ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/41102410

Herpesvirus-Associated Papillomatosis in a Green Lizard

Article *in* Journal of wildlife diseases · January 2010

DOI: 10.7589/0090-3558-46.1.257 · Source: PubMed

CITATIONS
17

READS
90

7 authors, including:

Viktoria Majlathova
Pavol Jozef Šafárik University in Košice
56 PUBLICATIONS 548 CITATIONS

SEE PROFILE

Igor Majláth
Pavol Jozef Šafárik University in Košice
41 PUBLICATIONS 368 CITATIONS

SEE PROFILE

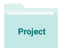
Pavel Kulich
Veterinary Research Institute, Brno
64 PUBLICATIONS 533 CITATIONS

SEE PROFILE

Eva Roubalova
Institute of Parasitology Biology Centre, ASCR
17 PUBLICATIONS 370 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



CENATOX View project

CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068), European Regional Development Fund. View project

All content following this page was uploaded by Viktoria Majlathova on 19 May 2014.

The user has requested enhancement of the downloaded file.

Journal of Wildlife Diseases, 46(1), 2010, pp. 257–261
© Wildlife Disease Association 2010

Herpesvirus-Associated Papillomatosis in a Green Lizard

I. Literak,[1] B. Robesova,[2] V. Majlathova,[3] I. Majlath,[4] P. Kulich,[2] P. Fabian,[5] and E. Roubalova[1,6]
[1] Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackeho 1-3, 612 42 Brno, Czech Republic; [2] Veterinary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic; [3] Parasitological Institute SAV, Hlinkova 3, 040 01 Kosice, Slovakia; [4] Department of Biology and Ecology, Faculty of Natural Sciences, P. J. Safarik University, Moyzesova 11, Kosice, Slovakia; [5] Department of Oncological and Experimental Pathology, Masaryk Memorial Cancer Institute, Zluty Kopec 7, 656 53, Brno Czech Republic; [6] Corresponding author (email: roubalovae@vfu.cz)

ABSTRACT: Papillomatous skin lesions from a green lizard (*Lacerta viridis*) were examined histologically, using electron microscopy and DNA was isolated from the lesions for herpesviral DNA detection. Histology confirmed the lesions to be squamous epithelial papillomas. Using electron microscopy, no virus particles were detected. The specific sequence of herpesviral DNA-directed DNA polymerase (EC 2.7.7.7) was amplified using degenerate primers in a nested format. The 235–base-pair (bp) sequence was sequenced and compared with previously published DNA-directed DNA polymerase sequences from various reptile herpesviruses. The sequence from the green lizard showed significant similarity with sequence of fibropapilloma-associated turtle herpesviruses from sea turtles.

Key words: DNA-directed DNA polymerase, electron microscopy, green lizard, herpesvirus, Hungary, *Lacerta viridis*, papilloma, skin lesions.

The green lizard (*Lacerta viridis*; Reptilia: Lacertidae) is a large lizard that lives in dry, bushy biotopes with limestone soil across S and SE Europe and Asia Minor (Barus and Oliva, 1992). Papillomatous skin lesions in a green lizard were first described in Northern Italy (Blanchard, 1890). Later on papillomas were found on the skin of a green lizard in the Leipzig Zoo (Germany; Schanbel, 1953). Papillomas also appeared in a group of green lizards in Northern Italy (Raynaud and Adrian, 1976). Histologic examinations confirmed that these lesions were papillomas. The nuclei of epidermal cells in the basal layer of epidermis were hypertrophic with a large amount of chromatin accumulated close to nuclear membrane. Inclusions were found in these cells and, using electron microscopy, three types of viruses were detected in these inclusions: viruses belonging to the families of Herpesviridae and Reoviridae, and viruses resembling members of the former Papovaviridae family. Papillomas in green lizards were associated with viral etiology in subsequent studies as well (Cooper et al., 1982), and recently, papillomatosis in a green lizard was described in the SE of Slovakia (Racka et al., 2006).

In the summer of 2007, fifty green lizards were studied in Hungary in the Godolo locality NE of Budapest (47°33′N, 19°21′E) and papillomatous lesions were recorded in two animals. With reference to previously published works indicating viral etiology of papillomatosis in green lizards (Raynaud and Adrian, 1976; Cooper et al., 1982) and to recent works associating papillomatosis in sea turtles with herpesviral etiology (Herbst et al., 2004; Greenblatt et al., 2005), we focused on detection and characterization of the herpesvirus in papillomatous lesions of one affected green lizard. The affected lizard was an approximately 2-yr-old male (weight 18.6 g, length 215 mm). Two papillomatous lesions (13×9×5 mm and 10×6×4 mm) were located in both sides of the body directly in front of the forelimbs (Fig. 1). No other lesions were detected at necropsy. Samples from both lesions, as well as from unaffected skin, were collected for a standard histologic examination, for electron microscopy, and for molecular detection of DNA-directed DNA polymerase gene.

