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FIBROPAPILLOMATOSIS OF ROE DEER (*CAPREOLUS CAPREOLUS*) IN HUNGARY

Thesis of Ph.D. dissertation

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1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

It has been established, that infectious agents play a significant role in the etiology of neoplastic diseases occurring in free ranging wildlife. These disease conditions are mostly species specific and they are often endemic, i.e. only present in certain animal populations. The best known diseases in this category are the skin tumours of cervids caused by papillomaviruses (Sundberg et al., 2001), fibropapillomatosis of sea turtles associated with herpesvirus infection (Lackovich et al., 1999), hepatocellular carcinomas of woodchucks caused by woodchuck herpesvirus (WHV) (Tyler et al., 1981), fibroma of cotton-tailed rabbits caused by a pox virus, the Shope fibroma virus (Robinson and Kerr, 2001), etc. Human induced pollution, radiation etc. may also be a significant causative factor in some cumulative neoplastic diseases, but their primary etiological role is often very hard to prove.

Permanent occurrence of skin tumours has been regularly observed in certain parts of the Hungarian roe deer population. According to Kocsner (1996) and our own earlier studies these lesions can be characterized as benign, cutaneous fibromas or fibropapillomas of viral aetiology, similar to the endemic skin tumours occurring in other cervid species. Hunters and game management personnel find these, sometimes fairly big, lesions very repulsive and the public is also concerned with the subject from time to time. There are many misconceptions regarding the aetiology of the disease from environmental pollution (e.g. waste incinerating plants) to radioactive radiation from the Chernobyl accident (Takács and Nagy-Bozsoky, 1998).

However, all circumstances and scientific evidence pointed to the potential viral origin of this disease. Based on the characteristics of the lesions and considering the overall species specificity of papillomaviruses we inferred, that the causative agent would belong to the *Deltapapillomavirus* genus containing the other cervid papillomaviruses causing fibropapillomatosis.

1.2. Objectives of the study

In order to elucidate the above issues and to comprehensively characterize roe deer (and red deer) fibropapillomatosis I have formulated the following research objectives:

- sequencing and description of the complete genome of the roe deer papillomavirus
- phylogenetic characterization of the roe deer papillomavirus
- comparison of viruses isolated from different geographical locations
- survey of the distribution of roe deer fibropapillomatosis in Hungary
- study of the pathology and significance of roe deer fibropapillomatosis
- analysis of the factors influencing the spatial distribution of the disease

and additionally for comparative studies:

- study of the aetiology and pathology of red deer fibropapillomatosis

2. MATERIALS AND METHODS

2.1. Pathology

Roe deer carcasses submitted for post-mortem diagnostic examination to the Central Veterinary Institute in the three year period between 2004 and 2006 were included in the study. Samples were taken from 42 roe deer examined in this period for histopathology and DNA extraction. Samples from suspect fibropapillomatous lesions were also received from foreign countries in the form of fresh or fixed tissues, or extracted DNA.

Red deer fibropapilloma samples originated from a one year old stag shot in 2004 in Lower Austria and one year old doe shot in 2007 for a routine health check at the Bőszénfa deer farm belonging to the Kaposvár University.

Formalin fixed and paraffin embedded tissues were sectioned and further processed by hematoxilin-eozin (HE) staining, immunohistochemistry (IHC) and in-situ hybridisation (ISH). Histological lesions, IHC and ISH reactions were examined and evaluated by light microscopy. We adapted a novel immunohistochemical method for the detection of viral antigen in tumour tissue (Erdélyi et al., 2009b).

The presence and localisation of papillomavirus DNA in tumour tissue sections was examined by in-situ DNA hybridization. A DIG-labelled 392 bp long DNA probe, synthesised by PCR from the L1 ORF region (nt 6407-6798; EF680235) of the CcPV1 genome, was used in the reaction.

2.2. Virology

DNA was extracted from homogenized skin and tumour tissue samples collected during post mortem examinations.

We designed a novel PCR, suitable for the detection of cervid delta-papillomaviruses, to demonstrate the presence of roe deer and red deer papillomavirus DNA in tumour tissue (Erdélyi et al., 2008). A consensus alignment of the three known cervid papillomavirus genomes available from GenBank at the time (DPV, NC_001523; EEPV, NC_001524 and REPV, NC_004196) was used as a template for primer design. The specific product amplified in the reaction was

approximately 730bp long, and it spans the neighbouring regions of the L1 and L2 ORFs).

