50 Years of transforming lives through dairying

NATIONAL DAIRY DEVELOPMENT BOARD
ANAND
GUJARAT

ANIMAL HEALTH UPDATES

Animal Health Group

VOLUME V ISSUE II | Quarterly Newsletter | July– Sept'16

IN THIS ISSUE:

Enzootic Bovine Leukosis (EBL) and its control

I. Introduction

Infection with Bovine Leukosis Virus (BLV) in cattle is referred to as Enzootic Bovine Leukosis (EBL). As per the Terrestrial Animal Health Code of World Organization for Animal Health (OIE), only semen from bulls maintained in an EBL free herd is eligible for international trade.

Though EBL is prevalent in India, reports on the same are scanty. NDP-I has embarked on an ambitious target of significantly increasing the semen production and AI coverage in the country. Strict biosecurity protocols are already in place that facilitates selection of disease free high genetic merit male calves being procured under various bull production programmes and, for the semen stations that are being strengthened under NDP-I. It is therefore important to look at EBL in this context as presently there are no protocols for its control under NDP-I.

II. EBL-the disease

EBL is caused by an exogenous C-type oncovirus of genus delta-retrovirus in Retroviridae family. Cattle may be infected at any age, including embryonic stage. Most infections are **sub-clinical**, but a proportion of cattle (~30%) **over 3 years of age** develop **Persistent Lymphocytosis (PL)** and, a smaller proportion (<5%) develop **lymphosarcomas** in various internal organs. Once an animal is infected, the infection **persists** for life. Natural infection has also been recorded in buffaloes, sheep and capybaras. Cattle with lymphosarcomas almost invariably die either suddenly, or weeks or months after the onset of clinical signs.

III. Prevalence in India

Presence of BLV in our country has been reported as early as 1987wherein 1511 sera samples from 37 herds across 15 States were tested. The average sero-positivity was **21%** (range between 2-41%), with 322 samples in 17 herds found positive for BLV antibodies.

IV. Modes of transmission

(i) Direct contact

Horizontal transmission is the usual method of virus spread. **Close physical contact** and exchange of **contaminated biological materials** are required for transmission. Highly cellular fluids are likely to be more infective.

An estimated threshold number of <100-1000 infected lymphocytes are required to establish infection. This variation can be explained by the infection load on lymphocytes which can vary from <5% to as high as 50% in animals with PL.

(ii) AI, frozen semen and natural service

Researchers have failed to find the virus in semen and, artificial insemination is **not** presently considered as a method of spread. It is however possible that **semen with infected lymphocytes** could serve as a source of the virus. Bulls at semen stations are required to

be **negative serologically** to BLV virus. BLV positive bulls used for **natural service** that had reproductive tract infections causing increased number of infective lymphocytes in the semen **could spread** infection to susceptible cattle.

(iii) Embryo transfer

Embryo transfer also is **not** reported to be a major source of vertical transmission . Embryos from BLV positive donors **do not** infect BLV negative recipients nor result in infected foetuses. However, embryos from either BLV positive or negative cows are at risk of **in-utero** infection if implanted in **BLV positive** recipients.

(iv) Iatrogenic

Transmission can occur via **infected blood** which contaminates **tagging machines, hypodermic needles** etc which are used on infected and then susceptible animals **without disinfection**. Amounts of blood as low as 0.1µl are capable of transmitting the infection. Thus infection can be even transmitted via tuberculin syringe during **intradermal test**.

Transmission via infective colostrum/milk is possible, though rare, in the first 3 days of life of the calf.

(v) Rectal palpation

Use of **blood contaminated sleeves** from sero-positive animals to palpate sero-negative animals has resulted in transmission of infection.

(vi) Insects

Blood sucking insects may be involved in transmission of the virus, especially ***Tabanus*** **(horse fly)** since they have relatively large mouth part volume compared to other flies to carry sufficient infected lymphocytes.

(vii) Congenital infection

Congenital infection may occur in **4-8%** of calves born from BLV seropositive cows.

V. Pathogenesis

The virus establishes a persistent infection in a sub-population of B-lymphocytes by integrating pro-viral DNA into the host cellular DNA. There are **four** possible outcomes of infection with BLV:

1. **Failure** of animal to become infected.
2. Establishment of permanent infection and development of detectable levels of antibodies. **(Latent carriers)**
3. Establishment of **permanent infection,** development of detectable levels of antibodies and Persistent **Lymphocytosis (PL).** PL is defined as the absolute lymphocyte count at least **three standard deviations** above normal mean count that persists for at least 3 consecutive months. This occurs in **around 30%** of the infection. This benign lympho-proliferative process is **not** a pre-clinical stage of lymphosarcoma.
4. Sero-positive animals that may or may not have been through a PL stage which develop neoplastic, malignant tumours- lymphosarcoma. This occurs in **<5%** of the infection.

