Re-Emerging Enzootic Bovine Leukosis in A Cattle Farm in Lithuania: A Case Report

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Abstract

Enzootic bovine leukosis (EBL) has a severe impact on bovine health and leads to significant economic losses in affected herds. The aim of this case study is to analyze possible sources of repeated occurrence of an EBL infection in a dairy cattle farm in Lithuania. Lithuania is EBL free since 2012; however, increased numbers of disease cases were notified during annual national surveillance in 2017–2019. This case report documents an assessment of a dairy herd with 2 EBL cases within 3 years: EBL was confirmed in 2017 and, after regaining disease free status one year later in 2018, EBL re-entered the same herd in 2019. The most common bovine leukemia virus infection routes were discussed according to the available official data, and iatrogenic transmission was considered as the most plausible reason. There is a constant EBL risk for cattle herds in southwest Lithuania, and this study highlights the need of knowledge about the reasons of confirmed EBL outbreaks to stop the spread of the disease.

Introduction

Enzootic bovine leukosis (EBL) is a disease of cattle caused by bovine leukemia virus (BLV), a member of *Deltaretrovirus* in the family *Retroviridae* (Frie *et al.*, 2015). BLV causes a persistent, life-long infection, induces immune dysregulation with increased susceptibility to other infections and, lastly, may progress to lymphomas in various internal organs (OIE, 2018). Cattle may be infected at any age, including the embryonic stage. Most infections are subclinical, but a proportion of cattle (~30%. Around 60% of infected animals remain asymptomatic and EBL cases in cattle are usually detected during active surveillance of the disease by using serological tests (Kabeya, Ohashi and Onuma, 2001). The modes of transmission of BLV are both vertical and horizontal. The main BLV transmission is horizontal, and any mechanism able to transmit blood or infected lymphocytes between animals can be considered, including the most frequent iatrogenic route with contaminated equipment (EFSA AHAW Panel, 2015) lifelong infection in a subset of B cells. Malignant tumours lymphomas. It has been estimated that movement of infected cattle plays a primary role in the EBL spread between herds, regions or countries (Kobayashi *et al.*, 2014). Vertical transmission via in utero and ingestion of colostrum from BLV-infected cows may account for 10–25% of infections (Mekata *et al.*, 2013). EBL is not a vector-borne disease, but hematophagous insects such as horse flies may contribute to the spread of BLV within a herd by mechanically transferring lymphocytes via biting (Kobayashi *et al.*, 2014).

EBL is a notifiable disease at a national and international level. In the European Union, 22 countries, including Lithuania, have successfully demonstrated freedom from EBL (EC, 2017). After Lithuania officially gained an EBL-free country status in 2012, 20% of bovine herds each year are tested. The annual sampling includes cattle ⩾24 months old, which should be tested at least once every five years. Risk-based sampling for serological EBL surveillance has been suggested in order to increase the sensitivity of surveillance detecting infected herds (Reist, Jemmi and Stärk, 2012). However, EBL still occurs sporadically in Lithuania, but the prevalence of infected herds does not exceed 0.2% annually. There were 21 EBL infected bovine herds in 2017, 27 in 2018, and 21 in 2019. They all were located in south west of Lithuania.

Methodology

The data regarding a confirmed EBL outbreak in a dairy cattle farm including herd structure, animal movement, laboratory diagnostics, and control and management strategy were used from the National Veterinary Information Management System and from the EU Animal Disease Notification System (ADNS). Laboratory testing was carried out from cattle blood by performing an enzyme-linked immunosorbent assay (ELISA) test at the National Food and Veterinary Risk Assessment Institute, which is the reference laboratory for animal diseases in Lithuania.

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EBL Case presentation

During 2017–2019, 41 of 69 EBL infected herds were found in Marijampolė county in the southwestern part of Lithuania. Since 2017 when risk-based surveillance for EBL was implemented, 100% of bovine herds in affected municipalities of Marijampolė county, including all cattle aged ⩾12 month in a herd, were tested, and increased numbers of BLV-positive animals were confirmed.

The bovine herd taken for this case study was tested for EBL since 2011. The first occurrence of EBL in this herd was in 2017, where 1 animal of 5 tested was confirmed as being positive (Fig. 1). The cow was introduced into the herd in 2008 and since then until 2017 was tested 3 times with negative results. After slaughtering the positive animal, the herd was tested BLV-negative 3 times and regained its disease-free status in April 2018. During the next herd test in February 2019, EBL was confirmed again. The test showed that 1 of 7 tested cows was positive for EBL. A BLV-positive cow was born in the herd in 2008 (Table 1). Between the outbreaks in 2017 and 2019, this cow was tested negative for EBL 3 times. During the period between the last herd test for EBL and the outbreak in 2019, no animals were introduced into the herd. During both EBL outbreaks, positive cows did not show any clinical symptoms of the disease. Both positive animals from the outbreaks in 2017 and 2019 as well as their calves were slaughtered under the supervision of veterinary authorities. All animals in the infected herd that were more than 12 months old were tested serologically at least 2 times within the year. The herd regained its official disease-free status in 2020.