The sequencing of the complete roe deer papillomavirus genome was achieved by multiple PCR assays. The remaining part of the circular genome was amplified by KOD Hot Start DNA polymerase with outward primers annealing to the ends of the originally detected sequence. This product (7900 bp) was used as a template for the sequencing of the remaining part of the genome. Genome segments were amplified by 27, partly degenerated primers and sequenced directly with the same primers or after cloning into pGEM-T plasmids (Promega).

The whole roe deer and red deer papillomavirus genome was also amplified by RCA (Rolling Cycle Amplification) reaction utilizing the Phi29 DNA polymerase for the amplification of circular DNA. The resulting DNA was linearized by a restriction enzyme (*Sac*I) with a single cleavage site in the virus's genome and subsequently cloned into a pUC19 plasmid.

The phylogenetic analysis of the roe deer papillomavirus was performed with both the complete L1 ORF sequence and a 3561 bp long, concatenated sequence of the four main ORFs (L1, L2, E1 and E2) against 70 selected papillomavirus genome sequences available from GenBank. The phylogeny of the red deer papillomavirus was reconstructed based on the partial L2 ORF nucleotide sequences. All analyses were conducted by neighbour-joining and maximum parsimony methods.

The characterisation of papillomaviruses is primarily based on the analysis of the virus genome sequence. It consists of the description of the genome length, identification and characterisation of the main ORF sequences, of the untranslated regulatory region (URR) and the identification of transcription factor binding sites, motifs and protein domains. Molecular masses of identified viral proteins and the similarities between the main ORFs of the roe deer papillomavirus and a selected set of other papillomaviruses were calculated. The transcription factor binding sites, motifs and protein domains were either searched manually or by on-line databases and software.

A real-time PCR method was designed and used to determine the number of papillomavirus genome copies present in the tumour tissue.

2.3. Ecology and epidemiology

A questionnaire study was used to assess the distribution of roe deer fibropapillomatosis in Hungary, and study its ecology. Questionnaires were mailed at the end of March 2006 to all 1201 game management units (GMU) registered in the National Game Management Database (OVA). The one page multiple choice questionnaire was accompanied by a single page covering letter explaining the purpose of the study and containing photographic illustrations of the lesions typical for the disease. A reminder was sent out to non-responding GMUs two weeks after the response deadline. The questionnaire was designed to collect categorical information about disease occurrence, trend and frequency of detection, on primarily affected age classes in both sexes, and the association of lesions with roe deer mortality. A question about the occurrence of skin tumours on red deer was also part of the questionnaire.

Additional data were collected on the occurrence of roe deer fibropapillomas throughout Europe, from institutions performing wildlife disease diagnostics or maintaining wildlife disease databases. Available databases were searched retrospectively for diagnoses of skin tumours in general and specifically papillomas, fibropapillomas or fibromas found in roe deer and for the total number of roe deer samples submitted for examination during the same time period. Institutions kindly providing these data were: AFSSA-LERRPAS France, Leibnitz Institute for Zoo and Wildlife Research Berlin, Germany, National Veterinary Institute Uppsala, Sweden, Veterinary Laboratories Agency Diseases of Wildlife Scheme, United Kingdom.

Roe deer population data (yearly population size estimates and hunting bag sizes for bucks, does and fawns) were obtained from the Hungarian National Game Management Database (OVA). Averages of the previous six years' data (2001–2006) were calculated for each GMU separately and used in the study.

Data of individual environmental categories were extracted from the Corine Land Cover 2000 database (CLC2000) (European Environment Agency) for every GMU territory and used for subsequent analyses.

Using the GMUs with known disease status and the proportion of CLC2000 classes within these GMUs a classifier was created based on random forest supervised machine learning algorithm (Breiman, 2001). This algorithm was used to predict the disease status of non-responding GMUs and estimate the wider distribution area of the disease based on population density and the CLC2000 environmental data. Logistic regression and Pearson's product moment correlation coefficient was used to quantify the association of disease occurrence, environmental and host population data.

