AVIPOXVIRUS INFECTION IN WILD BIRDS: NEW FINDINGS FROM SLOVAKIA AND POLAND

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Abstract

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From 1996 to 2000, wild birds from five locations in Slovakia and one in Poland were tested for the presence of avipoxvirus lesions. A total of 2,509 birds of 77 species were tested. A retrospective analysis of photographs made in 1984 of cutaneous lesions of birds from a Slovak location was also performed. Avipoxvirus infections were diagnosed by histopathological examination of cutaneous lesions of *Sylvia atricapilla* in Socovee (1999) and *Prunella modularis* in Tatranská Javorina (2000) in Slovakia, and of *Lanius collurio* in Krempna (2000) in Poland. An analysis of photographs revealed characteristic avipoxvirus nodular skin lesions in another two specimens of *Prunella modularis* from Socovee (1984). Analogous skin changes observed in *Prunella modularis* from Socovee (1999), in *Sylvia curruca* from Ruské (1997) and *Parus montanus* from Krempna (2000) were most probably also caused by avipoxvirus.

Vertebrates, disease, cutaneous lesion, virus

Viruses from the family Poxviridae may infect various vertebrates. In birds, the infection is caused by the genus Avipoxvirus. It is still, however, unclear how many species, strains or varieties exist within the genus (Bolte et al. 1999). Clinical and pathological changes caused by various avipoxviruses are nevertheless very characteristic, and the disease in birds may occur in cutaneous or diphteric/pharyngeal forms, or both. Avipoxvirus infections occur in all parts of the world. In a large recent review, about 232 species in 23 orders were reported to have acquired a natural poxvirus infection (Bolte et al. 1999).

Avipoxvirus infections in different species of wild birds have been reported from a number of European countries. Information from some European countries, however, is still missing and it is not clear whether that is because avipoxvirus infections do not occur there or because they have not been given sufficient attention. We identified avipoxvirus skin lesions in wild birds in two European countries from which only scarce information on avipoxvirus infections had been available. The aim of this paper is to describe the cases and characterize the prevalence of avipoxvirus infections in populations of wild birds in the locations investigated.

Materials and Methods

From 1996 to 2000, wild birds were trapped in different locations in Slovakia (Ruské, Socovce, Blatnica, Necpaly and Tatranská Javorina) and Poland (Krempna) using mist nets. The main objective of the trapping was to visually examine the birds for the presence of skin trematodes *Collyriclum faba*, because foci of their occurrence

had been identified in those areas (Literák and Sitko 1997; Literák and Honza 2000). Visual examination of some of the birds revealed pathological changes of the skin on toes and eyelids (and, exceptionally, also elsewhere) whose morphological character did not correspond to the kind of lesions caused by *C. faba*.

The lesions found there were described, photographed and, in some cases, extirpated. In the case of more extensive changes on legs, the toes affected were amputated. Samples of the tissues removed were fixed in 70% ethanol and further processed by standard histological techniques, i.e. embedded in paraffin, stained with hematoxylin-eosin. In indicated cases, the PAS reaction was also used.

Wild birds were also trapped as a part of an ornithological investigation at one of the Slovak locations (Socovce) investigated by us (Žďárek 1994). At that time, pathological changes in the skin on the legs were also found. Some of those lesions were photographed and recently analysed to determine their possible origin.

Site descriptions

Ruské (Snina district) - 49.07 N 22.21 E, 500 m above sea level

An area abandoned by its former inhabitants in the vicinity of the source of the Cirocha River. Birds were trapped in successive tree growths near the former village of Ruské. At trapping sites, areas with deciduous trees of different age with a predominance of beech *Fagus sylvatica* alternated with meadows. The birds were examined there in August and September in 1996 through 1999. A total of 1,242 birds of 56 species were examined.

Socovce (Martin district) - 48.57 N 18.52 E, 440 m above sea level

Birds were trapped in the lowlands along the Turiec River in the broad Turčianska kotlina basin. Birds were trapped in shrub, reed and meadow growths of the Kláštorské lúky Reserve. The most numerous among shrubs was the willow *Salix caprea*. Previous bird trapping took place here in the summer months of 1982 through 1988. Our research team trapped and examined 514 birds of 41 species here from 25 July to 1 August 1999.

Blatnica (Martin district) - 48.56 N 18.56 E, 940 m above sea level

The trapping site lies in the Veterná Valley of the Veľká Fatra Mountains near a stream lined mainly with growths of beech and spruce *Picea abies*. Birds were trapped between 2 and 8 August 1999. A total of 152 birds of 20 species were examined.

Necpaly (Martin district) - 48.59 N 18.58 E, 760 m above sea level

The trapping site lies near a stream in the Necpalská Valley in the Vel'ká Fatra Mts. The most numerous around the site were beech and spruce trees. Birds were trapped here from 25 July to 6 August 2000. A total of 101 birds of 20 species were examined.