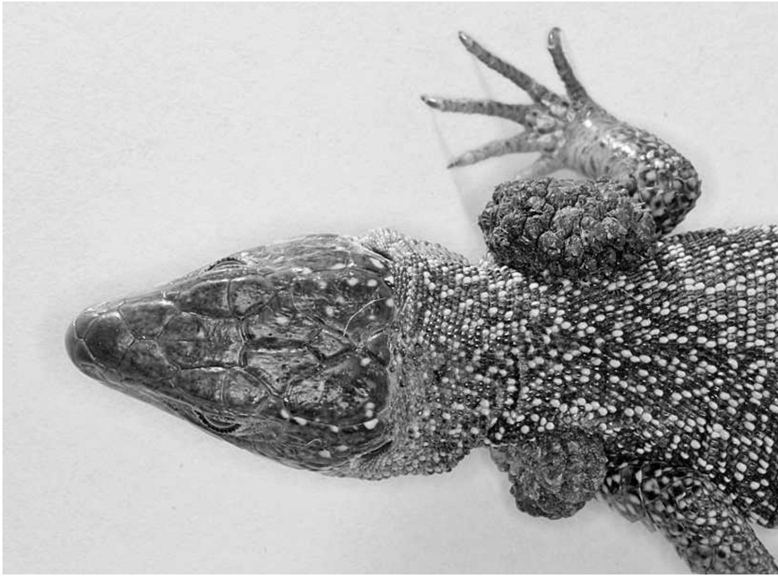


FIGURE 1. Papillomas on the body of a green lizard. (Photographed by Peter Podzemy)

On histologic examination, sharply demarcated, exophytically growing epithelial tumors with papillary arrangement were observed. Thin stromal papillae with complex hierarchical ramification were covered with stratified squamous epithelium of varying numbers of cellular layers (from four up to 15). In the neoplastic epithelium a functional stratification and differentiation from stratum basale to spinosum, granulosum, and corneum, respectively, was well-preserved. Marked hyperkeratosis without parakeratosis was a conspicuous feature. The uniform tumor cells had abundant, lightly eosinophilic cytoplasm, and a centrally placed oval nuclei with homogenous fine chromatin and with one or two inconspicuous basophilic nucleoli. Focally, mostly perinuclearly located clear intracytoplasmatic vacuoles were present. No inclusions were observed. Mitotic figures were quite frequent, although they were visible exclusively in the basal and parabasal layers. Atypical mitoses were not found. Within the stromal papillae, numerous pigmented cells were randomly distributed in addition to subtle capillaries. Many pigment-loaded dendritic cells with thin cytoplasmic branched projections were dispersed among the neoplastic epithelial cells. In the corneal layer, focal strong pigmentation was also frequent. A sharp demarcation between the tumor mass and the dermal fibrous tissue was observed with no signs of invasive growth. Skin papillomatosis was confirmed by histologic examination.

A Philips EM 208 electron microscope (Royal Phillips Electronics, Amsterdam, The Netherlands) was used for examination of samples stained by negative staining technique and for ultrathin sections examination. No virus particles were found using electron microscopy. Ultrathin section examination showed no abnormality in the majority of epithelial cells. A few nuclei were hypertrophic with centrally located heterochromatin. Several nuclei were vacuolated with centrally located chromatin or with rarefied chromatin located close to the nuclear membrane.

For polymerase chain reaction (PCR) amplification, sequencing, and sequence analyses, DNA from both the skin lesions

(Samples 1 and 2) and unaffected skin (Sample 3) was isolated using the NucleoSpin® Tissue kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. Polymerase chain reaction was carried out to detect a sequence of herpesviral DNA-directed DNA polymerase (EC 2.7.7.7) using degenerate primers in a nested format (VanDevanter et al., 1996). Secondary PCR products were visualized on 1% agarose gel containing 0.5 μg/ml ethidium bromide. Products of expected size (ca. 250 base pairs [bp]) were amplified in samples from both lesions, while no product was detected amplifying the sample from unaffected skin.