3. RESULTS

3.1. Pathology

3.1.1. Macroscopic and histopathology

From the 42 roe deer carcasses examined between 2004 and 2006, 14 showed macroscopic lesions consistent with skin fibropapillomatosis. Fibropapillomas of roe deer were found to be firm, round, multiple skin tumours of varying size. On average the lesions were 3–5 cm in diameter but sometimes they reached 11–15 cm. Although the number of fibropapillomas on one animal was usually less than 10, individuals carrying more than 150 tumours were also found. Tumours were typically localised on the head, neck, belly and legs of infected animals.

Some tumour surfaces exhibited moderate and transient papillary structure but the typical appearance was of a firm, hairless mass covered with smooth or verrucous epidermis. The epidermis was often pigmented and the surface of larger tumours was frequently eroded and ulcerated.

Histopathology revealed that the main tumour mass was located in the stratum reticulare of the dermis and it consisted of groups of proliferating fibroblasts embedded in a compact mesh of collagen fibres. The epidermis showed signs of acanthosis and hyperkeratosis.

The nodular lung fibrosis occasionally associated with DPV infection was not detected in roe deer with skin fibropapillomatosis, however in the two examined cases we detected moderate, diffuse interstitial fibrosis.

3.1.2. Immunohistochemistry and in-situ hybridisation

Both papillomavirus L1 antigen and DNA were visualised in the epidermis covering the tumour stroma. Papillomavirus DNA homologous to the L1 ORF sequence was present in a few keratinocyte nuclei of the stratum basale but the stratum spinosum and stratum granulosum had much larger numbers of keratinocytes containing papillomavirus L1 DNA in both their nuclei and cytoplasm. Papillomavirus major capsid antigen was predominantly localised in the cytoplasm

and nuclei of keratinocytes of the stratum granulosum, but antigen aggregations were often observed between the keratin layers of the stratum corneum as well.

3.2. Virology

3.2.1. Detection of Delta-papillomaviruses from cervids by PCR

The PCR developed for the detection of Delta-papillomaviruses was successfully used to detect both roe deer (CcPV1) and red deer papillomavirus (CePV). Sequences of the partial L2 ORFs obtained from eight geographically distinct roe deer samples were found to be identical.

Results of the RT-PCR showed that the number of viral genome copies in the epithelial layer of the roe deer fibropapilloma was $1.8–2.1\times10^{10}$ copies/μg of total DNA.

The complete circular CcPV1 and CePV genomes were successfully amplified in the RCA reaction and visualized after digestion by restriction enzymes (EcoRV and SacI). However, only CcPV1 was cloned successfully as confirmed by PCR and sequencing.

3.2.2. Characterization of the complete genome sequence of the roe deer papillomavirus (CcPV1)

The CcPV1 genome sequence was 8032 bp longand it was submitted to the GenBank database under accession number EF680235 and it can be also found in the collection of viral genomes (NC_011051 - Western roedeer papillomavirus 1).

All nine ORFs common to Delta-papillomaviruses were identified in the CcPV1 genome and molecular masses of putative viral proteins were calculated. At the end of the early-protein coding region we identified the E9 ORF typical for all the other cervid papillomaviruses described by Eriksson et al. (1994). The long control region (LCR) or untranslated regulatory region (URR) was 799 bp long and contained the elements of the CcPV1 origin of replication.

3.2.3. Phylogenetic analysis

Phylogenetic analysis of 71 papillomavirus genomes (Figure 1.) based on the comparison of complete L1 ORF sequences and a compound sequence alignment of the four major ORFs (L1, L2, E1 and E2) performed with the neighbour-joining (NJ) and maximum parsimony (MP) methods placed CcPV1 on a separate branch between OPV and the other deer papillomaviruses within the Delta-papillomavirus genus.

This result was confirmed by distance matrix and parsimony analysis in the Phylip software, resulting in identical branch topology and almost identical bootstrap values. Delta-papillomavirus ORFs (E6, E7, E1, E2, L1 and L2) and the URR sequences produced the same topology for CcPV1 as the concatenated sequence alignment.

