Acta virologica **64**: 409 – 416, 2020 doi: 10.4149/av_2020_404

Review

Herpesvirus diseases of domestic animals and game species in the Slovak Republic

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Received January 17, 2020; accepted August 21, 2020

Summary. – Herpesviruses are DNA viruses that cause serious latent infections in humans and animals. These pathogens significantly influence the animal health and economy of animal husbandry. The reduction of production parameters, abortions, birth of weak individuals and by costs associated with the elimination and monitoring of herpesvirus diseases are among the most serious harms caused by herpesviruses. In our review we focused mainly on herpesvirus diseases in pigs and cattle (Aujeszky's disease, bovine infectious pustular vulvovaginitis and balanoposthitis, infectious bovine rhinotracheitis) and herpesvirus diseases in red deer, dogs, and carps. In the past, these herpesvirus diseases have caused considerable economic losses in livestock. At present, several of these diseases are eliminated in the Slovak territory. Currently, a continuous monitoring is carried out not only in populations of domestic animals, but also in wild animals, which are the main reservoirs of the mentioned herpesviruses.

Keywords: herpesvirus; Aujeszky's disease; animals; fish; eradication; Slovak Republic

Introduction

Herpesviruses are highly successful pathogens infecting animals and humans. Although there is a wide variety of different herpesviruses with different biological characteristics, they share common basic properties such as virion morphology, highly regulated transcription and the ability of persistence in host tissues in the latent form. Herpesviruses are large enveloped viruses with double stranded DNA. Taxonomically, herpesviruses belong to the family *Herpesviridae* (Ryan and Ray, 2004; Sandri-Goldin, 2006; Mettenleiter *et al.*, 2008). Phylogenetic analyses showed an existence of a divergence in group of herpesviruses and subsequently contributed to inclusion of new families to the order *Herpesvirales*. Fish and amphibian herpesviruses belong to the family *Alloherpesviridae*, herpesviruses of molluscs to the family *Malacoherpesviridae* and herpesviruses of mammals, birds and reptiles belong to the family *Herpesviridae* (ICTV, 2011). The family *Herpesviridae* includes three subfamilies (*Alpha-*, *Beta-* and *Gammaherpesvirinae*) (McGeoch *et al.*, 1995). The subfamily *Alphaherpesvirinae* includes viruses characteristic by a wide host spectrum, relatively short replication cycle and fast spread from cell to cell. Alphaherpesviruses cause destruction of infected cells and the viruses persist in latent form, mainly in sensory ganglia of infected organism.

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Abbreviations: AD = Aujeszky's disease; ADV = Aujeszky's disease virus; BoHV-1 = bovine herpesvirus 1; BoHV-5 = bovine herpesvirus 5; CHV = canine herpesvirus; CvHV-1 = cervid herpesvirus 1; CyHV-3 = cyprinid herpesvirus 3; ELISA = enzyme-linked immunosorbent assay; IBR = infectious bovine rhinotracheitis; KHD = Koi herpesvirus disease; KHV = koi herpesvirus

The subfamily *Betaherpesvirinae* is characterized by viruses with limited spectrum of host species, long infection cycle, formation of cytomegalia and latency fixed to secretion glands, lymphoreticular cells, the epithelia of salivary glands and kidneys.

Members of the subfamily *Gammaherpesvirinae* infect limited spectrum of host species, and the viruses replicate in lymphoblast cells in the *in vitro* environment. The viruses infect specifically either B or T lymphocytes and induce latent infection in lymphoid cells, which consequently increase their proliferation or may be transformed (Kostrábová *et al.*, 2017).

Herpesviruses can be transmitted in different ways such as direct contact, indirectly via contaminated objects or substrates, transplacentally or by aerogenic transmission on a short distance. The most common is the direct transmission of infection through the mucous membranes. Herpesviruses, in general, show a limited viability under environmental conditions outside the host organism.

Herpesviruses in their hosts cause damage to the mucous membranes of the respiratory, digestive and genital apparatus, damage to the vascular epithelium, liver necrosis, and vesicular lesions of the superficial epithelium. Herpesvirus infections in pregnant animals can cause abortions (Mojžišová, 2006).

