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INTESTINAL YERSINIOSIS IN ANIMALS

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The article presents data from domestic and foreign scientific literature on the prevalence, clinical manifestation and diagnosis of infection Yersinia enterocolitica of animals of different species, including birds and humans. Analysis of the literature confirms the relevance of intestinal yersiniosis, demonstrates the need for further work, particularly in terms of epidemiological monitoring, development of new methods of diagnosis and highly effective means for control and prevention.

MOLECULAR ANALYSIS OF BLV ISOLATES – A LINK TO AN EPIDEMIOLOGICAL STUDY

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Recent studies have shown that bovine leukemia virus (BLV) sequences can be classified into seven distinct genotypes based on full gp 51 sequence. This classification was based on available sequence data that mainly represented the BLV population that is circulating in cattle from the US and South America. In order to aid with a global perspective inclusion of data from Eastern Europe and Siberia we examined 44 BLV isolates from different geographical regions of Poland, Belarus, Ukraine and Russia. Phylogenetic analysis based on a 444 bp fragment of *env* gene revealed that most of isolates belonged to genotypes 4 and 7. Furthermore, we confirmed the existence of a new genotype, genotype 8, which was highly supported by phylogenetic analysis. A significant number of amino acid substitutions were found in the sequences of the studied Eastern European isolates of which 71 % have not been described previously. The substitutions encompassed mainly the C-part of the CD4 + epitope, zinc binding peptide region, CD8 + T cell epitope and overlapping linear epitope E. These observations highlight the use of sequence data to both elucidate phylogenetic relationships and the potential effect on serological detection of geographically diverse isolates.

Epidemilogy of BLV infection. BLV serological surveys reveal that the infection is widely disseminated throughout the world with a high prevalence in North and South America, some Asiatic and Middle Eastern countries as well as Eastern Europe. Efforts in the implementation of control measures and programmes to eradicate BLV infection in Western European countries have been successful and nowdays most of EU member states are officially free of EBL. In contrast, the situation is different in Eastern Europe where the infected animals are still present in several countries (Bulgaria,Croatia, Estonia, Latvia, Poland, Romania, Ukraine, Russia). In Australia and New Zealand dairy herds began in the mid-1990s and more than 98 % of dairy herds were negative in 2005. Serological studies from 2007 revealed that 83.9 % of U.S. dairy herds were positive for BLV. In South America, individual infection rates between 34 and 50 % were reported in Colombia, Venezuela, Chile and Uruguay. In Argentina, individual and herd prevalence levels showed to be from 32.8 % to 84 %. In Brazil, the individual prevalence of BLV infection varies considerably among provinces and reaches about 50 %. The epidemiological situation in Asia showed that BLV is present in Indonesia, Taipei (China) and Mongolia. The seroprevalence rates in Japan were found to be 28.6 % and 68.1 % at the individual and herd levels, respectively. In Korea, individual seroprevalence rates reached 50 % whilst 86.8 % of dairy herds were infected. Recent data showed that BLV infection is noted in cattle from Turkey and Iran where the herd seroprevalence was 48.3 % and 64.7 %, respectively, while the individual seroprevalence in Iran was estimated between 17 and 24.6 %.

Phylogeny of BLV isolates. Characterisation of the global BLV genetic diversity is an ongoing international research effort. In such studies, phylogenetic analysis were conducted showing that the *env*-derived sequences could be grouped into three [18] or four [11, 19] different genetic subgroups. A study by Rodriguez et al [20] which integrated the available full gp 51 sequences of BLV from different geographic origins clearly showed that the sequences could be classified into seven distinct genotypes. However, as this classification was based on available sequence data, whilst it included sequences from Asia, Europe and Australia, it mainly represented the BLV population circulating in cattle from the Americas. There is a lack of comprehensive studies focusing on genetic characterization and classification of BLV isolates present in Eastern Europe and Russia. In this study we analysed phylogeneticaly *env* gene sequences from 44 BLV isolates from different geographical regions of Poland, Belarus, Ukraine and Russia.