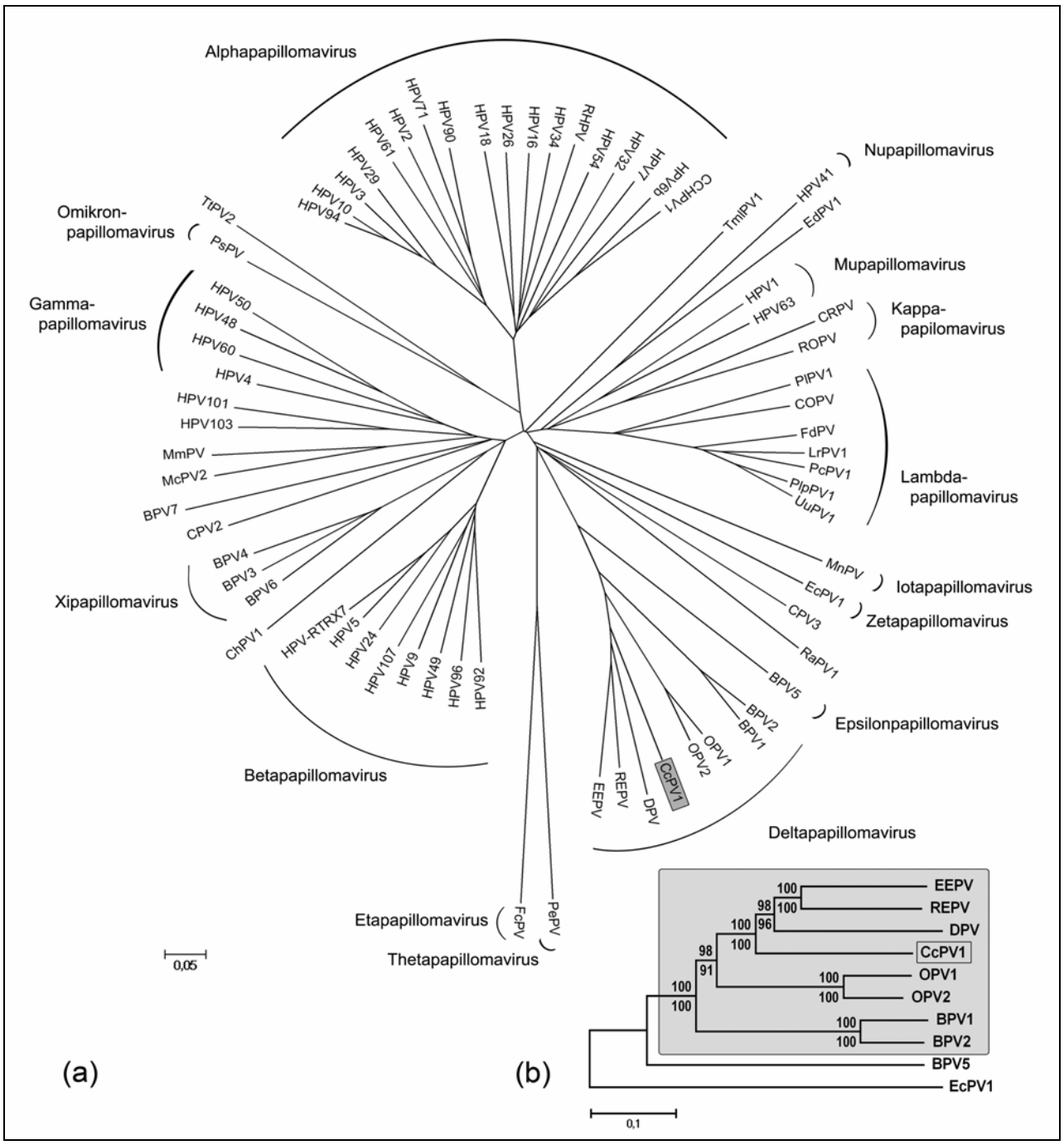

Figure 1. The evolutionary history of CcPV1 and another 70 papillomaviruses inferred from the 3861 bp long concatenated sequence of the E1–E2–L2–L1 ORFs using the neighbour-joining (NJ) method and (b) a NJ phylogram indicating the evolutionary distances between the members of the Delta-papillomavirus genus (shaded). The corresponding bootstrap values obtained for the identical topologies by the NJ (top) and maximum parsimony (below) methods are indicated on the NJ tree.