In the Slovak Republic, the most common herpesviruses in animal populations belong to the subfamily *Alphaherpesvirinae* of the genus *Varicellovirus*.

Aujeszky's disease, bovine infectious pustular vulvovaginitis and balanoposthitis, infectious bovine rhinotracheitis, herpesvirus diseases in dogs and cats are among the most important animal herpesvirus diseases. In the past, these herpesvirus diseases have caused considerable economic losses in livestock. Recently, several of mentioned diseases are eradicated in the Slovak Republic and some of these diseases are still monitored, not only in livestock holdings, but also in populations of game species, which are the main reservoir species of the mentioned herpesvirus diseases.

Suid herpesvirus 1

Suid herpesvirus 1 causes Aujeszky's disease (pseudorabies, ADV), an economically significant, highly infectious neurotrophic herpesvirus disease of swine. Infection caused by this virus in young swine results in symptoms of CNS involvement (bulbar paralysis or *'crazy itching'*) accompanied by high mortality. In older pigs, it causes diseases of respiratory and reproductive system (Anonymous, 2013).

In 1909, Weis found that the host reservoir (natural host) of the virus is domestic swine (*Sus scrofa domestica*) and the disease can be transmitted to ruminants (Ruminantia), especially cattle (*Bos taurus*), goat (*Capra aegagrus hircus*) and sheep (*Ovis aries*), cats (*Felis catus*), dogs (*Canis lupus familiaris*), horses (*Equus*), northern raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), house mouse (*Mus musculus*) and brown rats (*Rattus norvegicus*) (Verpoest *et al.*, 2014). The complete life cycle of the virus occurs only in domestic swine (*Sus scrofa domestica*) (Anonymous, 2015) meaning that these animal species are more or less only latent virus carriers (Anonymous, 2013; Hu *et al.*, 2016). Wild boar (*Sus scrofa*) is considered a natural reservoir species in which infection usually occurs asymptomatically (Müller *et al.*, 2000; Risco *et al.*, 2018; Carr *et al.*, 2019). In infected swine, ADV is isolated primarily from the nose and oropharynx; respectively, vagina, ejaculate, milk and urine (Cay and Letellier, 2009). ADV is transmitted mainly by individuals infected with a virus that persists in latency, e.g. after priming, after reactivation of the viral genome, resp. by vaccinated animals (Leuenberger *et al.*, 2007). Viral latency can develop in vaccinated sows with passive immunity (Hahn *et al.*, 1997; Hu *et al.*, 2015). ADV is most commonly transmitted horizontally by direct *´nose-to-nose´* contact or by vertical way during the mating, especially in wild boar (Romero *et al.*, 1997; Meier *et al.*, 2015).

Occurrence of Aujeszky's disease virus (ADV) in the Slovak Republic

Kalafa and Sabó (1990) summarized in their work the occurrence of ADV in 1971–1975 in the Slovak Republic. During this period 105–148 AD outbreaks were confirmed per year (15 644–23 719 pigs infected). Since 1976, 36 outbreaks have been confirmed, and in 1980, 10 outbreaks of AD. In the years 1981–1987 one, resp. 2 outbreaks per year in domestic pig farms were confirmed. In 1988 the disease occurred in 65 pigs. In addition to the swine population, the AD virus has also been confirmed several times in cattle. In average of 2–15 outbreaks per year were diagnosed in 1971–1977; 22–45 cases of AD in cattle were confirmed in 1978–1982; in the years 1983–1984 it was 14–16 AD outbreaks; 5–11 AD outbreaks in 1985–1987 and three AD outbreaks (42 heads) in cattle in 1988. In the territory of the Slovak Republic, as in other parts of Europe, a National recovery program of husbandry from Aujeszky's disease was carried out in order to heal domestic pig populations. By issuing Commission Decision no. 2007/603/EC, the Slovak Republic was included in the list of Aujeszky's disease-free regions. The condition of keeping Slovak Republic free of Aujeszky's disease is the continuity of monitoring of Aujeszky's disease.