For direct sequencing, secondary PCR products were purified using the QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany), eluted into 30 μl of H_2O and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) according to manufacturer's instructions. Sequencing was carried out using the ABI Prism 310 Genetic Analyzer (Applied Biosystems). Sequencing of both positive samples resulted in a 235-bp sequence (identical for both samples). Acquired sequence was stored in the GenBank database under accession number EU527336. Using NCBI-BLAST2 we searched for sequences with maximum similarity to our sequence and found a significant similarity with the group of DNA-directed DNA polymerase sequences from fibropapilloma-associated herpesviruses from sea turtles (*Chelonia mydas*, *Caretta caretta*, and *Lepidochelys kempii*; AY646889–AY646894), which confirmed the presence of herpesvirus in both of our papilloma samples.

To specify the relations between diverse reptile herpesviruses, our sequence was aligned with corresponding sequences of DNA-directed DNA polymerase gene of all available reptile herpesviruses acquired from GenBank, using Molecular Evolutionary Genetics Analysis (MEGA) version 4 (Tamura et al., 2007) and 183-bp alignment of 17 sequences was consequently used to construct phylogenetic trees. FindModel web site (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) was employed to determine the best model of evolution that fits to our data. Consequently all genetic distances were computed and phylogenetic trees were constructed using MEGA4 software employing neighbor-joining (NJ; Saitou and Nei, 1987) and maximum parsimony (MP) algorithms. In NJ analysis, Kimura 2-parameter (K2P) model with homogenous pattern among lineages and uniform rates among sites was used (Kimura, 1980). In MP analysis, only minimal trees were retained. Bootstrap analysis (1,000 replicates) was used in both NJ and MP algorithms to evaluate robustness of topology. As seen in Figure 2, phylogenetic analysis using NJ method placed our sequence to the closest proximity with the sequence of Varanid herpesvirus 2 (AB189433) with undefined manifestation. The phylogenetic tree obtained using MP method showed very similar topology to that of NJ tree (data not shown).

In turtles, two types of clinical manifestation of herpesviral infection were described: lung-eye-trachea disease (LETD), and fibropapillomatosis (FP) (Coberley et al., 2002). *Lacerta viridis* sequence was compared to corresponding sequences of herpesviruses representing both these groups. Pairwise distances were estimated using the K2P method in MEGA4. Analysis showed higher similarity (0,401 base substitutions per site) with the group of aforementioned FP-associated turtle herpesviruses from sea turtles (*Caretta caretta*, *Lepidochelys kempii*, and *Chelonia mydas*; Herbst et al., 2004), compared to LETD-associated herpesvirus sequence (AY124579) from *Chelonia mydas* (Coberley et al., 2002); these had significantly less homology with the *Lacerta viridis* sequence (1,441 base substitutions per site).

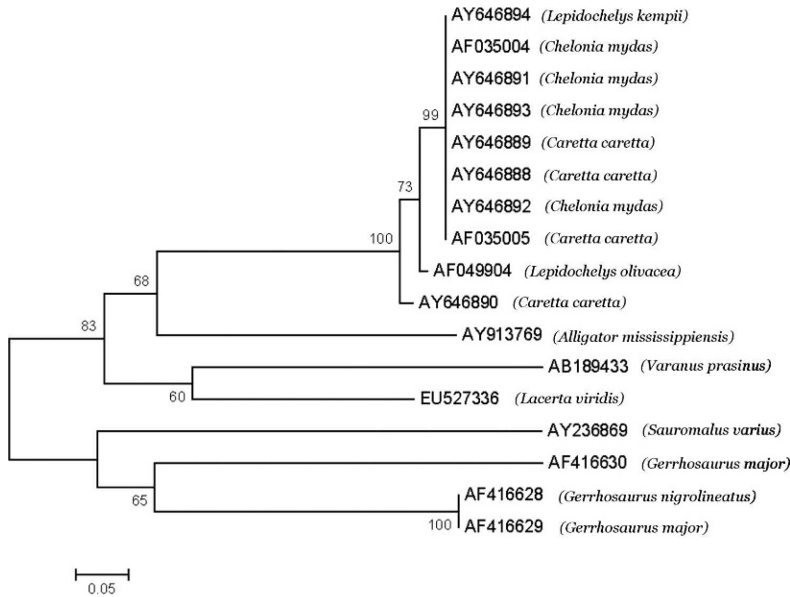


FIGURE 2. Results of phylogenetic analysis of the 183 bp sequence of herpesviral DNA directed DNA polymerase gene from a green lizard (*Lacerta viridis*) (EU 527336) compared to the sequences of this gene originated from other reptiles (GenBank and Quackenbush et al., 2002, Wellehan et al., 2003, 2004, Herbst et al., 2004, Greenblatt et al. 2005).