Tatranská Javorina (Kežmarok district) - 49.16 N 20.09 E, 1450 m above sea level

The trapping site lies in the Javorová Valley in the High Tatras Mts. just above the spruce forest line. Birds were trapped in a dwarf mountain pine *Pinus mugo* growth, and along a borderline between the dwarf pine growth and a mountain meadow. A total of 252 birds of 25 species were trapped there between 8 and 19 August 2000.

Krempna (Jasło district) - 49.30 N 21.29 E, 400 m above sea level

The trapping site was on the borderline between farms and large forests in the Beskid Niski Mts. The nets were situated near a stream along the edge of a deciduous forest, and in a shrub growth between farm buildings. A total of 248 birds of 31 species were examined there between 21 and 26 August 2000.

Results

1. Avipoxvirus infections in birds in Ruské

Changes corresponding to avipoxvirus skin lesions were found in one young *Sylvia curruca*. The bird trapped on 4 August 1997 exhibited a well-defined swelling of about 2 mm in diameter on its lower eyelid. The swelling resembled a small cyst of *C. faba* but it was a solid body without any trematodes or suppurative content. Between 1996 and 1999, a total of 8 *S. curruca* from Ruské were examined.

2. Avipoxvirus infections in birds in Socovce

2.1. Results of retrospective analysis of photographs of pathological lesions in the skin of birds trapped in the 1980's

Changes corresponding to avipoxvirus lesions were found in two specimens of *Prunella modularis*. In one of them (adult bird trapped on 5 August 1984), marked nodular changes on the toes of both legs were found (Plate III, Fig. 1). On individual toes, different phalanges were affected. The other young bird trapped on 11 August 1984 had a papule on the lower eyelid and a largish node on the dorsum of the beak (Plate III, Fig. 2).

2.2. Results of bird examination in 1999

Avipoxvirus changes were found in *Sylvia atricapilla* and *Prunella modularis*. The young *Sylvia atricapilla* trapped on 27 July 1999 had characteristic changes on the right upper

eyelid, in a corner of the beak, on the distal end of the right wing, on the toes of both of its legs, and on the tarsus of the right leg (Plate IV, Fig. 3). A large node of about 8 mm in diameter at the distal end of the middle toe of the right leg in the place of a missing claw was amputated and histopathologically examined.

The histopathological examination proved epidermal nodular hyperplasia with central and superficial necrosis of hypertrophic keratinocytes. Along the edges of the lesion, the hyperplasia had the character of an irregular papillomatosis resembling a neoplastic growth – a papilloma. The surface was covered with a thick serocellular crust, with a marked inflammatory reaction and mixed exudate in the surrounding corium. The hypertrophic keratinocytes contained conspicuous eosinophilic and amphophilic intracytoplasmic bodies (Plate IV, Fig 4.). The inclusions were bigger nearer the surface, until they took up almost the entire cytoplasm. The nuclei showed signs of karyopyknosis, karyorrhexis and karyolysis, with final degradation and cellular disintegration.

A total of 81 Sylvia atricapilla were examined at that location.

The young *Prunella modularis* trapped on 1 August 1999 had a grey-white papule on the left lower eyelid. A total of 8 *P. modularis* from the location were examined.

- 3. Avipoxvirus infections in birds in Blatnica and Necpaly
 - No cases of avipoxvirus changes were found at those two locations.
- 4. Avipoxvirus infections in birds in Tatranská Javorina

Avipoxvirus changes in the skin were found in an adult *Prunella modularis* trapped on 10 August 2000. The bird had cutaneous lesions on the toes of the left leg, with an epidermal nodular lesion of about 4 mm in diameter with suppurative necrotic centre in the place of a claw on the distal phalanx. The lesion was extirpated and examined. Histopathological examination of the amputated toe shows epidermal avipoxvirus lesions analogous to those found in the above *Sylvia atricapilla*, but the epidermal nodular hyperplasia was smaller, and the keratinocyte nekrosis was noticeable only under a thin crust on the lesion surface. Intracytoplasmic inclusions were smaller and did not push the nuclei to the periphery. The right toe had a node covered with a crust, and a similar dermal node of about 2 mm in diameter was also on the tarsus. A total of 63 *P. modularis* were examined.

5. Avipoxvirus infections in birds in Krempna

Avipoxvirus changes were found in *Lanius collurio*, a young bird trapped on 23 August 2000. The upper lid of one of its eyes was much swollen (swelling of about 3 mm in diameter), and the lesion continued through the conjunctiva to the eyeball. The tissue taken from the conjunctiva was a macroscopically amorphous grey and pink mass. From the histophathological point of view, it was characterized by clusters of desquamated keratinocytes and predominantly cellular crust with remains of nuclear chromatin. The keratinocytes contained conspicuous intracytoplasmic bodies. This *L. collurio* was the only bird of that species trapped at Krempna.

