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Avipoxvirus in blackcaps (Sylvia atricapilla)

Pavel Kulich^{1*}, Eva Roubalová², Lenka Dubská², Oldřich Sychra², Bedřich Šmíd¹, Ivan Literák²

¹*Veterinary Research Institute, Hudcova 70, Brno 621 00, Czech Republic*

²*Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, Brno 612 42, Czech Republic*

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*To whom correspondence should be addressed. Tel.: +420 732162163. Fax: +420 541211229.

E-mail: kulich.pavel@seznam.cz

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Avipoxvirus in blackcaps (*Sylvia atricapilla*)

Pavel Kulich^{1*}, Eva Roubalová², Lenka Dubská², Oldřich Sychra², Bedřich Šmíd¹, Ivan Literák²

Abstract

From July to September 2005, 1075 wild birds of 37 species were mist-netted at a location in the north-eastern part of the Czech Republic. The birds were examined for the presence of avipoxvirus lesions. This was demonstrated by electron microscopy in skin lesions in nine blackcaps (*Sylvia atricapilla*) of 244 examined (prevalence 4 %). Blackcaps skin biopsies were processed using the ultrathin section method. In skin biopsies, avipoxviruses were demonstrated in intracytoplasmic inclusions where, in addition to mature viruses, there were found lipids and filamentous structures concentrated into large circular formations. The so-called additional inclusions were also found. These did not contain any virus components, and they served as the precursor of A-type intracytoplasmic inclusions. Blackcap avipoxvirus was isolated by passage on chorioallantoic membrane (CAM) of 9-day-old chicken embryos. The virus was successfully adapted after 11 passages (each passage lasted 5–7 days), at which time a marked change in the form of tiny nodules 2–3 mm in diameter was produced on the CAM. Further identification of field isolates and of the cultured virus was carried out by the use of polymerase chain reaction (PCR) and sequencing. Sequences were compared to consensus sequences of both canarypox and fowlpox viruses. Our sequence was found to be 98.8 % identical to the canarypox consensus sequence, but only 63 % identical to the

fowlpox consensus sequence. Our avipoxvirus sequence was proven to be significantly more closely related to canarypox viruses than to fowlpox viruses also by phylogenetic analysis.

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Introduction

Avian poxviruses of the genus *Avipoxvirus* (family *Poxviridae*) cause infection in a broad spectrum of domestic and wild birds (Gough *et al.*, 1988; Tripathy *et al.*, 1997; Bolte *et al.*, 1999). At least 232 avian species of 23 orders are sensitive to avipoxvirus infection (Bolte *et al.*, 1999). The avipoxvirus genomes hitherto sequenced completely are genomes of fowlpox virus (FPV) infecting the domestic chicken (*Gallus gallus*) and turkey (*Meleagris gallopavo*) (Afonso *et al.*, 2000) and of canarypox virus (CNPV) (Tulman *et al.*, 2004). Avipoxviruses, which cause infection also in a number of other avian species, need to be characterised in greater detail both from the genetic and biological properties viewpoints.

Avipoxviruses are the causative agents of acute contagious disease characterised by proliferative skin lesions around the beak, on the eyelids, crest, feet and all other body areas relatively free of feathers, as well as by fibrous necrotic proliferative lesions on the mucous membrane of the oral cavity, oesophagus and upper section of the respiratory tract (Tripathy & Red, 1997). The systemic form of the avipoxvirus infection is rare. Avipoxvirus infection diagnostics are based mainly on clinical, histopathological and electron microscopy examinations. Electron microscopy is a very useful method for the direct demonstration of avipoxviruses.

The mature avian poxvirus is brick-shaped, 250 × 350 nm in size. On its surface, there is an envelope made of microfilaments (Carter & Cheville, 1981). On the cross section, the virion is made up of a centrally situated electron-dense biconcave core. There are two cavities in the core containing one lateral body each. The core consists of a nucleoid containing the linear double-stranded DNA (Tripathy, 1997).