Virus particles were found neither by ultrathin sections, nor by negative staining. Nevertheless, herpesviral DNA-directed DNA polymerase sequence was detected using nested PCR assay with degenerate primers. Because this PCR detects highly conservative herpesviral motif and is considered to be a reliable method for herpesvirus detection, we can conclude that herpesvirus was associated with both papillomatous lesions from the green lizard. Moreover, this sequence was amplified in both papilloma lesions, but not in the unaffected skin sample, so the presence of this previously unidentified herpesvirus is related to the tumor tissue. Phylogenetic analysis placed our sequence in close proximity to the sequences of herpesviruses causing FP in sea turtles, compared to LETD-associated virus AY124579 sequence from *Chelonia mydas*, which differed significantly from our sequence. This result is in accordance with clinical findings and implies a relationship of this green lizard herpesvirus to FP-associated herpesviruses from sea turtles.

Skin papillomas studied in this work were histologically similar to previously published findings in green lizards (Blanchard, 1890; Schanbel, 1953; Raynaud and Adrian, 1976; Cooper et al., 1982; Racka et al., 2006). Results presented in our study document the first case of papillomatosis in a green lizard in Hungary. Papillomatosis in green lizards seems to occur sporadically in the wider area of distribution of this species. If herpesviruses are associated with skin papillomas in green lizards, we can assume that these herpesviruses are highly specific for green lizard populations. Recurrence of papillomatous lesions could be associated with a variety of stressors, including co-infection with another agent, overcrowding, and temperature changes, as was proposed for animal herpesviral infections by Murphy et al. (1999).

This study was funded by grant MSM6215712402 from the Ministry of Education, Youth and Sports. B. Robesova was funded by grant MZe 0002716201 from the Ministry of Agriculture of the Czech Republic.

LITERATURE CITED

Barus, V., and O. Oliva. 1992. Plazi—Reptilia, fauna CSFR (Reptiles, fauna of Czechoslovakia, in Czech with a summary in English). Academia, Prague, Czech Republic, 222 pp.

Blanchard, R. 1890. Sur une remarquable dermatose causee chez le lezart vert par un champignos du genre *Selenosporium*. Memoirs Societe Zoologique de France: 241–245.

Coberley, S. S., R. C. Condit, L. H. Herbst, and P. A. Klein. 2002. Identification and expression of immunogenic proteins of a disease-associated marine turtle herpesvirus. Journal of Virology 76: 10553–10558.

Cooper, J. E., S. Gschmeissner, and P. E. Holt. 1982. Viral particles in a papilloma from a green lizard (*Lacerta viridis*). Laboratory Animals 16: 12–13.

Greenblatt, R. J., S. L. Quackenbush, R. N. Casey, J. Rovnak, G. H. Balazs, T. M. Work, J. W. Casey, and C. A. Sutton. 2005. Genomic variation of the fibropapilloma-associated marine turtle herpesvirus across seven geographic areas and three host species. Journal of Virology 79: 1125–1132.

Herbst, L., A. Ene, M. Su, R. Desalle, and J. Lenz. 2004. Tumor outbreaks in marine turtles are not due to recent herpesvirus mutations. Current Biology 14: R697–R699.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120.

Murphy, F. A., E. P. J. Gibbs, M. C. Horzinek, and M. J. Studdert. 1999. Veterinary Virology. Academic Press, San Diego, California, 629 pp.

Racka, K., S. Toth, I. Majlath, and V. Majlathova. 2006. Papilomatoza u jasterice zelenej (*Lacerta viridis*, Laurentus, 1768) v Narodnom Parku Slovensky kras [Papillomatosis in the green lizard (*Lacerta viridis*, Laurentus, 1768) in National Park Slovensky Kras, in Slovak]. *In* The 2nd conference "Infectious and Parasitic Diseases of Animals," A. Kocisova, J. Suli and J. Toporcak (eds.). University of Veterinary Medicine in Kosice, Slovakia, pp. 258–260.

Raynaud, A., and M. Adrian. 1976. Cutaneous lesions of papillomatous structure associated with viruses in green lizard (*Lacerta viridis* Laur). Comptes Rendus Hebdomadaires des Seances de l Academie des Sciences, Serie D 283: 845–847.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Schanbel, R. 1953. Papillome an einer Smaragdeidechse (*Lacerta viridis*). Ein Beitrag zur sogenannten Pockenerkrankung wechselwarmer Wilbertiere. Der Zoologische Garten 20: 270–278.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599.

Vandevanter, D. R., P. Warrener, L. Bennett, E. R. Schultz, S. Coulter, R. L. Garber, and T. M. Rose. 1996. Detection and analysis of diverse herpesviral species by consensus primer PCR. Journal of Clinical Microbiology 34: 1666–1671.

Received for publication 21 April 2008.