

# Emergence and expansion of roe deer (*Capreolus capreolus*) fibropapillomatosis in Slovakia

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Received: 15 February 2015 / Revised: 9 October 2015 / Accepted: 20 October 2015 / Published online: 26 October 2015  
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**Abstract** A study of roe deer fibropapillomatosis, a neoplastic disease with rising occurrence, was conducted in Slovakia during 1998–2014. The first documented case of the disease was identified in 1998, at the district of Senica, in the western part of the country bordering with the Czech Republic. The disease spread from the place of initial occurrence towards the south-eastern regions of Slovakia. During the 17 years of monitoring, the disease spread to 37 districts from the total of 72 districts in the Slovak Republic and 610 cases of roe deer fibropapillomatosis were registered. Examined cases were categorized according to the extent of the lesions as follows: animals with 1 to 10 tumours (53.28 %), 11 to 30 tumours (30.49 %) and more than 31 tumours (16.23 %). The size of tumours was categorized in 45.41 % of the individuals as small (10–50 mm) and in 46.72 % individuals as medium sized (51–100 mm). Large tumours (101 mm and larger) were rare. The predilection site of tumour development in both sexes of roe deer was the skin of the abdomen, followed by

forelegs and hind legs, the back and the head. Although the viral aetiology of the disease has been clarified previously, there are still open questions regarding the epidemiology of the disease, particularly about the role of vectors and other environmental factors in its expansion.

**Keywords** Roe deer · Fibropapillomatosis · Occurrence · Tumour morphology

## Introduction

Members of the *Cervidae* family have been found to be affected by benign skin tumours described as fibromas, papillomas, warts or fibropapillomas. Shope (1955), Lancaster and Sundberg (1982) reported these lesions in white-tailed deer (*Odocoileus virginianus*), Lancaster and Sundberg (1982) in mule deer (*Odocoileus hemionus*), Moreno-Lopez et al. (1981) in Eurasian elk (*Alces alces*), Moreno-Lopez et al. (1987) in reindeer (*Rangifer tarandus*), Moar and Jarrett (1985), Forejtek (2009), Sundberg (1987), McDiarmid (1975) and Erdélyi et al. (2009) in red deer (*Cervus elaphus*), and Kocsner (1996), Takács and Nagy-Bozsoky (1998), Erdélyi et al. (2008, 2009), Kureljušić et al. (2012) and others in roe deer (*Capreolus capreolus*).

Phylogenetic analysis clusters papillomaviruses inducing the development of cutaneous fibromas and fibropapillomas of cervids into the genus delta-papillomavirus together with bovine papillomavirus type 1 and 2 (BPV1 and BPV2) (Sundberg and Lancaster 1988). Kocsner (2001) demonstrated the presence of papillomavirus antigen by immunohistochemistry in tumours of roe deer. Findings of Erdélyi et al. (2008) definitively confirmed the viral origin of fibromatosis (fibropapillomatosis) of roe deer in Hungary. Eventually, roe deer papillomavirus (CcaPV1) was characterised by Erdélyi

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et al. (2009) as the causal agent of roe deer fibropapillomatosis, and it was acknowledged as a new papillomavirus species by the International Committee on the Taxonomy of Viruses (Bernard et al. 2010).

Cutaneous tumours can be transmitted by inoculation of tissue obtained from the tumour into scarified skin of a susceptible individual (Shope 1955). Successful transfer of cutaneous fibroma among white-tailed deer by intradermal and subcutaneous inoculation was reported by Sundberg et al. (1985) and O'Banion and Sundberg (1987). However, it is not precisely known how the infection is transmitted in nature. It is assumed that the virus spreads through the contact of impaired skin with infectious material either directly from an infected animal or from the contaminated environment (e.g. vegetation). Disease transfer would potentially also be possible by haematophagous insects.

Cutaneous tumours of the *Cervidae* family, and ungulates in general have only rarely been described in Slovakia. Špeník (1977) reported the existence of cutaneous tumours in chamois (*Rupicapra rupicapra*) and rare cases in red deer. Lešník and Vrtiak (1979) described this disease in red deer as a rarely occurring infectious neoplastic disease, which mostly affects the neck and head. However, there have been no reports on the presence of this neoplastic disease in roe deer (*Capreolus capreolus*) in Slovakia.