Poxvirus replication takes place in so-called virus factories (Novoa *et al.*, 1997; Boulanger, 2000). Virus factories are electron-dense zones in the cell cytoplasm containing

no cellular organelles (Dales & Siminovitch, 1961; Morgan, 1976). The first visible structure appearing in the course of virion replication is a membrane structure resembling a crescent. The crescent gets gradually longer and begins to encircle the fine, slightly electron-dense material. The opposite ends of the crescent meet, creating a circular structure that contains the core. These circular structures are called intracellular virions (IV). The virion core begins to elongate to form an ellipse, flattened at the sides by two lateral bodies. The virion begins to mature to its characteristic brick-shape, which is called the intracellular mature virion (IMV), and is infectious. The IMV leaves the virus factory and exits the cell by budding through the cytoplasmic membrane or by cellular cytolysis (Boulangier *et al.*, 2000, Hatano *et al.*, 2001).

In the present study, the prevalence of infection was investigated and electron microscopy observations and sequences analyses of avipoxviruses in blackcaps (*Sylvia atricapilla*) were performed. Avipoxvirus in blackcaps was first documented in 1999 by clinical and histopathological examination in Slovakia (Literák *et al.*, 2001) and later also in the Czech Republic (Rajchard, 2003). In this study, avipoxvirus was demonstrated by negative staining in native preparations of lesions from blackcaps, and the morphogenesis of this avipoxvirus was investigated using ultrathin sections of tissue samples fixed in glutaraldehyde. Avipox viruses from blackcaps were analysed using PCR and partially sequenced. Sequences were compared to another known avipox viruses sequences and phylogenetically analysed.

Material and methods

Location, examined birds, collection of material. Investigation of avipoxvirus lesions in birds was performed at Čerták (49° 34' N, 17° 59') in the north-eastern part of the Czech Republic, Sub-Beskydian Hills, which are the foothills of the Western Beskyds and the westernmost part of the Western Carpathian Mountains. It is a densely populated area with intensive agriculture. The area is covered with a mixed forest. The dominant woody species are maples (*Acer* spp.), European ashes (*Fraxinus excelsior*), oaks (*Quercus* spp.) and common spruces (*Picea abies*). Birds were captured in mist nets placed across a forest aisle near a stream. Some mist nets were placed along the forest edge adjoining cattle pastures. The shrub growth along the forest edge consisted mainly of black elder (*Sambucus nigra*), and the dominant species along the forest edge included blackthorn (*Prunus spinosa*) and dog rose (*Rosa canina*). The location lies 370–400 m above sea level.

Birds were netted from 29 July 2005 to 24 September 2005, and the nets were checked once every hour. The birds were determined, examined and released back to the wild. Each bird was visually and clinically examined with special attention given to proliferative skin lesions, especially on the legs, around the beak, the cloacae and on sparsely feathered parts of the body. Each skin lesion was recorded, and a bioplate 1 mm³ in size was taken from it. Bioplates were taken by an excision with a sterile scalpel and stored for ultra thin sections preparation in a microfuge tube containing 3 % glutaraldehyde. During the netting, two blackcaps with nodular lesions on their legs died. Immediately afterwards, the bird cadavers with suspect avipoxvirus infection were frozen at -20 C for subsequent electron microscopy examination, virus isolation and DNA analysis.

Electron microscopy. Bioplates 1 mm³ in size were taken from skin lesions from the two frozen cadavers for negative staining. Each bioplate was homogenised and suspended within a drop of distilled water. The resulting suspension was covered with a grid coated with

Formvar film (Sigma-Aldrich) and carbon. The grid was removed from the suspension after 10–15 sec, and the residual water was dried with a strip of filtration paper. A drop of NH_4MoO_4 was placed onto the grid for a few seconds, and excess stain was dried with filtration paper. Sections were observed under a Philips 208 electron microscope at 18000 x magnification and an accelerating voltage of 90 kV.