3.3. Ecology and epidemiology

3.3.1. The distribution of roe deer fibropapillomatosis in Hungary

Replies to the questionnaire were received from 539 GMUs. The overall questionnaire retrieval rate was 45.3% representing 50.9% of the total territory of all Hungarian GMUs, i.e. effectively half of the country. The occurrence of the disease was reported by 293 GMUs (54.4% of responders), characterised as intermittent occurrence by 200 (37.1%) and permanent presence by 93 (17.4%) GMUs. Regarding frequency of disease detection, an odd case over several years (DFI = 1) was reported by 126 GMUs (43% of positive territories), 1–2 cases per year (DFI = 2) by 109 (37.2%) and more than 2 cases per year (DFI = 3) by 58 (19.8%).

The trend of disease occurrence was characterised as declining in 60 (21.1%), stagnating in 187 (65.6%) and increasing in 38 (13.3%) GMUs. Bucks were found to be only slightly more affected (51.2%) by the disease than does (48.4%) but this difference was not statistically significant (McNemar's Chi-squared test with continuity correction, $p = 0.7657$). The distribution of primarily affected age classes within each sex (bucks/does) was 2.3/2.8% juveniles, 14.9/35.8% subadults, 56.6% middle aged adult bucks, 26.2% old adult bucks and 61.4% adult does. Differences detected in the age distribution of the disease within each sex were found to be significant by Pearson's Chi-squared test ($p < 0.001$). Fibropapillomas were seen on animals found dead in 34 GMUs, i.e. 11% of the 309 units answering this question.

3.3.2. Prediction of disease occurrence

Both random forest algorithms produced for disease occurrence and frequency data utilised the following variables: non irrigated arable land (CLC-code 211), pastures (CLC-code 231), broad-leaved forest (CLC-code 311), water courses (CLC-code 511) and roe deer density. As the preliminary visual assessment of disease distribution and random forest variable importance values suggested a potential effect of lowland areas and major waterways on PV distribution, we tested the association of disease occurrence with water courses, forested areas and roe deer population density data. Results of the logistic regression analysis showed that both roe deer density (OR: 1.284, 95% CI: 1.151–1.433, $p < 0.001$) and the presence of waterways

(OR: 1.318, 95% CI: 1.105–1.572, p = 0.002) increased, while broad-leaved forest cover decreased (OR: 0.974, 95% CI: 0.965–0.984, p < 0.001) the likelihood of infection by CcPV1. Pearson's product moment correlation coefficient analysis showed no association (R: 0.07, p = 0.12) between water courses and roe deer density, a weak negative (R: -.244, p < 0.001) association between broad-leaved forest cover and roe deer density, and a moderate, positive association between non-irrigated arable land cover and roe deer density (R: 0.303, p < 0.001).

3.3.3. European distribution

An extensive literature search and the results of a survey of European wildlife disease databases showed that the endemic presence of roe deer fibropapillomatosis has not been detected elsewhere in Europe. This can be declared based on the diagnostic records of specialist laboratories in France (SAGIR: 1986-2007; 10585 roe deer carcasses), Sweden (SVA Uppsala: 1947-2008; 6585 examined roe deer), Germany (IZW Berlin: 1972-2008; 231 roe deer cases) or United Kingdom (VLADoWS: 2000-2008; 70 roe deer cases).

3.4. The red deer papillomavirus

3.4.1. Epidemiology and pathology

The morphological features of the tumours from both cases were almost identical. They were oval, firm masses, with a rough, hairless, pigmented surface exhibiting a moderately pronounced papillary structure. Erosions were only seen on the surfaces of some of the Case 1 tumours. Similarly to roe deer tumours the cross-sections of the tumour masses were shiny, white and firm.

Histological examination of both samples determined that they were fibropapillomas showing marked proliferation of fibroblasts and connective tissue accompanied by hyperkeratosis, parakeratosis and acanthosis of the overlaying epidermis, and occasional foci of inflammation.

The presence of papillomavirus antigen was detected in both samples by IHC. Aggregation of viral particles was visible primarily in the nuclei and occasionally in the cytoplasm of koilocyte-like keratinocytes of the stratum granulosum and between the squamous cell layers of the stratum corneum of both cases.