VI. Risk factors

(i) Animal

The prevalence of infection in animals under 17-24 months of age is much lower than in adult cattle and **increases sharply** after 24 months of age.

A complex relationship exists between genetic merit, milk production, Bovine Lymphocyte Antigens (BoLA), genotype and susceptibility to PL. Cows with **high** genetic potential for milk and fat yields are **more** susceptible than low yielders.

A **highly** significant correlation was shown between BLV infection and persistence of *Trichophyton verrucosum* infection. The risk for other infectious diseases also seemed to be **greater.**

(ii) Lack of biosecurity

The introduction of **infected animals** into the herd has a significant effect of subsequent prevalence of infection and clinical disease. Iatrogenic transmission due to improper biosecurity protocols and improper fly control could also be potential risk factors.

VII. Clinical signs

(i) Enzootic Bovine Leukosis (EBL)

This form is seen in adults **above 3 years** of age. The usual incubation period is 3-6 years. It is rarely seen in animals below 2 years of age and most common in 4-8 years age group. In 5-10% of the clinical cases, the course is per-acute. In most cases however, the course is sub-acute and initiated by an unexplainable loss of body condition, appetite, decreased milk pro-

duction and muscular weakness.

Enlargement of **external lymph nodes** occur in ~25% of the tumourous animals. Enlargement of visceral lymph nodes is also common but remains sub-clinical unless other organs /nerves are compressed. Usually a tumour affecting **one anatomical region** predominates.

Hypertrophy of the peripheral lymph nodes in EBL.
Source : http://www.fmv.ulisboa.pt/atlas/linfoide/ind_linfneo_ing.htm

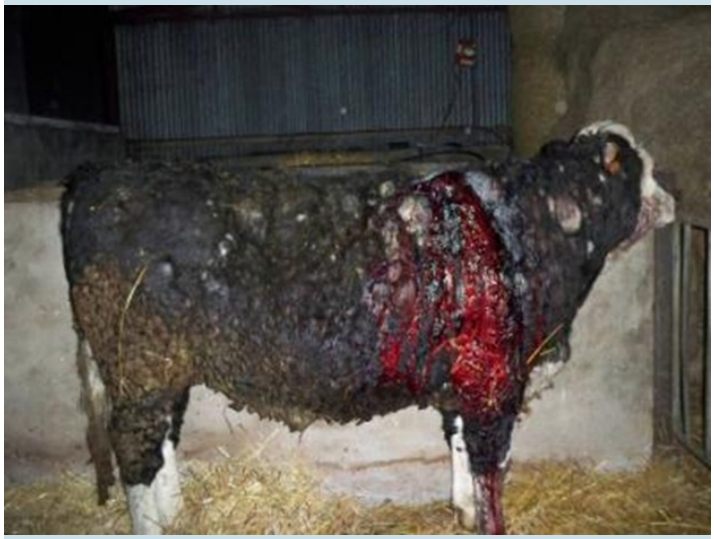
Digestive lesions (melaena, chronic moderate bloat, persistent diarrhoea), **cardiac lesions** (hydro pericardium, engorgement of jugular vein, brisket oedema) and **nervous lesions** (posterior paralysis, knuckling of fetlock, difficulty in rising and recumbency) relates to the **anatomical area** affected.

Hypertrophy of the mesenteric lymph nodes in EBL
Source : http://www.fmv.ulisboa.pt/atlas/linfoide/ind_linfneo_ing.htm

Less common lesions include dyspnea and stertor due to enlargement of retropharyngeal lymph nodes, exophthalmos due to involvement of peri-oribital tissues and lesions involving kidney and uro-genital system. Skin tumours appear **rarely** in adult lymphosarcoma cases, which are firm and may be either nodular or plaque-like.

(ii) Sporadic (Atypical) Bovine Leukosis (SBL)

SBL includes (i) Juvenile (ii) Calf (iii) Thymic and (iv) Cutaneous (skin form). All are **rare** and mostly occur in cattle **below 2 years** of age. They **rarely** caused by BLV infection and hence termed 'sporadic'. Most animals, but not all, with SBL are **BLV sero-negative**.



Cutaneous lymphoma in a calf with SBL
Source : https://www.agriculture.gov.ie/

(a) Juvenile and Calf lymphosarcoma

This occurs in calves from **birth to 18 months** of age. Juvenile form is mostly T-cell, and, Calf form, B-cell lymphosarcoma. It is manifested by gradual loss of weight and sudden and palpable enlargement of peripheral lymph nodes. There could also be recurrent bloat and dyspnea. Death usually occurs 2-8 weeks after onset of symptoms.