Glutaraldehyde-fixed pieces of skin were rinsed in Millonig phosphate buffer, post-fixed in 1 % OsO_4 solution (in Millonig phosphate buffer), dehydrated in acetone, embedded in Epon-Durcupan mixture (Epon 812 Serva, Germany; Durcupan, ACM Fluka, Switzerland) and polymerized at 60°C for 72 h. Ultrathin sections were cut with glass knives on LKB ultramicrotome. The sections were stained with uranyl acetate and lead citrate and examined under a Philips EM 208 electron microscope.

Virus isolation. Bioplates of skin lesions taken from the two blackcap cadavers were homogenised by grinding in a mortar and then suspended in phosphate buffered saline (PBS). The suspension was centrifuged for 30 min at 4000 x g and 4°C. The supernatant was filtered through a 450 nm filter. The filtrate was inoculated on specific pathogen free (SPF) chorioallantoic membrane (CAM) of 9-day-old chicken embryos. The embryos infected were incubated for 5–7 days at 37°C. They were then cooled for 3 hours and the chorioallantoic membrane was removed. Its thickness and the presence of characteristic nodules (pocks) were evaluated macroscopically. A section (1 cm²) of the CAM was homogenised and suspended in distilled water. The suspension was used for electron microscopy avipoxvirus assay. The rest of the CAM was homogenised, centrifuged and filtered through a 450 nm filter. A part of the filtered virus suspension (0.25 ml) was used for CAM inoculation for the next passage. In this way, a total of 11 passages were made. After each passage, CAM was studied under the electron microscope for the presence of avipoxvirus.

DNA isolation, PCR amplification, sequencing and sequences analyses. DNA was isolated from pox skin lesions from two blackcaps and from the CAM containing isolated virus culture after 11th passage. From both CAM and tissue specimens, the DNA was isolated using NucleoSpin® Tissue kit (MACHEREY-NAGEL), according to the manufacturer's instructions. Skin lesions were cut into small pieces prior to DNA isolation. Tissue samples were lysed overnight at 56°C in the presence of the lysis buffer and proteinase K.

Primers designed to amplify DNA from both fowlpox and canarypox viruses were used (Thiel *et al.*, 2005). These primers were used to amplify the 3' part (about one half) of the sequence of thymidine kinase (TK) gene (FPV086, CNPV113), small part of the 5' end of the gene encoding for the host targeting (HT) motif protein (FPV087, CNPV114) and the intergenic region between these two genes. PCR amplification was performed as described previously (Thiel *et al.*, 2005).

PCR products were purified using QIAquick® PCR Purification kit (Qiagen) and resuspended in 50 µl of redistilled H₂O. Purified PCR products were sequenced on both strands, using ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) and acquired sequence was submitted to GenBank.

Analyses of the acquired sequences, including alignments and phylogenetic analysis were carried out using Chromas Lite 2.01 (Technelysium), VectorNTI Advance® (Invitrogen) and Basic Local Alignment Search Tool (BLAST) (National Center for Biotechnology Information) (<http://www.ncbi.nlm.nih.gov/BLAST>) softwares.

GenBank accession number. EU124667

Results

Findings of nodular lesions and confirmation of avipoxvirus infection in wild birds at Čerták. Nodular lesions on unfeathered parts of the body, especially on the legs, around the eyes and cloacae suspected as avipoxvirus infection were observed in nine blackcaps (of 244 blackcaps examined), as well as in *Sylvia communis* (1 positive/13 examined), *Prunella modularis* (2/36), *Erithacus rubecula* (1/155) and *Motacilla cinerea* (1/5). Using the negative staining and ultrathin section techniques, avipoxvirus presence was demonstrated in the lesions of all nine blackcaps. Avipoxvirus prevalence in blackcaps was 4%. Neither avipoxviruses nor other viruses were demonstrated in nodular lesions of the other bird species.

