellis TM, Voigt V, Wilcox GE. Serological evidence of an Australian bovine lentivirus. Vet Microbiol. 1999; 68(1–2):171–7.
20. Burke DS. Recombination in HIV: an important viral evolutionary strategy. Emerg Infect Dis. 1997;3:253–9.
21. Calabro V, Daugherty MD, Frankel AD. A single intermolecular contact mediates intramolecular stabilization of both RNA and protein. Proc Nat Acad Sci USA. 2005;102(19):6849–54.
22. Carpenter S, Miller LD, Alexandersen S, Whetstone CA, Van Der Maaten MJ, Viuff B, Wannemuehler Y, Miller JM, Roth JA. Characterization of early pathogenic effects after experimental infection of calves with bovine immunodeficiency-like virus. J Virol. 1992;66:1074–83.
23. Carpenter S, Vaughn EM, Yang J, Baccam P, Roth JA, Wannemuehler Y. Antigenic and genetic stability of bovine immunodeficiency virus during long-term persistence in cattle experimentally infected with the BIVR29 isolate. J Gen Virol. 2000;81:1463–72.
24. Cavirani S, Donofrio G, Chiocco D, Foni E, Martelli P, Allegri G, Cabassi CS, De Iaco B, Flamming CF. Seroprevalence to bovine immunodeficiency virus and lack of association with leukocyte counts in Italian dairy cattle. Prev Vet Med. 1998;37:147–57.
25. Chen H, Wilcox G, Kertayadnya G, Wood C. Characterization of the Jembrana disease virus tat gene and the cis-and trans-regulatory elements in its long terminal repeats. J Virol. 1999;73: 658–66.
26. Cho KO, Meas S, Park NY, Kim YH, Lim YK, Endoh D, Lee SI, Ohashi K, Sugimoto C, Onuma M. Seroprevalence of bovine immunodeficiency virus in dairy and beef cattle in Korea. J Vet Med Sci. 1999;61(5):549–51.
27. Cockerell GL, Jensen WA, Rovank J, Ennis WH, Gonda MA. Seroprevalence of bovine immunodeficiency-like virus and bovine leukemia virus in a dairy cattle herd. Vet Microbiol. 1992;31:109–16.
28. Coffin JM. Genetic diversity and evolution of retroviruses. Curr Top Microbiol Immunol. 1992;176:143–64.
29. Cojocariu M, St-Louis MC, Archambault D. Bovine immunodeficiency virus: identification of a long terminal repeat sequence with enhanced promoter activity. Arch Virol. 2009;154(7):1163–7.
30. Cooper CR, Hanson LA, Diehl WJ, Pharr GT, Coats KS. Natural selection of the *pol* gene of bovine immunodeficiency virus. Virology. 1999;255:294–301.
31. Deng G, Su Y, Mu J, Sha R, Geng Y, Qiao W, Chen Q. Molecular basis of the internalization of bovine immunodeficiency virus Tat protein. Virus Genes. 2008;36(1):85–94.
32. Desrosiers R. A review of some aspects of the epidemiology diagnosis and control of *Mycoplasma hyopneumoniae* infections. J Swine Health Prod. 2001;9(5):233–7.
33. Flamming K, Van Der Maaten M, Whetstone C, Carpenter S, Frank D, Roth J. Effect of bovine immunodeficiency-like virus infection on immune function in experimentally infected cattle. Vet Immunol Immunopathol. 1993;36:91–105.
34. Gardner MB, Luciw PA. Animal models of AIDS. FASEB J. 1989;3:2593–606.
35. Garvey KJ, Obsrste MS, Esler JE, Braun MJ, Gonda MA. Nucleotide sequence and genome organization of biologically active proviruses of the bovine immunodeficiency-like virus. Virology. 1990;175:391–409.
36. Gonda MA, Oberste MS, Garvey KJ, Pallansch LA, Battles JK, Pifat DY, Bess IW Jr, Nagashima K. Development of the bovine immunodefiency like virus as a model of lentivirus disease. Dev Biol Stand. 1990;72:97–110.
37. Gonda MA, Wong-Staul F, Gallo RC, Clements JE, Narayan O, Gilden RV. Sequence homology and morpholgic similarity of HTLV-111 and visna virus a pathogenic lentivirus. Science. 1985;227:173–7.
38. Gonda MA, Braun MJ, Clements JE, Pyper JM, Wong-Staal F, Gallo RC, Gilden RV. Human T-cell lymphotrophic virus type III shares sequence homology with a family of pathogenic lentiviruses. Proc Natl Acad Sci USA. 1986;83:4007–11.