In-situ hybridization with the method used for the detection of CcPV1 demonstrated the presence of CePV DNA in both of the above samples. The localisation of viral DNA was identical to that of CcPV1. To our surprise however, apart from the epidermis, CePV DNA was also detected in the nuclei of fibroblasts located in the connective tissue of the main tumour mass of Case 2.

3.4.2. Characterization of the red deer papillomavirus DNA sequence

Only the PCR reaction performed from the DNA of frozen tissue sample from Case 1 produced a positive result. We suspect that the unsuccessful PCR of Case 2 was the consequence of DNA damage related to formalin fixation. The amplified DNA product was sequenced in a length of 588 bp and deposited in GenBank under the accession number EU881493. As expected, the sequence covered the 3' terminal segment of the L2 ORF. Phylogenetic analysis of the partial L2 ORF sequence alignment of 9 Delta-papillomaviruses and equine PV (EcPV1) by both neighbour-joining (NJ) and maximum parsimony (MP) method confirmed that the Red deer PV is very closely related to the Roe deer papillomavirus (Figure 2.)

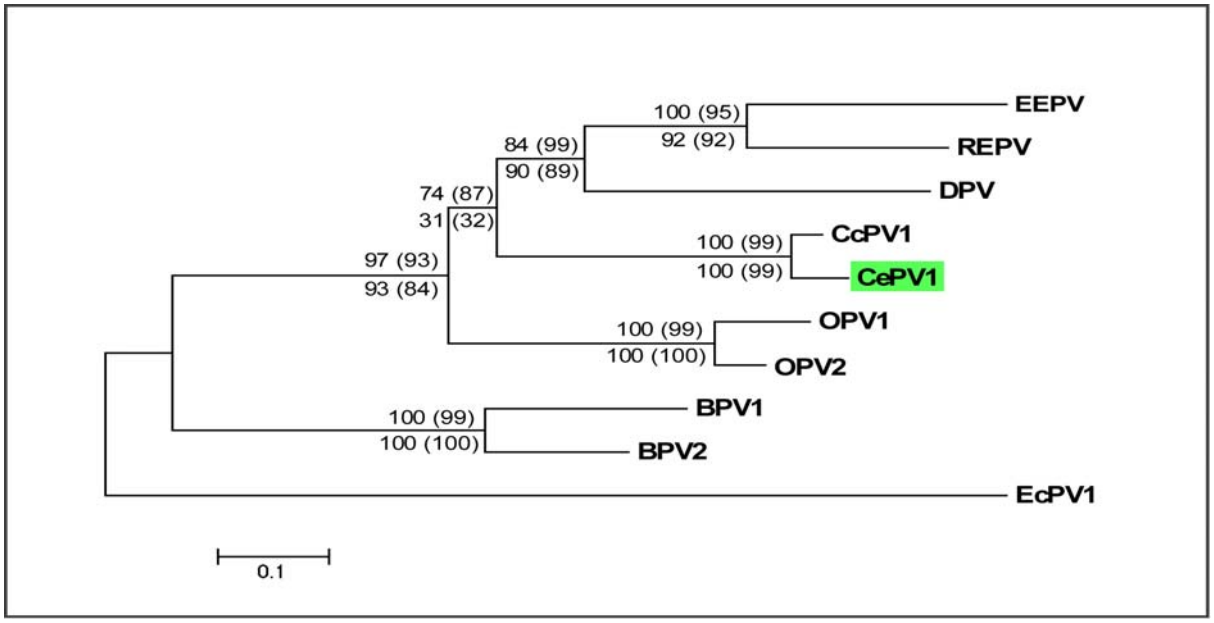

Figure 2. A neighbour-joining (NJ) phylogram indicating the evolutionary distances inferred from the alignment of the partial L2 ORF nucleotide sequences of nine Delta-papillomaviruses. The bootstrap values obtained for the identical topologies by the NJ (top) and maximum parsimony (below) methods are indicated on the NJ tree for both nucleotide and amino acid sequence alignments (in parentheses).