Discussion

According to the scientific literature, bovine movement, iatrogenic transmission, role of hematophagous insects are the most commonly discussed routes of BLV transmission (EFSA AHAW Panel, 2015)lifelong infection in a subset of B cells. Malignant tumours (lymphomas. These possible routes were observed according to the available official data, and the findings suggest the following hypothesis:

1) Hypothesis of EBL spread with the bovine movement

BVL spread between herds is believed to be facilitated by the movement of infected animals (EFSA AHAW Panel, 2015)lifelong infection in a subset of B cells. Malignant tumours (lymphomas. In our case study, after tracing all possible contacts of both in-

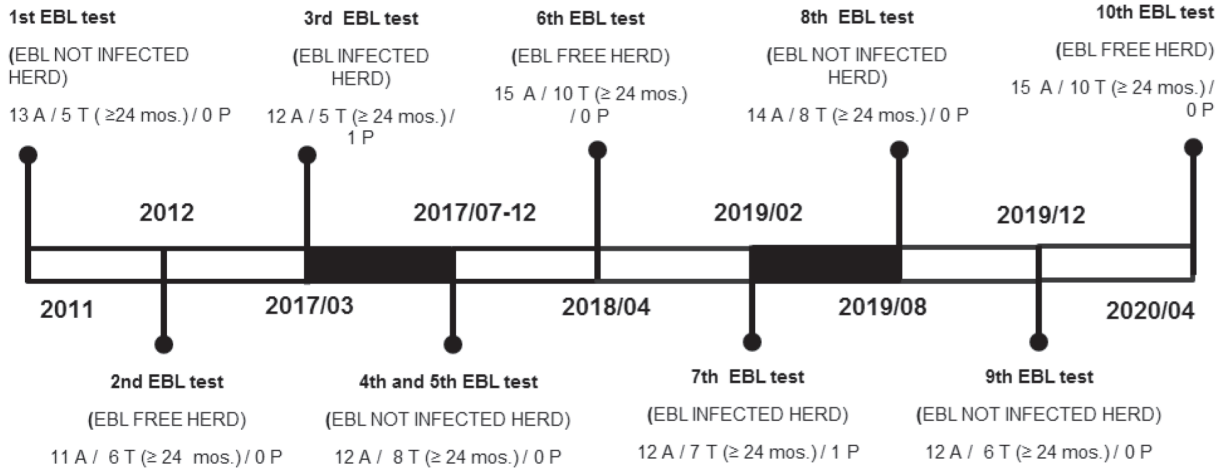

Fig. 1. EBL testing timeline of the outbreak herd.
A – total number of animals, T – tested animals, P – BLV-positive

Table 1. Descriptive information of EBL infected animals in 2017 and 2019 EBL outbreaks in the farm

EBL positive cattle characteristics	Case of 2017	Case of 2019
Age (in years)	8.6	11.2
Movement	1 time	0 times
Born in the herd / moved into the herd	Moved into the herd	Born in the herd
Number of offspring and their EBL status	7 (4 not tested, 3 negative)	7 (3 not tested, 4 negative)
Duration between the last negative EBL test and outbreak	4 years 1 month	9 months

fected cows, no connections between them and other BLV-positive cows from other herds were detected. The owner could not have brought BLV in with infected cows as the purchased cows originated from officially disease-free herds and had several EBL negative tests after movement.

2) Hypothesis via iatrogenic transmission

a. Via diagnostics and treatment. Often the virus is transmitted using blood-contained needles, dehorning instruments or contaminated gloves during rectal palpation (EFSA AHAW Panel, 2015)lifelong infection in a subset of B cells. Malignant tumours (lymphomas. In our case study, there was no accessible information about the treatment or the procedures that were carried out in this farm during the studied period. Considering the fact that the herd is being serviced by a visiting veterinarian who also manages other herds in the same region, iatrogenic transmission between herds through contaminated appliances cannot be excluded.

b. Via artificial insemination. The positive cow from the outbreak in 2017 was artificially inseminated in 2015 only. Since the calf of that cow was tested and was negative for EBL, this infection route for the 2017 EBL-positive cow was not likely. The positive cow from the outbreak in 2019 was artificially inseminated in March 2018. Since then, the cow was tested serologically for EBL being about 2 months pregnant and the result was negative. There is no available information about the calf since it was slaughtered soon after the birth without being tested for EBL due to not relevant age for testing.

Hypothesis of the role of horse flies (Tabanus spp.) in BLV transmission

There is a lack of research regarding the impact of hematophagous insects to BLV transmission within and between cattle herds in natural conditions (Panei *et al.*, 2019)the most common neoplastic disease in cattle. The horn fly, a major hematophagous pest of cattle, is able to transmit different diseases in cattle. However, its implication in BLV transmission under a natural environment is still discussed. The objectives of this work were to determine the presence of BLV in horn flies (by sequencing. According to the recent study of entomologists, *Tabanus spp.* insects are present in Lithuania, but the investigated EBL cases occurred during the time when horse flies are still in the stage of larvae (Turčinavičienė, 2018; Mehlhorn *et al.*, 2010). Nevertheless, we have to point out that the possible infection via a mechanical vector could happen during the time between cattle testing. We also assume that, in the case of *Tabanus spp.* involved in the spreading of EBL as a source of infection, there would possibly be more than one individual EBL case in the herd during an outbreak.

Conclusions

In our case study, no connection was found between the EBL outbreak in 2017 and the outbreak in 2019. We assume that in this particular case vertical transmission, mechanical vectors such as horse flies, animal movement or trade cannot be confirmed as being the causes of EBL cases. The most probable cause of EBL in this bovine herd could be related to iatrogenic transmission. This case report could encourage veterinarians and veterinary epidemiologists to perform a precise outbreak investigation of EBL cases considering all possible infection routes, in order to identify the most plausible source of infection and stop the spread of the disease within the regions at risk and at a national level.

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