

CASE REPORT

AVIAN LEUKOSIS VIRUS TYPE J (ALV-J) IN THE CZECH REPUBLIC

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The paper describes the first occurrence of myelocytomatosis in the Czech Republic. A group of hens from the breeding flocks of the meat type aged 27 and 50 weeks with increased mortality and tumorous disease occurrence was examined. Necropsy and pathohistology proved myelocytomatosis. The presence of the ALV subtype J virus was proved using PCR.

Breeding flocks, myelocytomatosis, ALV subtype J

The avian leukosis virus type J (ALV-J) has become highly prevalent in poultry flocks world-wide during the last 10 years. The virus representing a new ALV subgroup causes in breeding flocks of the meat type not only decreased yield but also increased mortality rates due to myeloid leukosis (myelocytomatosis) and other tumors. The infection has also been found in commercial broiler flocks (Payne et al. 1991; Payne 1998).

In this paper we describe the first case of this disease in the Czech Republic.

In June 1998 we performed examinations of a group of meat type (ROSS 208) hens aged 27 and 50 weeks originating from two breeding flocks with long-lasting increased mortality. The hens were of good nutritional state showing no clinical signs of disease. The animals were euthanized and subjected to post-mortem examination and pathohistology.

There were found nodules up to a chestnut size in the skin and subcutis localized caudally to the comb and laterally on the neck, clearly marked, of solid consistency in section, homogenous dark red colour. Lesions were diagnosed to be organising hematomas. There were also pea-sized skin cysts filled with keratin and cellular detritus, which were determined to be inclusion follicular cysts with sporadic ruptures and subsequent chronic granulomatous dermatitis. Their pathogenesis was probably traumatic. In one case we found a solid mass of a small apple size on the neck base.

Parenchymatous organs (liver, spleen, kidney, lung, ovary) showed different degrees of edematous enlargement, only in a part of the kidney and ovary there were protruding grey white nodules.

Liver and spleen enlargement was of the hepatosplenomegaly character with grey-red coloration and fragile consistency. In one case we observed a sharply demarcated nodule of a walnut size in the mesovarium, which was of grey pink colour in section, and with fascicullar structure, and a shapeless grey structure in the area of pancreas.

The organ enlargement was caused by infiltration of tissues by masses of cohesively connected tumorous cells with high mitotic index and islands of necrotic cells. Liver affected to a lesser extent had tumorous infiltration concentrated within portal areas, whereas more intensive affection was characterised by diffuse infiltration and intrasinusoidal infiltration.

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Atrophy of the lymphatic perielipsoidal lymphatic tissue and colonisation of the red and white pulp by tumorous cells was evident in the spleen. Tumorous cells infiltrated intertubular tissue in the kidney as well as the ovarian stroma and connective septa between parabronchi and lung capillaries causing subsequent atelectasis. The invasive growth of tumorous cells destroyed the parenchyma of affected organs.

Most tumorous cells were cytologically mature myeloid cells with marked eosinophilic granules in the cytoplasm and large, vesicular, excentric nuclei and one or two nucleoli. The cells resembled normal granular myelocytes of the bone marrow.

Besides the granular myelocytes, we occasionally found in the liver and spleen various quantities of cells with slightly basophilic cytoplasm and almost concentric large nucleus with several nucleoli typical of normal myeloblasts. Markedly dark basophilic cytoplasm, perinuclear halo and large oval nucleus characterised erythroblasts.

We occasionally found pancreatic adenocarcinoma and leiomyoma of the mesosalpinx.

Solitary and multiple grey white nodules on body cavity serosae were frequent. We observed plaques or protruding shapeless, grey white tumorous masses on the periosteum of outer and inner surface of sternum, pelvis and costochondral junction. Sporadic grey green masses in the form of bands parallel to the muscle fibres were found in breast muscles. Histologically it was proved that it is the same tumorous proliferation of myelocytes as in the parenchymatous organs and invading the periosteum and the surface area of the compacta of flat bones of the pelvis and ribs.

Tumorous lesions of organs were not infrequently complicated by ischaemia caused by ruptures of the enlarged liver and intraperitoneal hematomas under various stages of resorption and organisation, atrophy, hemorrhages into the ovarian stroma and follicles. Yolk masses in the body cavity and fibrinous pleuroperitonitis and pericarditis were frequently found.

On the basis of necropsy, histology and cytology of tumorous cells we diagnosed the changes as myelocytomatosis of poultry.

For the purpose of proving the presence of the ALV-J we used the PCR technique. DNA was isolated from the tissue of a solid tumor – approximately 0.05 g of frozen tissue was transferred into 500 µl of TEN buffer and 1 µl of RNA-se A (Sigma, St. Louis, MD, USA; 10mg/ml), 25 µl of 10% SDS and 5 µl of proteinase K (Sigma, St. Louis, MD, USA; final concentration of 0.1mg/ml) were added. DNA was purified using phenolchloroform extraction with subsequent precipitation by ethanol and then dissolved in 50 µl nuclease-free water.

Oligonucleotide primers H5 and H7 were used in the PCR (Smith et al. 1998). Amplifications were performed in 25 µl PCR mixture containing 100 mM Tris-HCL, 15 mM MgCl₂, 500 mM KCl (pH 8.3) and 25 mM MgCl₂, 250 mM dNTPmix, 20 U/µl Taq polymerase (PCR Core Kit, Boehringer Mannheim GMBH, Germany), 20 pmol corresponding primers and 300 ng of template DNA. A “touch down” PCR program (Don et al. 1991) consisting of the following steps was used for the amplification: denaturation at 93 °C for 1 min, annealing at 60 °C for 1 min decreasing by 1 °C in each cycle and extension at 72 °C for 1 min 20 s for 13 cycles, followed by 30 cycles of 93 °C for 1 min, 48 °C for 1 min, 72 °C for 1 min 20 s. Samples were evaluated using electronic dual-light transilluminator (Ultra-Lum, Inc.) following electrophoresis (85 V/45 minutes) on 2% agarose gel with ethidium bromide; marker 100 bp DNA Ladder (Promega Corp., Madison, WI, USA) served as a control. Positive samples (Plate XIII, Fig. 1) resulted in the formation of a PCR product of 545 bp which was specific for the ALV-J (Smith et al. 1998) and proved the presence of the avian leukosis virus type J in the tumor examined.

Virus aviární leukózy typu J (ALV-J) v České republice

Práce popisuje první výskyt myelocytomatózy v České republice. Byla vyšetřována skupina slepic z rozmnožovacího chovu masného typu ve věku 27 a 50 týdnů, kde došlo k výskytu nádorového onemocnění a zvýšení mortality. Patologickým a patohistologickým vyšetřením byla zjištěna myelocytomatóza. Přítomnost viru ALV subtypu J byla potvrzena technikou PCR.

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Plate XIII

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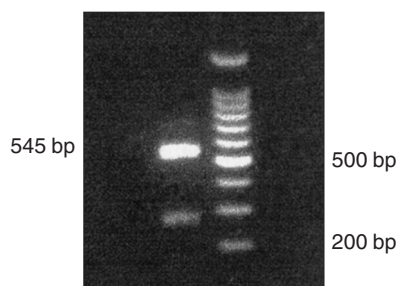


Fig. 1. Ethidium bromide stained agarose (2%) gel showing PCR specific product (545 bp). In the PCR we used DNA isolated from cells of a solid tumor of diseased hens and primer couples H5 and H7 amplifying only the ALV-J proviral DNA (Smith et al. 1998).