The objective of the present study was to evaluate the occurrence and spread of fibropapillomatosis in roe deer in the Slovak Republic during 1998–2014 and describe basic morphological characteristics of the studied tumours.

## Materials and methods

Fibropapillomatosis in roe deer was examined in the Slovak Republic over a 17-year period, from the first record of this disease in 1998 to 2014. Monitoring data were obtained in cooperation with authorities of the State Veterinary and Food Administration of the Slovak Republic and the Slovak Hunters' Chamber from the whole territory of the country. Diagnoses were based on macroscopic and microscopic examinations. In this study, we evaluated data obtained through the examination of 610 roe deer (464 shot and 146 found dead) showing signs of fibropapillomatosis. Following the confirmation of fibropapillomatosis cases by pathological examination, we also evaluated the spatial spread, and manifestation of the disease. The dynamics of disease spread was assessed from the number of newly infected districts in individual years, which were depicted on the map of the Slovak Republic. We determined the sex and age structure of the affected individuals together with the evaluation of lesions and disease symptoms. The age structure of affected roe deer was only analysed for males according to the aging system used in the trophy evaluation procedure (I. age class—1 year

old, II. age class—2 to 5 years old, III. age class—6 years and older). Positive cases were divided into three categories depending on the number of tumours on the given animal: (1) 1–10 tumours, (2) 11–30 tumours and (3) 31 and more tumours. The size of tumours localized on the body of examined individuals was also evaluated. Two measures of each tumour were taken and subsequently an average was calculated. With regard to the fact that we noted large numbers of very small tumours in a number of sick individuals, we decided to individually count only tumours with a diameter larger than 10 mm. Each positive roe deer was assigned to one of the three categories according to the average size of measured tumours: 10–50 mm (small), 51–100 mm (medium) and 101 mm and more (large). The topographic localization of tumours on the body surface of affected individuals was recorded for the following regions: head, neck, forelegs, hind legs, abdomen, flanks and back. Since tumours occur both as solitary and multiple ones, in the later cases the most affected body part (considering tumour size and frequency) was named as the principal site of the infection. The number of sites affected by tumours on the animal's body was also recorded for each evaluated case. The software Statistica 10 was used for statistical evaluation of results. The chi-square test for independence was used to confirm or disprove the association between individual qualitative characteristics, and to calculate the probability of a random phenomenon ( $P$ ). To determine the strength of the dependence between the characteristics we also calculated Chuprov's association coefficient ( $K$ ).

Removed tumour samples were conserved and archived in 10 % neutral buffered formalin solution for histopathology. At the same time, they also serve for educational purposes in the archives of pathological findings in game at the Faculty of Forestry of the Technical University in Zvolen.

## Results

### Pathomorphology

Tumours on the examined roe deer had a light grey to black epithelial surface (Figs. 1 and 2). The typical cross section of tumours revealed an elastic to firm, shiny fibromatous mass, with a white or pink-coloured centre (Fig. 3). Individual tumours were well defined, without signs of infiltration into neighbouring tissues and they could be easily excised (Fig. 4). The tumours were restricted to the skin; there were no lesions in tissues under the skin (i.e. the subcutis or underlying muscles). The epidermis was mostly pigmented and the surface of larger tumours was often affected by injuries, erosions, suppurating processes or necrosis. Histopathological examinations confirmed that the main part of the tumour consisted of collagen fibres containing groups of proliferating



**Fig. 1** Tumours on examined roe deer with *light grey to black surface*

fibroblasts, covered by the epithelial layer showing signs of acanthosis and hyperkeratosis.

### Occurrence of fibropapillomatosis

Data on the number of roe deer fibropapillomatosis cases diagnosed in Slovakia during the years 1998–2014 are presented in Table 1.