At present time, Aujeszky's disease does not rise in domestic pigs in the Slovak Republic. The last reported

Table 1. Virological and serological monitoring of ADV infection in the Slovak Republic (modified according to the Veterinary Institute Zvolen, Slovak Republic)

Year	Examined species	Virus detection		Serological examination	
		Number of samples examined	Number of samples positive	Number of samples examined	Number of samples positive
2014	Pig	1	0	4 793	0
	Wild boar	25 077	4	2	0
	Dog	4	2	1	0
2015	Pig	1	0	4 679	0
	Wild boar	27 078	6	3	0
	Dog	8	2	0	0
2016	Pig	2	0	4 525	0
	Wild boar	28 822	13	0	0
	Dog	4	2	0	0
2017	Pig	3	0	4 649	0
	Wild boar	12 203	4	6	4
	Dog	2	1	0	0
2018	Pig	29	0	4 237	88
	Wild boar	12 515	0	0	0
	Dog	6	3	0	0
	Red fox	1	0	0	0
	ZOO animals	0	0	1	0
Summary	Pig	36	0	22 883	88
	Wild boar	105 695	27	13	4
	Dog	24	10	1	0
	Red fox	1	0	0	0
	ZOO animals	0	0	1	0

cases of AD in pigs were in 2003 from the districts of Martin, Spišská Nová Ves, Galanta, Levice, Trebišov and Svidník. As part of AD prevention, the Aujeszky's Disease Reference Center, Veterinary Institute Zvolen, carries out laboratory tests of susceptible animal species every year. Results of laboratory examinations by species and number of positive samples examined in 2014–2018 in Veterinary Institute Zvolen, reference laboratory for AD are given in Table 1.

In 2014, 29 878 samples from the whole territory of Slovakia were examined for the presence of the virus and presence of antibodies. From these, 25 077 samples originated from wild boar, 1 sample from domestic pig and 4 samples originated from dogs. For the presence of total antibodies, 4 793 samples of domestic pigs, 2 samples of wild boars and 1 sample derived from a dog were examined. Of the total number of examined samples, four AD virus strains were isolated and identified from wild boar caught in the districts of Prievidza and Rimavská Sobota; in 2 cases, Aujeszky's virus was isolated and identified from dogs that came from the districts of Trnava and Zvolen.

In 2015, out of 31 769 samples from Slovakia examined for the presence of virus 27 078 samples originated from wild boar, 1 sample from domestic pig and 8 samples from dogs. For the presence of antibodies, 4 679 samples of domestic pigs and 3 samples of wild boars were examined. Of the total number of samples examined, 6 strains of AD virus were isolated and identified from wild boar caught in the districts of Malacky, Nové Mesto nad Váhom, Rimavská Sobota, Levice, Trnava and Banská Štiavnica. In 2 cases, Aujeszky's virus was isolated and identified from dogs that came from the Malacky and Stará Ľubovňa districts.

In 2016, 30 353 samples delivered from the whole territory of Slovakia were screened for the presence of the ADV within the field diagnostic activity. From these, 28 822 samples were from wild boar, 2 samples from domestic pigs and 4 samples from dogs. 4 525 samples of domestic pigs were examined for the presence of total antibodies. Of the total number of samples examined, 13 strains of AD virus were isolated and identified from wild boars caught in the districts of Krupina, Rimavská Sobota, Levice, Trnava, Nové Zámky, Veľký Krtíš and Zlaté Moravce. In 2 cases, Aujeszky's virus was isolated and identified from dogs that came from the districts of Prievidza and Zvolen.

In 2017, 16 863 samples from Slovakia were screened for the presence of the virus and 12 203 samples were from wild boar caught, 3 samples from domestic pigs and 2

samples from dogs. For the presence of total antibodies 4 649 samples of domestic pigs and 6 samples of wild boar were examined. Of the total number of examined samples, 4 strains of AD virus were isolated and identified from wild boar caught in Humenné and Prešov districts. In one case, Aujeszky's virus was isolated and identified from a dog that came from Lučenec district. Antibodies were detected in wild boars caught in Lučenec district.