Nodular lesions were not found in *Turdus philomelos* (45 examined), *T. merula* (26), *Parus major* (44), *P. caeruleus* (24), *Phylloscopus collybita* (39), *Aegithalos caudatus* (23), *Ficedula hypoleuca* (23), *Troglodytes troglodytes* (18), *Sylvia borin* (16), *Parus montanus* (14), *Emberiza citrinella* (13), *Certhia familiaris* (12), *Lanius collurio* (12), *Muscicapa striata* (12), *Parus palustris* (10), *Pyrrhula pyrrhula* (9), *Fringilla coelebs* (8), *Sitta europaea* (7), *Locustella naevia* (6), *Phylloscopus trochilus* (6), *Ficedula albicollis* (5), *Phylloscopus sibilatrix* (4), *Phoenicurus phoenicurus* (4), *Regulus regulus* (3), *Coccothraustes coccothraustes* (2), *Hippolais icterina* (2), *Sylvia curruca* (1), *Acrocephalus palustris* (1), *Dendrocopos major* (1), *Picus canus* (1), *Alcedo atthis* (1) and *Crex crex* (1).

Visual inspection of the two blackcap cadavers showed that the birds were emaciated, with frequent proliferative skin lesions on the legs, digits and around the beaks and the eyes. Findings on the remaining seven blackcaps included nodular lesions, sometimes with necroses, on legs, digits, at the flexure of the wing underside, on the abdominal skins under the breastbone

Electron microscopy. In negative staining, virus particles appeared as heavily electron dense bodies. Virus particles were oval in shape and 200×350–400 nm in size. The virion surface was covered with randomly arranged microfilaments (Figure 1A). Enveloped virus particles without surface microfilaments were observed sporadically (Figure 1B).

In ultrathin sections, extracellular virus particles with membranes on their surfaces (EEV– extracellular enveloped viruses) and intracellular particles with a single membrane (IMV– intracellular mature viruses) were observed (Figure 1C). Both types of virus particles contained the core (Figure 1C - co), which was biconcave in shape (i.e. barbell-shaped), and two lateral bodies (Figure 1C - lb).

The ultrathin sections captured some stages of the developmental cycle of avipoxviruses. In addition to intracellular virus particles (Figure 1D - 4), we have observed A-type cellular inclusions (ATI) (Figure 1E, G, H) containing viruses, large amounts of filamentous structures arranged in circular formations (Figure 1H) and lipid matrix. Cells contained also circular formations without virus particles (additional inclusions) formed by Golgi complex (Figure 1D-Go). Additional inclusions contained only protein material (Figure 1D - AI). The so called virus factories (Figure 1D, E - vf) were also captured using ultrathin sections. The virus factory (Figure 1D) contained crescent (Figure 1D - 1), circular structure containing fine granular material (Figure 1D - 2), condensation of nucleoid inside of the circular structure (Figure 1D - 3) and intracellular mature virus (Figure 1D-4). Mature viruses are shown to bind the additional inclusion (1D - arrow). Virus particles leaving the host cell by budding through cytoplasmic membrane are shown on Figures 1E (arrows) and 1F.

Virus isolation. Avipoxviruses from blackcaps were not demonstrated in the first seven passages on CAMs. The membranes were not thickened, nor did they show pocks characteristic for pox viruses. Negative staining and ultrathin sections analysis techniques were equally unable to demonstrate avipoxvirus in CAMs in the first seven passages. The changes on CAMs in the form of pocks was first manifested at the eighth passage, when uniquely 2–3 white nodules 0.5 mm in size were observed on one CAM without the CAM thickening. Marked proliferative lesions were observed at the tenth passage, the CAM was thickened, nodules had grown to 1–2 mm in size, and their number had quadruplicated. At 11th passage, the CAM was markedly thickened and congested, with a large number of overlapping nodules 2 mm in diameter (Figure 2). Electron microscopy demonstrated avipoxvirus particles using the negative staining technique.

We have isolated total DNA from two samples of the pox skin lesions and from sample of the CAM containing the 11th passage of the virus. Isolated DNA was amplified and sequenced. The acquired sequences were mutually compared and differences were found neither between the two field isolates nor in the sequence of the passaged virus. The 404 bp long sequence was deposited in the GenBank (accession number: EU124667).