39. Gonda MA, Braun MJ, Carter SG, Kost TA, Bess JW Jr, Arthur LO, Van Der Maaten MJ. Characterization and molecular cloning of a bovine lentivirus related to human immunodeficiency virus. Nature. 1987;330:388–91.
40. Gonda MA, Oberste MS. AIDS- The human immunodeficiency virus: molecular and structural aspects of its biology. In: Kurstak E, editor. Control of virus diseases. New York: Marcel Dekker; 1992. p. 3–31.
41. Gonda MA, Luther DG, Fong SE, Tobin GJ. Bovine Immunodeficiency virus molecular biology and virus-host interactions. Virus Res. 1994;32:155–81.
42. Gonda MA, Oberste MS, Garvey KJ, Pallansch LA, Battles JK, Pifat DY, Nagashima K. Contemporary developments in the

biology of the bovine Immunodeficiency like virus. In: Schlloakens H, Horzinke M, editors. Animal models in AIDS. Amsterdam: Elsevier; 1990. p. 233–55.
43. Gradil CM, Watson RE, Renshaw RW, Gilbert RO, Dubovi EJ. Detection of bovine immunodeficiency virus DNA in the blood and semen of experimentally infected bulls. Vet Microbiol. 1999;70(1–2):21–31.
44. Greene WK, Meers J, Chadwick B, Carnegie PR, Robinson WF. Nucleotide sequences of Australian isolates of the feline immunodeficiency virus: comparison with other feline lentiviruses. Arch Virol. 1993;132:369–79.
45. Guo HY, Ma YG, Gai YM, Liang ZB, Ma J, Su Y, Zhang QC, Chen QM, Tan J. Bovine HEXIM1 inhibits bovine immunodeficiency virus replication through regulating BTat-mediated transactivation. Vet Res. 2013;44(1):21.
46. Hidalgo G, Flores M, Bonilla JA. Detection and isolation of bovine immunodeficiency-like virus (BIV) in dairy herds of Costa Rica. Zentralbl Veterinarmed B. 1995;42(3):155–61.
47. Hirari N, Kabeya Ohashi K, Sugimoto C, Onuma M. Detection of antibodies against bovine immunodeficiency like virus in dairy cattle in Hokkaido. J Vet Med Sci. 1996;58(5):455–7.
48. Horner GW. Serologic evidence of bovine immunodeficiency-like virus and bovine syncytial virus in New Zealand. Surveillance. 1991;18(2):9.
49. Horzinek M, Keldermans L, Stuurman T, Black J, Herrewegh, Sillekens P, Koolen M. Bovine Immunodeficiency virus: immunochemical characterization and serological survey. J Gen Virol. 1991;72:2923–8.
50. International Committee on Taxonomy of Viruses (ICTV), 2011.
51. Isaacson JA, Roth JA, Wood C, Carpenter S. Loss of *Gag*-specific antibody reactivity in cattle experimentally infected with bovine immunodeficiency-like virus. Viral Immunol. 1995;8:27–36.
52. Kertayadnya G, Wilcox GE, Soeharsono S, Hartaningsih N, Coelen RJ, Cook RD, Collins ME, Brownlie J. Characteristics of a retrovirus associated with Jembrana disease in Bali cattle. J Gen Virol. 1993;74(9):1765–78.
53. Lechner F, Machado J, Bertoni G, Seow HF, Dobbelaere DA, Peterhans E. Caprine arthritis encephalitis virus dysregulates the expression of cytokines in macrophages. J Virol. 1997;71:7488–97.
54. Levy JA. Pathogenesis of human immunodeficiency virus infection. Microbiol Rev. 1993;57:183–289.
55. Li Y, Carpenter S. *Cis*-acting sequences may contribute to size variation in the surface glycoprotein of bovine immunodeficiency virus. J Gen Virol. 2001;82:2989–98.
56. Liu ZQ, Sheridan D, Wood C. Identification and characterization of bovine immunodeficiency like virus tat gene. J Virol. 1992; 66(8):5137–40.
57. Malmquist WA, Van der Maaten MJ, Boothe AD. Isolation, immunodiffusion and immunofluorescence and electron microscopy of a lymphosarcomatous and apparently normal cattle. Cancer Res. 1969;29:188–200.
58. Mansky LM. Retrovirus mutation rates and their role in genetic variation. J Gen Virol. 1998;79:1337–45.
59. Martin SJ, Neill PO, Billello JA, Eiseman. Lymphocyte transformation abnormalities in bovine immunodeficiency-like virus infected calves. Immunol Lett. 1991;27:81–4.
60. McNab WB, Jacobs RM, Smith HE. A serological survey for bovine immunodeficiency like virus in Ontario dairy cattle and association between test results production records and management practices. Can J Vet Res. 1994;58:36–41.