In 2018, out of 16 789 samples from Slovakia screened for the presence of the virus 12 515 samples were from wild boar, 29 samples from domestic pigs, 6 samples from dogs and one sample from fox. For the presence of total antibodies 4 237 samples of domestic pigs and 1 sample taken in the zoological garden were screened. Of the total number of examined samples, 3 AD virus strains were isolated and identified from dogs caught in the districts of Prievidza, Stropkov and Rožňava. Pigs bred on one farm of domestic pigs in the district of Dunajská Streda were serologically positive. The presence of the virus on the farm was not confirmed.

In 2018–2019, we conducted a targeted serological survey in wild boar populations, which is the main reservoir species. Of the 199 samples examined, 71 were seropositive, which represents 37.68%. Compared to other European countries, we belong to the countries with moderate seroprevalence of Aujeszky's disease. In Central and Eastern European countries such as Slovenia, the prevalence of ADV was 31% (Vengust *et al.*, 2006), Austria 38% (Steinrigl *et al.*, 2012), Czech Republic 30% (Sedlak *et al.*, 2008) and North-eastern Germany up to 29% (Kaden *et al.*, 2009; Leschnik *et al.*, 2012).

Bovine herpesviruses

Alphaherpesviruses of ruminants form a numerous group of pathogens. Among others, cattle, goats, sheep and several species of wild ruminants belong to the susceptible hosts (Santman-Berends *et al.*, 2018).

Bovine herpesvirus 1 (BoHV-1) is the most important and the best studied member of the group (Zhu *et al.*, 2018). Its closest relative herpesvirus is cervine herpesvirus 1 (Inglis *et al.*, 1983).

BoHV-1 and bovine herpesvirus 5 (BoHV-5) are closely related viruses with high affinity to cattle. Animals are naturally infected through the respiratory system as viruses primarily replicate in the nasal mucosa. Neuroinvasiveness of BoHV-1 and BoHV-5 differs. BoHV-1 infection usually persists in neurons of the trigeminal ganglion, where the virus is present in a latent state. BoHV-5 is able to infect various areas of the brain and cause meningoencephalitis, especially of young cattle (Rola *et al.*, 2017).

BoHV-1 is a causative agent of infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis and balanoposthitis associated with abortions, infertility, conjunctivitis, encephalitis, mastitis, enteritis and dermatitis (Straub, 2001).

In the territory of the Slovak Republic, IBR occurs usually as a latent infection. BoHV-1 in the latent form is detectable in trigeminal ganglions and in pharyngeal tonsils. After primary infection, BoHV-1 can be detected in the conjunctival tissue and in the oronasal mucosa. After infection of reproductive organs, BoHV-1 can be found in sacral ganglions (Ackermann and Wyler, 1984; Winkler *et al.*, 2000).

Under favourable circumstances (immunosuppression, multifactorial stress), latently persisting BoHV-1 in the tissues of infected animals can be reactivated and excreted periodically (Thiry *et al.*, 1985, 1987; Turin *et al.*, 1999; Winkler *et al.*, 2000; van Drunen Little-van den Hurk, 2006; Jones and Chowdhury, 2010).

During the 1980s, several countries implemented control and eradication programs of the IBR (Ackermann *et al.*, 1990; Nylin *et al.*, 2000; Trapp *et al.*, 2003; Nuotio *et al.*, 2007; Åkerstedt *et al.*, 2010).

Recently, many European countries apply IBR eradication and control measurements targeted on cattle holdings. Elimination of infected animals combined with vaccination of cattle by marker vaccines is used for eradication of BoHV-1. In the field, the most complicated task is to eradicate a causative agent of the IBR in regions bordering with endemic territories (Ackermann and Engels, 2006; Blickenstorfer *et al.*, 2010; Raaperi *et al.*, 2014). Euthanasia of all seropositive animals is the most efficient method for eradication of BoHV-1 in the cattle holdings with a low rate of positivity (Ackermann and Engels, 2006). This elimination strategy was implemented successfully in Finland, Sweden, Norway, Denmark, Austria, Italy and in Switzerland (Nylin *et al.*, 2000; Ackermann and Engels, 2006; Nuotio *et al.*, 2007; Åkerstedt *et al.*, 2010; Blickenstorfer *et al.*, 2010).