In order to study the phylogenetic relationship between CcPV1 and CePV and their host species, we compared the phylogenetic trees of Delta-papillomaviruses and their ruminant hosts. The phylogeny of the host species was derived from the comprehensive study of 4510 mammalian species by Bininda-Emonds (2007). The two phylogenetic trees exhibited parallel topology, pairing up the host species and its respective virus, indicating a host linked evolution of these papillomaviruses. The exception to this rule was the roe deer papillomavirus. The recent divergence of CePV and CcPV1 and the incongruence of the CcPV1 and the roe deer phylogeny points to the highly likely possibility that CcPV1 evolved through a host-switch event from a common ancestor with CePV, i.e. an ancient red deer papillomavirus.

3.5. New scientific results

1. Detection of the novel roe deer papillomavirus (CcPV1) by PCR, sequencing and characterisation of its genome
2. Demonstration of the presence of roe deer papillomavirus (CcPV1) genomic DNA in infected tissues by in-situ DNA hybridisation
3. Detection of the novel red deer papillomavirus (CePV) by PCR, demonstration of the presence of its capsid antigen by immunohistochemistry and its genomic DNA by in-situ DNA hybridisation in infected tissues
4. Phylogenetic characterisation of both roe deer (CcPV1) and red deer papillomavirus (CePV)
5. Establishment of the geographical distribution of roe deer fibropapillomatosis, characterisation of its ecology and epidemiology
6. Development of a geographical prediction model of roe deer fibro-papillomatosis

4. conclusions and suggestions

4.1. Pathology

Based on our study, we conclude, that the pathological and epidemiological characteristics of roe deer fibropapillomatosis are very similar to the DPV infection of white-tailed deer and other North-American cervid species. Apart from the absence of nodular lung fibromatosis, the pathological lesions were found to be identical. However, unlike DPV infection which causes lesions in young animals, roe deer fibropapillomatosis was found to predominantly affect older age classes of both sex, similarly to the papillomavirus infection of European elk and North American Moose. This characteristic may indicate a cumulative tendency of the disease and a slow regression of lesions. However, the length of the course of the disease cannot be estimated from our data.

Macroscopic and histopathological features, and the localisation of PV antigen in the epithelial layer covering red deer fibropapillomas was similar to earlier descriptions (Moar and Jarrett, 1985). The cross-reaction of anti-BPV1 antibodies with the Red deer papillomavirus confirms the close antigenic relatedness of Delta-papillomaviruses. No evidence of tumour metastasis and no sign of malignancy was detected in neither roe deer nor red deer fibropapillomas.

4.2. Virology

Based on the phylogenetic and pathological studies we can establish that roe deer fibropapillomatosis is caused by the novel roe deer papillomavirus (CcPV1). As it was expected, the successful amplification of CcPV1 confirmed, that similarly to other Delta-papillomaviruses its genome is present in an episomal form in infected cells.

This virus belongs to the Delta-papillomavirus genus of the Papillomaviridae family and it is sufficiently distinct from all of its' presently known nearest relatives to represent a new papillomavirus type.

Although the degree of nucleotide identity (71.2 and 70.3%) between the L1 ORF sequence of CcPV1 and the closely related EEPV and REPV, respectively, exceeded the limit set for the definition of a novel papillomavirus species (de Villiers

et al., 2004), the robust topology of the phylogenetic tree lends support to our opinion that CcPV1 may eventually represent a separate papillomavirus species.

The phylogenetic analysis and the consistent pathological findings indicate that CePV belongs to the Delta-papillomavirus genus. Thus, our data support the findings of Moar and Jarrett (1985) regarding the close relatedness of BPV1/BPV2 and the Red deer PV established by Southern blot analysis. The exact extent of the inferred close relatedness of CcPV1 and CePV shall be determined by the sequencing of the complete CePV genome.

4.3. Ecology and epidemiology

Results of the questionnaire survey confirmed the presence of roe deer fibropapillomatosis in areas identified by all our earlier case records and with the data presented by Kocsner (1996, 2001) and Takács and Nagy-Bozsoki (1998). Many infected areas were identified for the first time and were confirmed by additional samples submitted after the survey.

The positive statistical correlation of disease distribution with both host density and waterways, and the fact that there was no correlation between these two variables suggests that waterways may have an independent effect on the distribution of roe deer fibropapillomatosis. One of the possible explanations, which should be experimentally tested, is that the habitats in question may be especially suitable for arthropod vectors which might play a significant role in the ecology of CcPV1. However, the potential effect of an increased, seasonal roe deer aggregation induced by these habitats in an agricultural landscape should not be excluded either.