Sequence analysis. Regions of seven fowlpox sequences (AF396867.1; AJ581527.1; AF198100.1; X52860.1; AJ223385.1; D00321.1; M16617.1) and of four canarypox sequences (D86731.1; D78347.1; AY318871.1; AY631870.1) obtained using BLAST, corresponding to our sequence, were aligned and consensus sequences of canarypox virus and of fowlpox virus were established separately. Our sequence was then compared to both canarypox and fowlpox consensus sequences. Our sequence was found to be 98.8 % identical to the canarypox consensus sequence, but only 63 % identical to the fowlpox consensus sequence. Our sequence differed from the canarypox consensus sequence only in five out of

404 base pairs (positions 34, 59, 65, 221 and 262 of our sequence). All the variations from the canarypox consensus sequence appeared in previously sequenced canarypox isolates.

To specify the relations between the particular viruses, the above-mentioned sequences were aligned and the phylogenetic tree was designed using the multiple sequence alignment application of the Vector NTI advance[®] software, AlignX, that carries out the phylogenetic tree construction based on the Clustal W algorithm using the Neighbor Joining method. As seen on Figure 3, all the canarypox virus sequences clustered together, being separated by a long branch from all the fowlpox virus sequences. Our avipoxvirus sequence was proven to be significantly more closely related to canarypox viruses than to fowlpox viruses.

To find whether and how the studied sequences differ on the protein level, we have translated our sequence and consensus sequences of both canarypox and fowlpox viruses using Vector NTI advance[®] software. Comparing the translation of our sequence (83 AA), which represents the 5' end of the TK gene, to the translations of consensus sequences of both canarypox and fowlpox viruses showed that our sequence was 97.6 % identical to canarypox consensus translation. It differed only in two amino acids (isoleucine instead of threonine on position corresponding to position 107 of CNPV113 and isoleucine instead of methionine on position corresponding to position 169 of CNPV113). Both these changes also appeared in some of the aminoacid sequences of previously described canarypox isolates.

The other above-mentioned nucleotide substitutions did not result into changes of amino acids, because they were either synonymous substitutions (changes on positions 59 and 65 of our sequence) or were not in the coding region (changes on position 262 of our sequence). In contrast, our sequence differed from the corresponding region of fowlpox consensus translation in 24 positions (72 % identity).

Discussion

Blackcaps are common passerine birds nesting in Europe. They usually winter in the Mediterranean and further down in Africa and to the south of the Sahara. Blackcaps regularly and abundantly nest over the entire territory of the Czech Republic, from lowlands to the upper forest border (Hudec *et al.*, 1983). They leave their nesting grounds for wintering grounds in August and September. A majority of birds from the populations in the Czech Republic migrate in a south-easterly direction across Asia Minor, and Cyprus, that ranks among countries with the highest number of findings of birds ringed in the Czech Republic. Findings from the winter months are an indication that birds from Czech populations winter in southern Mediterranean regions. It is probable that both locally nesting birds as well as migrating birds were examined in our study, and for that reason it was impossible to determine the place of origin of the infected birds or identify the source of their infection with any degree of accuracy.

Lesions found in blackcaps were characteristic for the cutaneous form of avipoxvirus infection. Proliferative skin lesions on the distal parts of legs may hinder birds in their movement, particularly when collecting food. This handicap can therefore have even fatal consequences for the infected birds, especially at the time of increased energy expenditure during nesting and migration. No specific reasons for the deaths of two infected blackcaps were discovered, although the birds were emaciated. They were handled in the same manner as the other birds netted, and the others survived all the handling without any consequences. The overall ill health and debilitation caused by the avipoxvirus infection may have conditioned death of the two blackcaps during our field research.

Birds with slight skin lesions may recover, whereby a major role in virus elimination will be played by cellular immunity. In some birds, avipoxviruses may exist in a latent form (Deem, 1997; Ciganovich, 1999) and the virus may survive for months or even years in dry skin crusts (Ritchie, 1995). It therefore appears very likely that the virus may survive in some members of the population and, through vectors (Proctor & Owens, 2000), or possibly even through direct contact between parents and their young, may persist in sensitive bird populations.