(b) Thymic lymphosarcoma of young cattle

This is seen in animals **6-24 months of age** characterized by massive enlargement of thymus and lesions in bone marrow and regional lymph nodes. This form is more common in beef cattle. This form is also suggested to have a **congenital mode** of transmission.

(c) Cutaneous lymphoma

This is rare and occurs in cattle **below 30 months** of age and usually affects **BLV sero-negative** animals. It

is manifested by cutaneous plaques. Their body condition deteriorates over 6 to 12 months. Scores of skin nodules are present in this form which allow clinical differentiation from adult lymphosarcoma wherein skin lesions are few.

VIII. Diagnosis

(i) OIE prescribed tests for EBL

(a) Enzyme Linked Immunosorbent Assay (ELISA)

Either an indirect or blocking ELISA may be used.

(i) Indirect ELISA - Milk ELISA: This method is suitable for antibody detection in pooled milk samples.

(ii) Blocking ELISA - Serum ELISA : This method is suitable for antibody detection in single or pooled serum samples.

(b) Agar gel immunodiffusion test (AGID)

The AGID test is a specific, but not very sensitive test for detecting antibody in serum samples from individual animals. It is, however, unsuitable for milk samples (except first colostrums) because of lack of specificity and sensitivity.

The diagnosis of lymphosarcoma is done by histopathology of lymph node biopsies or aspirates.

(ii) Diagnosis of SBL

Histopathological examination of the lymph node biopsies or aspirates would give a confirmative diagnosis in all forms of SBL.

IX. Differential Diagnosis of EBL

Tuberculosis (enlargement of peripheral lymph nodes without fever), **Johne's disease** (digestive lesions), **TRP** (cardiac lesions- however, there is absence of fever, toxaemia and neutrophilia), **dumb form of rabies** (spinal nerve lesions), **Actinomycosis** (enlargement of retropharyngeal lymph node)

X. Control

1. **Test and removal** /segregation of sero-positive animals.
2. Regular **re-testing**

NDDB R&D has the testing facility for detecting BLV in animals by ELISA and PCR.

False positive results may occur in calves up to six or seven months of age if they have received colostrum/ milk with antibodies against BLV. These passive antibodies gradually decay during the first half year of the calf's life. However, not all sero-positive calves are false positive; **4-8%** of calves from sero-positive dams in naturally infected herds are infected with BLV at birth. These infections are probably **acquired transplacentally.**

False negative results could be due to the following:

- Undetectable antibody levels during **early phases** of infection.
- **Poor** antibody response to infection.
- Infected cows tested 2 to 6 weeks **before or after parturition.**

XI. Prevention

1. **Eliminate** movement of blood from infected (sero-positive) to naïve animals.
2. **Use single use, disposable** needles for vaccination, blood collection and TB testing.
3. **Use fresh rectal sleeve** for each animal.
4. **Introduce only BLV sero-negative** animals into the herd.
5. **Disinfect** instruments between uses.
6. **Control fly** population.

Sources

1. Veterinary Medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses: Radostits, O. M., Gay, C.C., Blood, D. C., Hinchcliff, K. W., 10th edition. Pp. 1209-1221.
2. Rebhun's Diseases of Dairy Cattle. Thomas J Divers., Simon F Peek., 2nd edition, Saunders Elsevier Pp. 623-633
3. http://www.oie.int
4. Singh, V.P., Bansal , M.P., Singh, K.P. Sero-epidemiological studies of bovine leukaemia virus infection in Indian cross-bred zebu cattle. Rev. sci. tech. Off. Int. Epiz., 1987, 6 (1) , Pp. 225-231.
5. The Merck Veterinary Manual, 10th Edition, Merck and Co., Inc. USA. Pp.671 -674.

Significant animal diseases reported to OIE (Jul–Sept'16)

Disease	Country
Bluetongue	Bosnia and Herzegovina, Brazil, Croatia, Italy, Montenegro, Serbia
Rabies	Bulgaria
Lumpy skin disease	Burundi, Kazakhstan, Montenegro, Namibia, Saudi Arabia
Anthrax	France, Italy, Russia Kazakhstan, Romania, Sweden
Foot and Mouth Disease (FMD)	Mauritius, Mongolia
Infectious Bovine Rhinotracheitis (IBR/IPV)	Palestine Autonomous Territories

For further details please contact : Dr.A V Hari Kumar , Sr. Manager (AH), NDDB, Anand, Phone : 02692 226244 E mail:avhk@nddb.coop