The first case of roe deer fibropapillomatosis in Slovakia was documented in 1998 in a 3-year-old male. The disease was confirmed in another five males during the following year (1999). In 2000, the infection was diagnosed for the first time in female roe deer. The number of individuals suffering from fibropapillomatosis increased during the following years. A more than twofold increase in the number of positive individuals was recorded in 2004 compared to 2003. This dramatic increase in the number of affected animals continued over the following years and since 2007 the number of positive individuals reached more than 40 per year. The highest number of cases, 65 affected individuals, was registered in 2010.

An interesting phenomenon was observed regarding the distribution of positive cases among the sexes. Although only males were found to be infected during the first 2 years of the study, the number of registered female cases also increased soon after. Thus, the sex ratio gradually changed within the set of positive individuals. While, for example until 2000 inclusive, the sex ratio among positive individuals was strongly



**Fig. 2** Tumours on examined roe deer with *light grey to black surface*



**Fig. 3** The cross section of the tumour typically contains a *white or pink-coloured central area*

in favour of males (94.1 % (16 individuals) to 5.9 % females (1 individual)), it eventually declined by 2014 to 74.43 % of affected males (454 individuals) and 25.57 % of affected females (156 individuals). The age distribution of the positive 454 males was the following: 149 (32.82 %) were 1 year old (I. age class), 249 (54.85 %) were between 2 and 5 years old (II. age class) and 56 (12.33 %) were 6 years old and older (III. age class). The significant predominance of young males correlates with the naturally higher representation of younger age categories in the population's age pyramid. Positive association was found between the age of roe bucks and the size of tumours ( $P=0.000001$ ,  $K=0.234$ ) and between the age of roe bucks and number of tumours ( $P=0.010707$ ,  $K=0.159$ ).

### Spatial distribution of fibropapillomatosis

The spatial and temporal changes in the distribution of roe deer fibropapillomatosis in the Slovak Republic are summarized on Fig. 5. The first recognized fibropapillomatosis cases of roe deer in the Slovak Republic originated from the Senica district, in the western part of the country, bordering with the Czech Republic. From 1988, the disease spread southeast and by 2007 disease presence was confirmed in 13 districts. The epizootic situation changed in 2008. Until that time, all cases



**Fig. 4** Individual tumours are well defined, without signs of infiltration into neighbouring tissues and they can be easily removed



**Table 1** Number of fibropapillomatosis positive individuals of roe deer registered on the territory of the Slovak Republic during the years 1998–2014

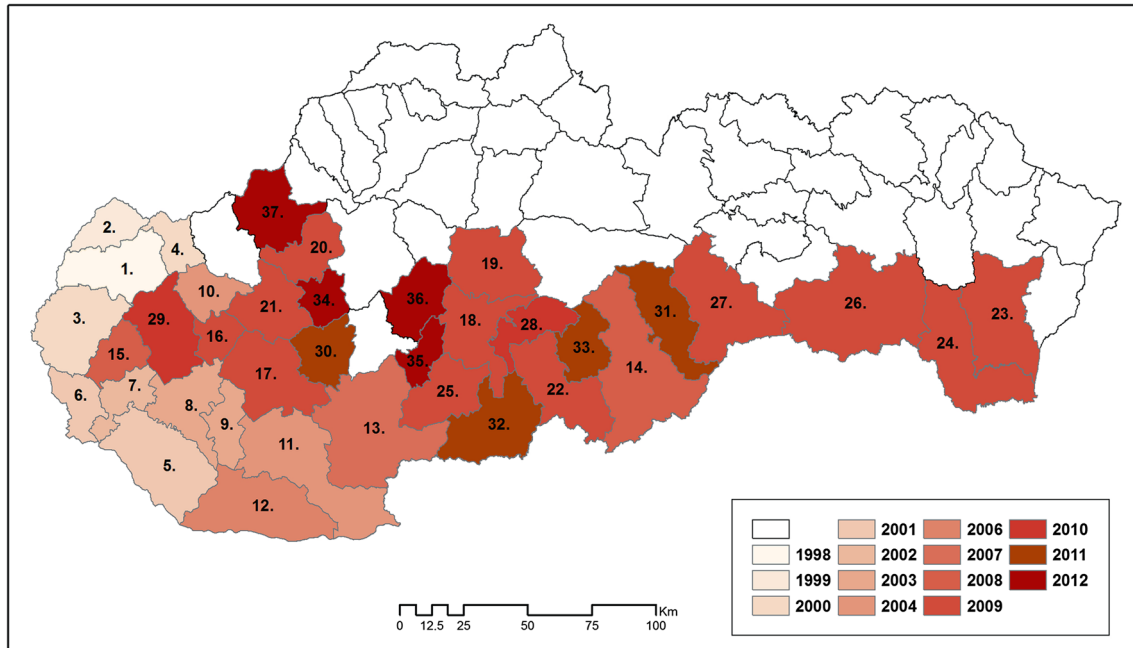
Years	Number of positive females	Number of positive males	Number of positive individuals (females and males in total)
1998	0	1	1
1999	0	5	5
2000	1	10	11
2001	1	15	16
2002	1	17	18
2003	2	20	22
2004	2	44	46
2005	3	25	28
2006	4	27	31
2007	6	48	54
2008	7	52	59
2009	20	42	62
2010	31	34	65
2011	25	29	54
2012	18	24	42
2013	19	31	50
2014	16	30	46
Total	156	454	610