In the young *Parus montanus* trapped on 24 August 2000 we found an approximately 2 mm papule distally from the corner of the beak. On palpation it resembled a small skin *C. faba* cyst, but it was a solid body which, when cut, contained no trematodes or a suppurative content. A total of 16 *P. montanus* were examined at the location.

Discussion

A histopathological examination of lesions found in *Sylvia atricapilla* in Socovce (in 1999), *Prunella modularis* in Tatranská Javorina (2000) and *Lanius collurio* in Krempna (2000) revealed morphological characteristics that are patognomic for the cutaneous form of avian poxvirus infection. Cutaneous lesions are mainly found in sparsely feathered areas and are macroscopically characterized by papules, temporary vesicles and pustules, quite large nodules with a superficial crust and an inflammatory reaction in the surrounding area.

Appendix: List of birds examined for the presence of macroscopic pathological changes.

Species Ruské Socovce Blatnica Necpaly Tatranská Kre						Krempna
•	1996-1999	1999	1999	2000	Javorina 2000	2000
Accipiter gentilis	1	-	-	-	-	-
Accipiter nisus	7	-	-	-	-	-
Acrocephalus palustris	2	83	1	-	-	1
Acrocephalus schoenobanus Aegithalos caudatus	12	11 8	-	-	-	-
Alcedo atthis	12	3	-			3
Anthus trivialis	26	1	_	2		-
Asio otus	3	-	-	-	_	_
Bonasa bonasia	1	-	-	-	1	-
Buteo buteo	10	1	-	-	-	-
Carduelis canabina	-	8	-	-	-	-
Carduelis carduelis	3	8	-	-	-	-
Carduelis chloris Carduelis spinus	-	3	1	1	1	-
Certhia familiaris	5	-	1	2	2	2
Cinclus cinclus	2	_	3	-		-
Coccothraustes coccothraustes	1	7	-	-	-	-
Crex crex	1	-	-	-	-	-
Cuculus canorus	1	-	-	-	-	-
Delichon urbica		-	-	-	1	-
Dendrocopos leucotos	1		-	-	-	-
Dendrocopus major	5 2	1	-	-	-	-
Dendrocopus minor Dryocopus martius	$\frac{2}{3}$	-	_	1 - 1		
Emberiza citrinella	11	7	-		-	13
Emberiza schoeniclus	-	18	_	_	_	-
Erithacus rubecula	229	5	26	38	27	27
Ficelula albicolis	9	-	-	-	-	-
Ficedula hypoleuca	4	-	-	-	2	-
Ficedula parva	10	-	-	1	1	1
Fringilla coelebs	35	3	6	-	3	27
Garrulus glandarius	9	- 2	-	-	-	-
Hippolais icterina	5	3 16	-	-		3 15
Hirundo rustica Jynx torquilla	-	16		1 [1	15
Lanius collurio	8] - [-	1
Locustella fluviatilis	3	1	-	-	-	-
Locustella naevia	-	9	-	-	-	1
Luscinia luscinia	6	-	-	-	-	-
Motacilla cinerea		i - 1	11	2	-	
Muscicapa striata	15	1	-	-	-	4
Nucifraga caryocatactes	4 2	-	- 1	-	24	-
Parus ater Parus caeruleus	69	12	1 2	-	24	1 5
Parus cristatus	- 09	12	-		2	-
Parus major	105	3	-	1	-	11
Parus montanus	29	7	1	-	2	16
Parus palustris	33	2	-	2	-	6
Passer montanus	-	1	-	-	-	-
Pernis apivorus	-	1	-	-	- 2	- 1
Phoenicurus ochruros	- 4	-	-	1 -	3	1
Phoenicurus phoenicurus Phylloscopus collybita	256	31	19	2	26	33
Phylloscopus sibilatrix	7	J1 -	-		- 20	2
Phylloscopus trochilus	37	49	4	-	1	-
Picus canus	3	-	-	-	-	-
Prunella modularis	7	8	35	10	63	6
Pyrrhulla pyrrhulla	21	-	6	2	3	8
Regulus ignicapillus	-	-	-	-	- 61	1
Regulus regulus Saxicola rubetra	1	1		1	64 1	-
Saxicola rubetra Saxicola torquata	-	3	-	_	1	-
Scolopax rusticola	3	-	-	-	_	-
Sitta europaea	5	3	1	-	-	1
Strix aluco	3	-	-	1	-	-
Sturnus vulgaris	-	17	-	-		
Sylvia atricapilla	103	81	28	20	15	34
Sylvia borin	3	28	1	1	1	4
Sylvia communis Sylvia curruca	24 8	15 4	-	-	1	11
Sylvia curruca Sylvia nisoria	8	1				2
Troglodytes troglodytes	20		1	2	1	6
Turdus merula	49	22	-	8	-	1
Turdus philomelos	14	13	3	3	1	i
Turdus pilaris	-	15	-	-	-	-
Turdus torquatus		-	1	1	5	-
Turdus viscivorus	1	-	-	-	-	-
Total	1242	514	152	101	252	248