No avipoxvirus lesions were found in any other bird species netted at that locality, including a large number of birds of the Sylviidae family or *Sylvia* genus. This appears to indicate that the avipoxvirus found is species-specific for the blackcap.

Avipoxvirus infections with typical nodular lesions in blackcaps have so far been found and histologically confirmed only in one of 81 blackcaps tested in Slovakia in July 1999 and in one of 14 blackcaps in the Czech Republic from a location different than that used in August 1999 (Literák *et al.*, 2001; Rajchar & Rachač, 2001). In the present study, avipoxvirus infection in blackcaps in the Czech Republic was scientifically demonstrated by avipoxvirus assay.

Avipoxvirus infections will probably be much more frequent, in Central Europe at least, than might be assumed from papers published to date. Lesions characteristic for the skin form of an avipoxvirus infection have been photo-documented by a number of field ornithologists operating in Central Europe. Blackcaps with probably avipoxvirus nodular lesions have been found in central Germany (September 2001, A. Goedecke, personal communication), south-east Poland (September 2004, M. Jendra), Slovakia (July 2003, P. Žďárek; April 2004, P. Procházka) and in other places in the Czech Republic (August 2002, J. Stolarczyk; July 2003, V. Kovář; August 2003, M. Hromádko; August and September 2003, J. Klápště; August 2004, M. Haluzík & E. Pernická; August 2007, J. Bartoníček). In

one of the latter cases, avipoxvirus etiology was confirmed by histological testing (not published).

Most of the findings that are demonstrably or probably avipoxvirus nodular lesions were observed from the July to September, i.e. from the post-nesting to migration period. This may be due to a degree of infection dynamics among Central European blackcap's populations as well as increased activity of field ornithologists – bird ringers – at the time of post-nesting migrations. From the view point of avipoxvirus infection prevalence dynamics and the importance of avipoxvirus infection in blackcaps, it seems desirable to monitor blackcaps of a defined population at a location with avipoxvirus prevalence from their arrival from wintering grounds, through the nesting period, and until they depart again for their wintering grounds.

Transmission electron microscopy allowed us to monitor different maturation stages of avipoxviruses and their behaviour in cells during a natural infection in blackcaps. In most respects, individual stages of avipoxvirus replication in blackcaps are consistent with results of existing studies on CNPV and FPV (Sadasiv *et al.*, 1985; Boulanger *et al.*, 2000).

We have observed the formation of envelopes and of virus inclusions. Mature avipoxvirus particles acquire an additional Golgi complex-derived membrane from cytoplasmic vesicles (Stannard *et al.*, 1998; Boulanger *et al.*, 1999). A virion thus enveloped is released from the cell by budding through the cytoplasmic membrane. Another possibility of acquiring a membrane is the virion's binding on extended Golgi complex containing a lipid matrix and filamentous structures (Stannard *et al.*, 1998). Viruses entering a Golgi complex acquire a membrane and form an intracytoplasmic inclusion of the A-type. Stannard *et al.* (1998) described additional inclusions in infected cells that did not contain virus particles but only electron dense protein material. Viruses enter such additional inclusions and transform them into A-type inclusions (ATI).

During our examinations, we have found a large number of ATI. Very interesting was the discovery of a large number of filamentous structures arranged in circular formations inside of ATI. Figure 1H (fi) shows the first electron microscopy image of these filamentous structures. The findings of additional inclusions correspond to results described by Stannard *et al.* (1998). We have observed binding of virus particles on that additional inclusion, but we did not observe any virus particles leaving a virus inclusion. Virus particles are probably released from the inclusion only after cellular cytolysis. We have sporadically recorded viruses leaving cells by budding.