of fibropapillomatosis occurred in the south-western part of Slovakia. However, in 2008 the disease was also reported from Rimavská Sobota, the south of central Slovakia. By 2014, 17 years from the first recognized occurrence, the disease had been confirmed in 37 districts of Slovakia, representing more than half of the territorial-administrative units (72 districts) of the Slovak Republic.

### Characteristics of the number, size and localization of tumours

Considering tumour numbers on infected males, the majority (236/51.98 %) was assigned to the first category (1–10 tumours), 140 (30.84 %) to the second category (11–30 tumours) and 78 (17.18 %) qualified for the third category (31 and more tumours). Similarly, 89 roe does (57.05 %) were assigned to the first category, 46 (29.49 %) to the second category and 21 (13.46 %) to the third category. There was no correlation between sex and the number of tumours per animal ( $K=0.054$ ,  $P=0.823347$ ). The overall distribution of positive individuals (both sexes) among categories was as follows: first category 325 individuals (53.28 %), second category 186 individuals (30.49 %) and third category 99 individuals (16.23 %).

The data on tumour sizes measured on affected individuals is summarized in Table 2. On average, medium-sized tumours



**Fig. 5** Dynamics of the spatial distribution of fibropapillomatosis in roe deer over the territory of the Slovak Republic during the years 1998 to 2014. (“1” numerical order of the district according to sequence of disease occurrence, “Senica” name of the district, colour code year of first occurrence in the given district/see legend). 1. Senica, 2. Skalica, 3. Malacky, 4. Myjava, 5. Dunajská Streda, 6. Bratislava, 7. Senec, 8. Galanta, 9. Šaľa, 10. Piešťany, 11. Nové Zámky, 12. Komárno, 13.

Levice, 14. Rimavská Sobota, 15. Pezinok, 16. Hlohovec, 17. Nitra, 18. Zvolen, 19. Banská Bystrica, 20. Bánovce nad Bebravou, 21. Topoľčany, 22. Lučenec, 23. Michalovce, 24. Trebišov, 25. Krupina, 26. Košice, 27. Rožňava, 28. Detva, 29. Trnava, 30. Zlaté Moravce, 31. Revúca, 32. Veľký Krtíš, 33. Poltár, 34. Partizánske, 35. Banská Štiavnica, 36. Žiar nad Hronom, 37. Trenčín

**Table 2** Division of infected roe deer according to tumour size categories

Sex	Size category of tumours		
	Small 10–50 mm	Medium 51–100 mm	Large >100 mm
Females ( <i>n</i> =156)	76 (48.72 %)	59 (37.82 %)	21 (13.46 %)
Males ( <i>n</i> =454)	201 (44.27 %)	226 (49.78 %)	27 (5.95 %)
Females and males in total ( <i>n</i> =610)	277 (45.41 %)	285 (46.72 %)	48 (7.87 %)

dominated among males, namely in 49.78 % of individuals, while most females (48.72 %) were affected by smaller tumours. At the same time, large tumours were more often recorded on females (13.46 %) compared to males (5.95 %). However, there was no correlation between the sex of the affected roe deer and the size of tumours ( $K=0.153$ ,  $P=0.005606$ ). On the whole, the proportion of small tumours (45.41 %) and medium size tumours (46.72 %) was balanced in both sexes.