Blood samples from Russia and Ukraine were originally collected by collaborating laboratories from these countries and then sent to the NVRI. Blood samples from Poland were selected by national reference laboratory during EBL monitoring programme. Two archived samples from BLV positive cows were received from national reference laboratory in Minsk, Belarus. The primers for nested PCR amplification were described previously by Beier et al [12] their sequence is as follows: env 5032 (5'-TCTGTGCCAAGTCTCCCAGATA-3'); env 5608 (5'-AACAACAACCTCTGGGAAGGGT-3') and env 5099 (5'-CCCACAAGGGCGGCGCC GGTTT-3'), env 5521 (5'-GCGAGGCCGGG TCC AGAGCTGG-3'). Amplification was performed with 500 ng of genomic DNA. Sequence data were analysed using the BioEdit sequence alignment editor and subsequently were aligned using the Geneious Alignment module within Geneious Pro 5.3 Software (Biomatters

Ltd). Phylogenetic trees were constructed using Mr Bayes method with GTR substitution model and the neighbour-joining (NJ) method with Tamura-Nei model of nucleotide substitution.Mean nucleotide distances within (intra-genotype) and among (inter-genotype) BLV genotypes were estimated by adopting the Tamura Nei model in MEGA 4.

Conclusion: All 44 BLV sequences including isolates from Poland, Russia, Ukraine and Belarus were assigned to three genotypes: G4, G7 and a new genotype, G8. We found that most of these isolates (25/44) clustered within G4, fifteen were classified in G7 while four in a new genotype 8. The existence of distinct subgroups within G7 (A, B and C) comprised exclusively of Russian, Ukrainian and Polish isolates, respectively fully corresponded to their geographical origin, since each subgroup contained isolates from one country only. A new genotype, G8 included four isolates from distinct regions of Ukraine and one isolate from Russia. The pair-wise genetic distances analysis within genotype 8 ranged between 0.0–2.3 % and it was relatively lower than those observed for G4 and G7. Surprisingly, this low level of genetic diversity did not reflect the broad geographical origin of these isolates, perhaps indicating the same origin of infection.

Amino acid diversisty of gp51 within BLV isolates. Studying the genetic diversity of viruses could help to gain the correlation between variation in genotype and disease progression, differences in infectivity or potential effect of viral variability on diagnostic assays. We analysed whether nucleotide mutations affected amino acid composition and possibly the conformational structure of envelope glycoprotein gp51.

Amino acid (aa) sequences of the 44 BLV isolates were aligned to aa sequence of BLV-FLK. We noted 21 different amino acids substitutions. Furthermore, although a variety of single aa substitutions were evident over the full length of the analysed part of gp51 some amino acids changes (R121H, H142R, I144T, I176L) were observed in multiple samples. These substitutions encompassed mainly the C-part of CD4+ epitope, zinc binding peptide region, CD8+ T cell epitope and overlapping linear epitope E. We noted the highest numbers of aa substitutions in isolates belonging to G4.

Conclusion. It was interesting that more than half (71%) of all aa substitutions have not been described previously. The biological significance of these changes is unknown, although the histidine replacement (H) by arginine (R) at position 142 could be speculated. In some BLV isolates this histidine was replaced by tyrosine (Y) or leucine (L) [20] and it was shown that this histidine is one of the three histidine residues which are present in the zinc-binding region, which is an essential component of zinc-binding proteins together with cysteine. Taking into account that the region of SU localised between residues 137–156 affects fusion and infectivity of BLV *in vivo* this mutation may be crucial for virus infectivity. Also, the substitution phenylalanine to serine at position 146, found in one isolate, shows that this substitution is exposed on the surface of the second neutralizing epitope and it would diminish immunoreactivity of this epitope.

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МОЛЕКУЛЯРНИЙ АНАЛІЗ ІЗОЛЯТІВ ВІРУСУ ЛЕЙКОЗУ ВРХ – ПОСИЛАННЯ НА ЕПІДЕМІОЛОГІЧНІ ДОСЛІДЖЕННЯ

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У статті наведені дані щодо дослідження 44 ізолятів вірусу лейкозу, виділених з різних географічних регіонів Польщі, Білорусі, України та Росії. Проведено філогенетичний аналіз, за допомогою якого було підтверджено наявність нового генотипу 8.

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PREVALENCE OF ENTEROTOXIN-PRODUCING STRAINS OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM COW'S UDDER IN SERBIA

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Staphylococcus aureus is a major cause of food poisoning, due to the production of heat resistant enterotoxins, which when consumed cause vomiting and diarrhea [1]. Enterotoxins are low-molecular weight proteins (26900–29600 Da). To date, 23 different staphylococcal enterotoxins have been described besides toxic-shock syndrome toxin-1, including staphylococcal enterotoxin A to staphylococcal enterotoxin like V (SEA to SEIV) and they can be divided into five phylogenetic groups [2, 3, 4]. All share superantigenic activity, whereas, only few of them (SEA to SEI, SER, SES, and SET) have been proved to be emetic [4, 5, 6]. SE and SEI have been classified based on their amino acid sequences [3, 4, 7].