The introduced DNA data show that both analysed field isolates obtained from blackcaps have the same sequences, suggesting that both analysed viruses could belong to the same strain. The analysed sequences have closer relation to canarypox viruses than to fowlpox viruses. This finding is consistent with the fact that blackcaps belong to the same order as canaries (order Passeriformes), contrary to domestic chicken and turkey (FPV hosts), that belong to order Galliformes.

The fact that sequence of DNA isolated from the passaged virus did not differ from the sequences of field isolates shows, that the analysed region is probably not important for virus host specificity. We presume, that the sequence of the passaged virus might differ in other regions, such as one of the HT motives (e.g. CNPV114), but this assumption has to be confirmed by further investigation on this field.

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Figure 1. *Avipoxviruses from blackcaps. Morphogenesis documented by electron microscopy:*

A – B: electron micrographs, negative staining method. A – enveloped virus, his surface is covered with randomly arranged microfilaments characteristic for avipoxviruses;

B – enveloped virus without surface microfilaments;

C– H: electron micrographs, ultrathin sections. C – intracellular mature virus with core (c), lateral bodies (lb) and envelope (arrow); D – stages of development cycle of virus particles in intracelular compartment described as virus factory: crescent (1), circular structure with fine granular material (2), condensation of nucleoid inside of the circular structure (3), intracelLular mature virus (4), the additional cellular inclusion containing protein material, viruses binding to the inclusion (arrow), Golgi complex (Go) ; E – A-type intracytoplasmatic inclusion (ATI) containing viruses, microfilaments, lipid matrix, virus factories (vf) and viruses budding from the cell (arrows); F – virus particle obtaining an external surface envelope while leaving the host cell; G – A-type intracytoplasmatic inclusion, containing mature virus particles (IMV) and lipid droplets; H – detailed view of intracytoplasmatic inclusion containing large amount of filamentous structures (fi), lipid droplets (ld) and of intracelular mature viruses (IMV).

Figure 2. *Pock lesions on chicken chorioallantoic membrane (CAM) during 11th passage: CAM markedly thickened, congested and with numerous pocks caused by blackcap avipoxvirus.*

Figure 3. *The phylogenetic tree of an alignment of 404 bp region of DNA sequences from twelve avipoxviruses (nucleotide databases accession numbers are referred-to), including our isolate (EU124667). The calculated distance values are specified in parenthesis following the sequence accession number.*

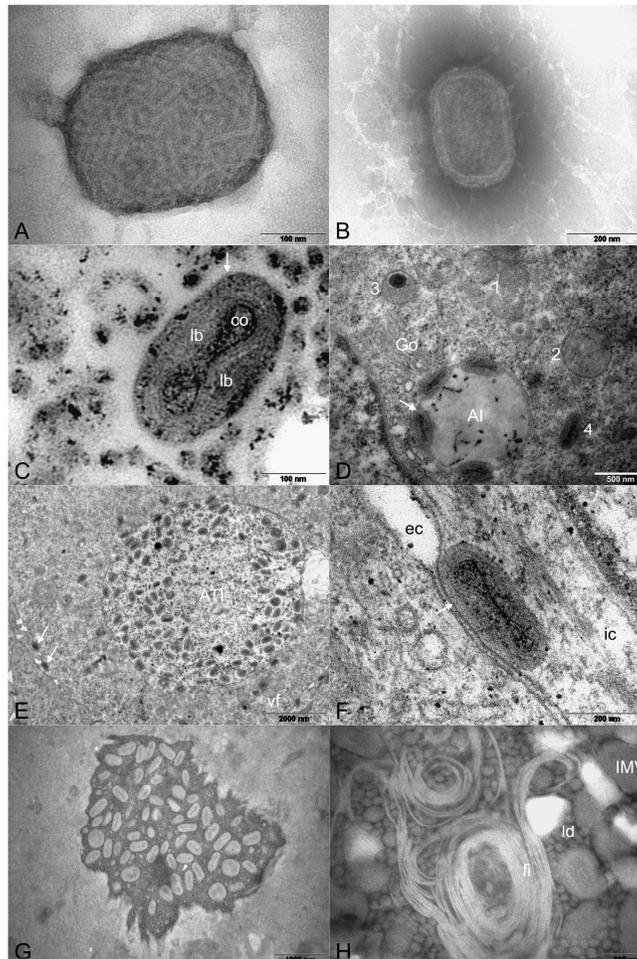


Fig. 1.