The localization of tumours on the body is described in Table 3. Although in most cases, tumours occurred at multiple body regions at the same time, the abdomen was the dominant predilection site for tumour development on females (24.36 %) while in roe bucks, the most affected parts of the body was the abdomen (20.04 %) and the forelegs (20.04 %). The flanks were the least affected body region in both sexes. The main sites of tumour occurrence in both male and female roe deer were the skin of the abdomen, followed by forelegs and hind legs, the back and the head, respectively. The differences in the localization of tumours between males and females were not significant ( $K=0.059$ ,  $P=0.985583$ ).

Animals with only one or a small number of tumours localized next to each other at one part of the body were rare (5.01 %). Similarly, cases with tumours occurring at all seven regions of the body were also rare (6.15 %). Most animals developed generalized fibropapillomatosis that occurred over multiple body regions. Cases with tumours on three (30.92 %) and two (24.47 %) body regions were seen most often. Correlation was found between the tumours' size and their localization on the body ( $K=0.187$ ,  $P=0.00004$ ) as well as correlation between the number of tumours and their

localization on the body ( $K=0.207$ ,  $P=0.000001$ ). The order of anatomic predilection sites according to tumour size was the following: abdomen, neck, head, back, hind legs, forelegs and flanks. The number of tumours decreased over the anatomic predilection sites in the following order: hind legs, forelegs, head, back, abdomen, neck and flanks.

The number of tumours on animals found dead was used to assess the potential mortality effect of the disease in roe deer. Of the 146 animals found dead, 23 (15.8 %) individuals carried 31 and more tumours, 112 (76.7 %) had 11–30 tumours while 11 (7.5 %) fell into the category of scarce tumour occurrence. In comparison, from the 464 shot animals that were affected by fibropapillomatosis 76 (16.4 %) individuals had 31 or more tumours, 74 (15.9 %) had 11–30 tumours and 314 (67.7 %) were assigned to the category of scarce tumour occurrence.

## Discussion

The study documents the spatiotemporal expansion of fibropapillomatosis in roe deer over the territory of the Slovak Republic in the period from the first detection of the disease in 1998 until 2014. Since the initial occurrence of fibropapillomatosis in roe deer was recorded from the district of Senica in the western part of Slovakia neighbouring the Czech Republic, the presence of this disease may also be anticipated in this neighbouring country. Specific data on the occurrence of fibropapillomatosis in roe deer in the neighbouring countries during the years of this study were available only in literature from Hungary (Kocsner 1996;

**Table 3** Localization of tumours on the body of roe deer

Localization of tumours	Females ( <i>n</i> =156)	Males ( <i>n</i> =454)	Females and males in total ( <i>n</i> =610)
Head	25 (16.03 %)	59 (13.00 %)	84 (13.77 %)
Neck	18 (11.54 %)	41 (9.03 %)	59 (9.67 %)
Forelegs	24 (15.38 %)	91 (20.04 %)	115 (18.85 %)
Hind legs	29 (18.59 %)	85 (18.72 %)	114 (18.69 %)
Abdomen	38 (24.36 %)	91 (20.04 %)	129 (21.15 %)
Flanks	3 (1.92 %)	10 (2.20 %)	13 (2.13 %)
Back	19 (12.18 %)	77 (16.97 %)	96 (15.74 %)

Takács and Nagy-Bozsoky 1998; Erdélyi et al. 2008). Kocsner (2001) reported that although fibropapillomatosis in roe deer was already detected in Hungary during the 1960s, increasing occurrence of fibropapillomatosis was not noticed before the 1990s. Erdélyi (2010) confirmed that the disease was concentrated in several areas, but it occurred over large parts of Hungary. However, these studies do not provide data on the temporal scale and the dynamics of fibropapillomatosis epidemiology.