The coincidence of genetic differentiation between European roe deer populations and the endemic, geographically limited distribution of roe deer fibropapillomatosis lends further support for the potential existence of genetically determined susceptibility differences of host populations (Erdélyi et al., 2008).

Apart from our phylogenetic studies, the likelihood of a host-switch event in the evolutionary history of CcPV1 is also supported by the phylogeography of CePV and CcPV1. While red deer fibropapillomatosis (i.e. CePV) is occurs sporadically throughout Europe, the distribution of roe deer fibropapillomatosis (CcPV1) is

endemic, limited to a genetically distinct roe deer population within the Carpathian basin.

Despite its practicality, the drawback of our survey approach is that classical epidemiological indicators (e.g. disease prevalence or incidence) cannot be calculated from the categorical data provided, and that certain bias may be inherent in the estimates provided by interviewees. This may be most pronounced in the answers provided by those GMUs where disease occurrence is scarce.

Nevertheless, results of this study indicate that there is a certain niche for questionnaire surveys in the study of wildlife disease ecology. Although skin tumours are special because of their more memorable and easily recognizable physical aspect, we believe that territorially organised game management and nature conservation systems may be used to obtain baseline data on the occurrence, distribution and the estimated or perceived impact and importance of wildlife diseases. Results of such initial enquiries could be used to establish targeted research and surveillance of specific pathogens or host populations.

5. PUBLICATIONS

5.1. Publications in scientific journals

Erdélyi, K., Bálint, Á., Dencső, L., Dán, Á., Ursu., K. (2008): Characterisation of the first complete genome sequence of the roe deer (*Capreolus capreolus*) papillomavirus. *Virus Research*, 135 (2) 307-311. p.

Erdélyi, K., Dencső, L., Lehoczki, R., Heltai, M., Sonkoly, K., Csányi, S., Solymosi, N. (2009a): Endemic papillomavirus infection of roe deer (*Capreolus capreolus*). *Veterinary Microbiology*, 138 (1-2) 20-26. p.

Erdélyi, K., Gál, J., Sugár, L., Ursu, K., Forgách, P., Szeredi, L., Steineck, T. (2009b): Papillomavirus associated fibropapillomas of red deer (*Cervus elaphus*). *Acta Veterinaria Hungarica*, 57 (2) 337–344. p.

5.2. Book chapter

Erdélyi, K. Roe deer and other ungulate papillomaviruses. In: Gavier-Widén, D., Duff, J. P., Meredith, A. (eds) Infectious Diseases of Wild Mammals and Birds in Europe, Wiley-Blackwell, In-Press

5.3. Publications in conference proceedings

Erdélyi, K., Ursu, K. (2005): Molecular evidence for the existence of a roedeer (*Capreolus capreolus*) papillomavirus. 42nd International Symposium on Diseases of Zoo and Wildlife, Prague, Proceedings, 42, 212-213. p.

Erdélyi, K., Ursu, K., Steineck, T. (2006): Identification of a red deer (*Cervus elaphus*) papillomavirus by molecular methods. Proceedings of the 7th Meeting of the European Wildlife Disease Association, St. Vincent, Italy.

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Rajský, D., **Erdélyi, K.**, Sugár, L., Krajniak, E. (2009): Najnovšie doplnky (november 2009) k prognóze rozširenia fibromatózy (fibropapilomatózy) v rámci zástupcov podčeľade *Cervinae* na Slovensku. Výživa a veterinárna problematika chovu jeleňovitých, Zborník referátov z medzinárodnej konferencie, Nitra, 12.11.2009. pp. 88-89. p.

5.4. Other conference papers

Erdélyi, K. (2008): Szarvasfélék deltapapillomavírusainak evolúciója. Paraziták és más patogének evolúciója és ökológiája. A Magyar Biológiai Társaság Ökológiai Szakosztályának, Környezet és Természetvédelmi Szakosztályának, Magyar Ökológusok Tudományos Egyesületének és a Magyar Parazitológusok Társaságának közös előadóülése. 2008. november 14., Magyar Természettudományi Múzeum, Budapest.