Figure 1. Avipoxviruses from blackcaps. Morphogenesis documented by electron microscopy: A and B: electron micrographs, negative staining method. A: enveloped virus, his surface is covered with randomly arranged microfilaments characteristic for avipoxviruses; B: enveloped virus without surface microfilaments; C, D, E, F, G and H: electron micrographs, ultrathin sections. C: intracellular mature virus with core (c), lateral bodies (lb) and envelope (arrow); D: stages of development cycle of virus particles in intracellular compartment described as virus factory: crescent (1), circular structure with fine granular material (2), condensation of nucleoid inside of the circular structure (3), intracellular mature virus (4), the additional cellular inclusion containing protein material, viruses binding to the inclusion (arrow), Golgi complex (Go) ; E: A type intracytoplasmic inclusion (ATI) containing viruses, microfilaments, lipid matrix, virus factories (vf) and viruses budding from the cell (arrows); F: virus particle obtaining an external surface envelope while leaving the host cell; G: A type intracytoplasmic inclusion, containing mature virus particles (IMV) and lipid droplets; H: detailed

view of intracytoplasmic inclusion containing large amount of filamentous structures (fi), lipid droplets (ld) and of intracellular mature viruses (IMV).

209x296mm (300 x 300 DPI)

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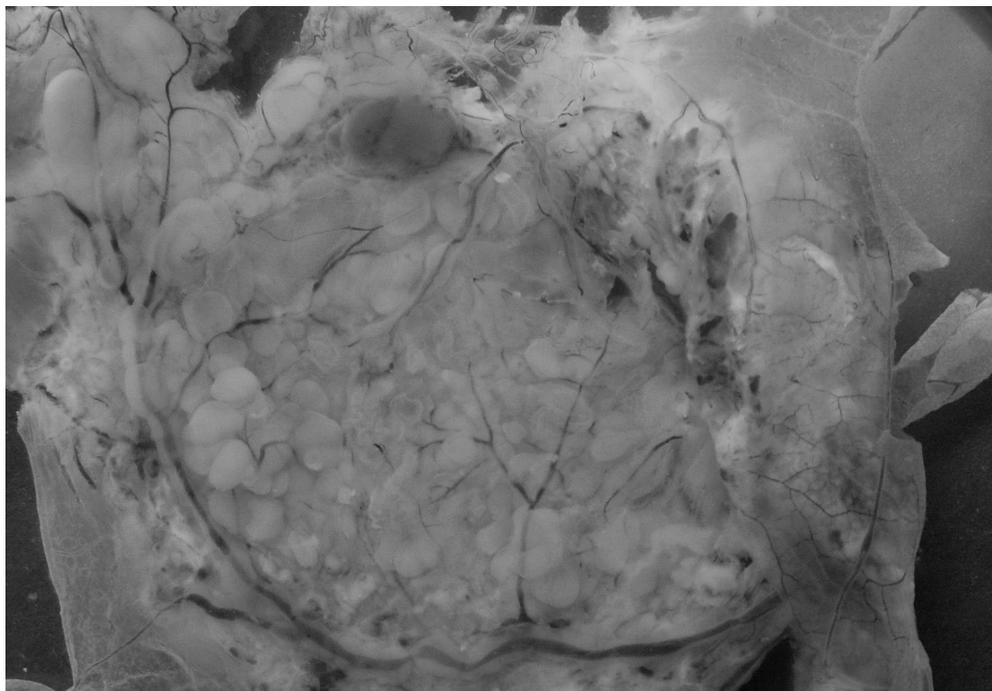


Figure 2. Pock lesions on chicken chorioallantoic membrane (CAM) during 11th passage: CAM markedly thickened, congested and with numerous pocks caused by blackcap avipoxvirus.
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