It is not possible to unambiguously explain why the disease has occurred predominantly in southern and western regions, and why the north and east of the country are almost free of the disease. Regions without disease occurrence are predominantly the mountainous parts of the country where roe deer population densities are lower. The population density of roe deer is well characterised by the roe deer hunting bag data from different districts. At the southern and western districts of Levice, Galanta, Šal'a, Piešťany, Myjava, Skalica, Senica, Trenčín and Kysucké Nové Mesto, the number of shot roe deer was 8.1–15.3 individuals/1000 ha in the 2011 hunting season. The southern and western districts of Dunajská Streda, Nové Zámky, Nitra, Zlaté Moravce, Lučenec, Krupina, Veľký Krtíš, Hlohovec, Topoľčany, Trnava, Pezinok, Nové Mesto nad Váhom, Ilava, Púchov and Čadca followed with 6.1 to 8.0 roe deer shot/1000 ha. Even lower roe deer hunting bags (below six individuals/1000 ha) were recorded in the districts of central, northern and eastern Slovakia. The lowest roe deer hunting bags of only 0–2 animals per 1000 ha were reported from the districts of Ružomberok, Brezno, Poprad, Spišská Nová Ves, Levoča, Gelnica, Sobrance and Snina (Bučko et al. 2012). Hunting bag data show that roe deer live in highest numbers in the districts of southern and western Slovakia. During the study, we noticed that the outbreak of disease is more common in hunting grounds with high density of game. Many authors, e.g. Cote et al. (2004), Gortázar et al. (2006), Acevedo et al. (2007), point out the disease risks which are the consequence of overpopulation of game. Erdélyi et al. (2009) also found association of high fibropapillomatosis occurrence with high host population densities. The sex ratio among affected individuals was approximately three males to one female.

Most individuals (53.28 %) of both sexes carried one to ten tumours, which were predominantly solitary tumours. Only 16.23 % of the examined animals had more than 30 fibropapillomas. This fact might be explained either with a mortality effect of the disease or by eventual recovery following tumour regression. The fairly low number of animals found dead in the category of 31 and more tumours could be due to the majority of animals dying or recovering before such extensive lesions develop. An extreme case of a shot roebuck with 156 tumours was also recorded.

According to average tumour size, 45.41 % of affected roe deer was assigned to the category with small and

46.72 % of the animals to the category with medium-sized tumours. Occurrence of large tumours was rare (7.87 %). The largest fibropapilloma measuring 121 mm×190 mm and weighing 1080 g was detected in a roe buck. Erdélyi et al. (2008) reported a 170 mm diameter roe deer fibropapilloma from Hungary, while Kureljušić et al. (2012) described a tumour measuring 120 mm from Serbia.

The evaluation of tumour localization in roe deer showed that the predilection site of tumour occurrence in both sexes is the skin of the abdomen, followed by forelegs and hind legs, back and head. Erdélyi et al. (2008) reported the most frequent occurrence of tumours on head, neck, abdomen and extremities of roe deer. Methods for the therapy of this disease in roe deer are unexplored and not under consideration under natural conditions. Vaccination is a successful method to control papillomatosis in cattle; however, its practical utilization in free living roe deer may not be efficient and treatment of sick individuals in nature is not envisaged. Garaj and Kropil (2010) stress the importance of eliminating as many of the clinically ill individuals as possible. Surgical treatment is feasible only in individual cases with scarce or solitary tumours at breeding farms (Rajský and Garaj 2008).

During the 17 years of this study, both hunting effort and the effort made by hunters to report evident disease conditions to relevant authorities were more or less constant. This constant effort and the high visibility of the tumours to hunters support the conclusion that the apparent spread of the disease across the country during the study period documented in this study represents true geographic expansion of the disease during the study period.

Despite the quickly accumulating knowledge on this subject, many aspects of roe deer fibropapillomatosis still require further extensive studies, particularly regarding the epidemiology, mechanisms of spread and pathogenesis.

**Acknowledgments** This work was supported by the Slovak Research and Development Agency under the contract No. APVV-14-0637.

**Compliance with ethical standards** The authors declare that to their knowledge there are no potential conflicts of interest involved with their research and that they adhered to ethical guidelines both during research and manuscript preparation. The manuscript is being submitted with the informed consent of all co-authors.

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