61. Meas S, Ohashi K, Sugimoto C, Onuma M. Phylogenetic relationships of bovine immunodeficiency virus in cattle and buffaloes based on surface envelope gene sequences. Brief report. Arch Virol. 2001;146:1037–45.
62. Meas S, Seto J, Sugimoto C, Bahksh M, Riaz M, Sato T, Naeem K, Ohashi K, Onuma M. Infection of bovine immunodeficiency virus and bovine leukemia virus in water buffalow and cattle populations in Pakistan. J Vet Med Sci. 2000;62(3):329–31.
63. Meas S, Ryas J, Faria NA, Usui T, Teraoka Y, Mulenga A, Chang KS, Masuda A, Madryga CR, Ohash K, Omma M, Ruas Faias J. Seroprevalence and molecular evidence for the presence of bovine immunodeficiency virus in Brazilian cattle. Jpn J Vet Res. 2002;50(2–3):145.
64. Meas S, Nakayama M, Usui T, Nakazato Y, Yasuda J, Ohashi K, Onuma M. Evidence for bovine immunodeficiency virus infection in cattle in Zambia. Jpn J Vet Res. 2004;52(1):3–8.
65. Miller JM, Miller LD, Olson C, Gillette KG. Virus like particles in phytohaemagglutinin stimulated lymphocyte culture with reference to bovine persistence lymphosarcoma. J Natl Cancer Inst. 1969;43:1297–305.
66. Mordow S, Hahn BH, Shaw GM, Gallo RC, Wong-staal F, Wolf H. Computer-assisted analysis of envelope protein sequences of seven human immunodeficiency virus isolates: prediction of antigenic epitopes in conserved and variable regions. J Virol. 1987;61:570–8.
67. Muluneh A. Seroprevalence of bovine immunodeficiency virus (BIV) antibodies ion cattle population in Germany. Zentralbl Veterinaramed B. 1994;41(10):679–84.
68. Nadin-Davis SA, Chang SC, Smith H, Jacobs RM. Detection of bovine immunodeficiency-like virus by the polymerase chain reaction. J Virol Methods. 1993;42(2–3):323–36.
69. Oberste MS, Willliamson JC, Greenwood JD, Nagashima K, Copeland TD, Gonda MA. Characterization of bovine immunodeficiency virus rev cDNAs and identification and subcellular localization of the Rev protein. J Virol. 1993;67:6395–405.
70. Oberste MS, Greenwood JD, Gonda MA. Anaplysis of the transcription pattern and mapping of putative rev and *env* splice junctions bovine immunodeficiency like virus. J Virol. 1991; 65:3932–7.
71. Onuma M, Koomto E, Furuyama H, Yasutomi Y, Taniyama H, Iwai H, Kawakami Y. Infection and dysfunction of monocytes induced by experimental ioculation of calves with bovine immunodeficiency like virus. J Acquir Immune Defic Syndr. 1992;5:1009–15.
72. Pallansch LA, Lackman Smith CS, Gonda MA. Bovine immunodeficiency-line virus encodes factors which trans activate the long terminal repeat. J Virol. 1992;66:2647–52.
73. Patil SS, Pattanaik B, Mishra N, Banumathi N, Dubey R, Pradhan HK. Detection of proviral genomic sequence of bovine immunodeficiency virus in Indian cattle. Curr Sci. 2003;84:563–6.
74. Pezo V, Wain-Hobson S. HIV genetic variation: life at the edge. J Infect. 1997;34:201–3.
75. Polack B, Schwartz I, Berthelemy M, Belloc C, Manet G, Wuillaume A, Baron T, Gonda MA, Levy D. Serologic evidence for bovine immunodeficiency virus infection in France. Vet Microbiol. 1996;48(1–2):165–73.
76. Rasmussan L, Battles JK, Ennis WH, Nagashima K, Gonda MA. Characterization of virus-like particles produced by a recombinant baculovirus containing the *gag* gene of the bovine immunodeficiency virus. Virology. 1990;178:435–51.
77. Setiyaningsih S, Desport M, Stewart ME, Hartaningsih N, Wilcox GE. Sequence analysis of mRNA transcripts encoding Jembrana disease virus Tat-1 in vivo. Virus Res. 2008;132(1–2):220–5.
78. Snider TG, Hoyt PG, Jenny BF, Coats KS, Luther DG, Storts RW, Battles JK, Gonda MA. Natural and experimental bovine immunodeficiency virus infection in cattle. Vet Clin North Am Food Anim Pract. 1997;13:151–76.
79. Starcich BR, Hahn BH, Shaw GM, cNeely PD, Modrow S, Wolf H, Parks ES, Parks WP, Josephs SF, Gallo RC, Wong-Staal F. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell. 1986;45:637–48.