The crusts gradually grow and fall off. From the histopathological point of view, they represent nodular hyperplasia of the epidermis and the epithelium of feather follicles with keratinocyte hypertrophy. Nodes that are being formed gradually merge into quite large nodular formations (epitheliomas), whose centre is subject to dystrophic changes. A superficial crust is also formed. Dystrophic keratinocytes contain conspicuous, pathognomic intracytoplasmic and eosinophilic inclusion (Bollinger bodies). The dermatitis is superficial, diffuse and non-suppurative. Pustules are subject to regression and leave tiny marks in the skin (Tripathy and Reed 1997).

In their initial period, lesions caused by the *Collyriclum faba* trematodes are characterized by an onset of a subcutaneous granulomatous reaction and a formation of a fibrous capsule. A pseudocyst containing paraziting trematodes is gradually formed. It looks like a subcutaneous node several mm in size. A macroscopic examination would, however, reveal its difference from avipoxvirus changes (Blankespoor et al. 1982).

A comparison of macrophotographs of skin lesions examined histopathologically suggests that the lesions recorded in the two *Prunella modularis* trapped in 1984 in Socovce were of avipoxvirus origin. And analogically, the changes found in the *P. modularis* from Socovce in 1999, in the *Sylvia curruca* from Ruské in 1997 and in the *Parus montanus* from Krempna in 2000 were probably of avipoxvirus origin as well.

Very little is known about the incidence of avipoxvirus infections in wild birds in Poland. Pathological lesions on legs and other parts of the body were reported in birds trapped during their autumn migration in 1963 (Szulz-Olechova 1964). The description of lesions on legs, near the eye and near the beak in *Turdus merula*, *Sylvia borin*, *S. curruca* and *Prunella modularis* seem to confirm the diagnosis of avipoxvirus infection. In other species - and particularly in *Fringilla coelebs* - a different aetiology of the pathological lesions described cannot be ruled out. Although the report mentions a specialist veterinary examination it does not say what methods were used or in which of the birds an correct diagnosis was made.

In the past, we did not come across any reports of the incidence of avipoxvirus infections in wild birds in Slovakia, and even recent reviews published on the topic mentioned no finds of the infection in Slovakia (Hubálek 1994, Bolte et al. 1999). Our results seem to be the first report of the avipoxvirus infection in *Sylvia atricapilla* and *Prunella modularis* in Slovakia.

Prunella modularis seems to be a species particularly sensitive to avipoxvirus infections. In the past, cases of infected Prunella modularis were reported in France (Mercier and Poisson 1923), the U.K. (Edwards 1955) and Poland (Szulz-Olechova 1964). Only very few reports, on the other hand, have mentioned the infection among birds of the family Sylvidae. To date, the avipoxvirus infection has been demonstrated only in Sylvia curruca and S. borin (Szulz-Olechova 1964, Bolte et al. 1999). An unambiguous demonstration of the avipoxvirus infection in Sylvia atricapilla from Slovakia has extended the list of affected species of the family Sylvidae. The family Laniidae has not been mentioned among affected families at all (Bolte et al. 1999). The demonstration of the avipoxvirus infection in the Lanius collurio from Poland extended not only the spectrum of species known to be affected by the infection but also the spectrum of thus affected families of Passerines.

Avipoxvirová infekce u volně žijících ptáků: nové nálezy na Slovensku a v Polsku

V letech 1996-2000 byli na pěti lokalitách Slovenska a jedné lokalitě v Polsku vyšetřováni volně žijící ptáci na přítomnost avipoxvirových lézí. Bylo vyšetřeno 2509 ptáků 77 druhů. Retrospektivně byly také analyzovány fotografie kožních lézí u ptáků z jedné slovenské lokality z r. 1984. U histopatologicky vyšetřených kožních lézí byly u jedinců *Sylvia atricapilla* v Socovcích (r. 1999) a *Prunella modularis* v Tatranské Javorině (2000) na Slovensku a u *Lanius collurio* v Krempne (2000) v Polsku prokázány následky

avipoxvirové infekce. Analýzou fotografické dokumentace byly charakteristické neštovičné kožní léze dokumentovány u dalších dvou jedinců *Prunella modularis* v Socovcích (1984). Analogické kožní změny pozorované u *Prunella modularis* v Socovcích (1999), u *Sylvia curruca* v Ruském (1997) a *Parus montanus* v Krempne (2000) byly s velkou pravděpodobností způsobeny rovněž avipoxvirem.

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