80. St-Louis MC, Abed Y, Archambault D. The bovine immunodeficiency virus: cloning of a tat/rev cDNA encoding a novel Tat protein with enhanced transactivation activity. Arch Virol. 2005;150:1529–47.
81. Suarez DL, Whetstone CA. Identification of hypervariable and conserved regions in the surface envelope gene in the bovine lentivirus. Virology. 1995;212:728–33.
82. Suarez DL, Whetstone CA. PCR diagnosis of the bovine immunodeficiency-like virus. Methods Mol Biol. 1998;92:67–79.
83. Suarez DL, Whetstone CA. Size variation within the second hypervariable region of the surface envelope gene of the bovine lentivirus BIV in experimentally and naturally infected cattle. J Virol. 1997;71:2482–6.
84. Suarez DL, Van Der Maaten MJ, Wood C. Isolation and characterization of new wild type isolates of bovine lentivirus. J Virol. 1993;67:5051–5.
85. Suarez DL, Van der Maaten MJ, Whetstone CA. Improved early and long-term detection of bovine lentivirus by a nested polymerase chain reaction test in experimentally infected calves. Am J Vet Res. 1995;56(5):579–86.
86. Truyen U, Parrish CR, Harder TC, Kaaden OR. There is nothing permanent except change. The emergence of new virus diseases. Vet Microbiol. 1995;43:103–22.
87. Turelli P, Guiguen F, Mornex JF, Vigne R, Querat G. dUTPase-minus caprine arthritis-encephalitis virus is attenuated for pathogenesis and accumulates G-to-A substitutions. J Virol. 1997; 71:4522–30.
88. Van Der Maaten MJ, Boothe AD, Seger CL. Isolation of a virus from cattle with persistent lymphocytosis. J Natl Cancer Inst. 1972;49:1649–57.
89. Wakiyama M, Kaitsu Y, Muramatsu R, Takimoto K, Yokoyama S. Tethering of proteins to RNAs using the bovine immunodeficiency virus-Tat peptide and BIV-TAR RNA. Annu Biochem. 2012;427(2):130–2.
90. Walder R, Kalvatchev Z, Tobin GJ, Barrios MN, Garzaro DJ, Gonda MA. Possible role of bovine immunodeficiency-like virus in bovine paraplegic syndrome: evidence from immunochemical, virological and seroprevalence studies. Res Virol. 1995; 146(5):313–23.
91. Wannmuehler Y, Issacson J, Wannmuehler M, Wood C, Roth JA, Carpenter S. In vitro detection of bovine immunodeficiency like virus using monoclonal antibodies generated to a recombinant *gag* fusion protein. J Virol Methods. 1993;44:117–28.
92. Whetstone CA, Van Der Maaten MJ, Black JW. Humoral immune response to the bovine immunodeficiency-like virus in experimentally and naturally infected cattle. J Virol. 1990;64:3557–61.
93. Whetstone CA, Suarez DL, Miller JM, Pesch BA, Harp JA. Bovine lentivirus induces early transient B-cell proliferation in experimentally inoculated cattle and appears to be pantropic. J Virol. 1997;71:640–4.
94. Wu D, Murakami K, Morooka A, Jin H, Inoshima Y, Sentsui H. In vivo transcription of bovine leukemia virus and bovine immunodeficiency-like virus. Virus Res. 2003;97:81–7.
95. Xuan C, Qiao W, Li J, Peng G, Liu M, Chen Q, Zhou J, Geng Y. BTat, a trans-acting regulatory protein, contributes to bovine immunodeficiency virus-induced apoptosis. Cell Microbiol. 2008;10(1):31–40.
96. Zhang S, Wood C, Xue W, Krunkenberg SM, Minocha HC. Immune suppression in calves with bovine immunodeficiency virus. Clin Diagn Lab Immunol. 1997;4:232–5.
97. Zhang S, Troyer DL, Kapil S, Zheng L, Kennedy G, Weiss M, Xue W, Wood C, Minocha HC. Detection of proviral DNA of bovine immunodeficiency virus in bovine tissues by polymerase chain reaction (PCR) and PCR in situ hybridization. Virology. 1997;236:249–57.
98. Zheng L, Swanson M, Liao J, Wood C, Kapil S, Snider R, Louahin TA, Minocha HC. Cloning of the bovine immunodeficiency virus *gag* gene and development at a recombinant protein based Enzyme-linked immunosorbent assay. Clin and Diagn Lab Immunol. 2000;7:557–62.
99. Zheng L, Zhang S, Wood C, Kapil S, Wilcox GE, Loughim TA, Minocha HC. Differentiation of two bovine lentiviruses by a monoclonal antibody on the basis of epitope specificity. Clin Diagn Lab Immunol. 2001;2:283–7.