In countries with high seroprevalence, animals are vaccinated using attenuated or inactivated marker vaccines (Vonk Noordegraaf *et al.*, 2004; Ackermann and Engels, 2006; van Drunen Little-van den Hurk, 2006; Jacevičius *et al.*, 2008). Due to the absence of one or more glycoproteins that are present in the BoHV-1 street virus in the vaccine, marker vaccines allow distinguishing infected and vaccinated animals (van Oirschot *et al.*, 1996, 1997).

Eradication of BoHV-1 in the Slovak Republic

Due to endemic circulation of BoHV-1 in cattle herds in Slovakia, a long-term IBR eradication and cattle holdings recovery program was established in 1995 by the State Veterinary and Food Administration of Slovak Republic. The elimination of seropositive animals in cattle holdings was chosen as a key strategy.

Table 2. The overview of eradication program targeted on BoHV-1 in animal holdings in the Slovak Republic (modified according to the State Veterinary and Food Administration of the Slovak Republic (Eradication programme, 2018)

Year	Incidence of BoHV-1 (officially free of occurrence)			Incidence of BoHV-1 (free of occurrence)			Holdings in the recovery process			Holdings not examined		
	Holdings			Holdings			Holdings			Holdings		
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
2010	3950	552	129	15	28	53	311	342	907	2314	142	6
2013	2810	501	161	81	113	118	226	346	784	2561	208	54
2017	3001	563	205	94	101	331	195	330	499	1869	185	74

A serosurvey using ELISA test targeted on detection of specific antibodies to BoHV-1 in cattle is carried out in Slovakia from 2006. Within this campaign a total of 87 208 cattle from 4 598 farms were examined. Specific antibodies were detected in 27 600 animals from 1 560 farms. Laboratory examinations in vaccinated holdings with the IBR *gE* ELISA marker vaccine were performed on 489 farms, where 20 790 cattle were examined; of these, 4 862 cattle coming from 296 farms were positive for the IBR *gE*.

The IBR recovery status of cattle holdings for the years 2010, 2013 and 2017 is shown in the Table 2 (Eradication programme, 2018).

Cervid herpesvirus 1

Cervid herpesvirus 1 (CvHV-1) was described for the first time in 1982 after successful isolation from red deer (*Cervus elaphus*) in the Great Britain (Gavier-Widen *et al.*, 2012). A primary site of infection is conjunctival mucosa and mucosa of respiratory tract (Reid *et al.*, 1986). The virus is transmitted by ocular and nasal secrets (Reid *et al.*, 1986) and a veneral transmission was also described (Tisdall and Rowe, 2001). CvHV-1 causes an ocular disease in red deers (Inglish *et al.*, 1983).

A positive serological detection of antibodies against CvHV-1 in sera of red deer from the Czech Republic was published by Pospísil (Pospísil *et al.*, 1996). In Poland, both, wild red deer and red deer bred in farms showed seropositivity after natural CvHV-1 infection (Rola *et al.*, 2017). On the basis of these results it can be suggested, that the circulation of CvHV-1 is probable in the Slovak population of red deer.

Canine herpesvirus 1

Canine herpesvirus (CHV) is the most important in the population of dogs and was first characterized as a herpesvirus in 1965. CHV was described as the causative agent of a fatal haemorrhagic disease of puppies and an upper respiratory tract in adult dogs (Carmichael *et al.*, 1965). CHV belongs to the genus *Varicellovirus* (the subfamily *Alphaherpesvirinae*, the family *Herpesviridae*) (Pellett *et al.*, 2012). On the basis of several studies it has been proven, that CHV is antigenically monotypic (Manning *et al.*, 1988).

The canine herpesvirus (CHV) has been isolated and seropositivity in dogs was confirmed in various countries around the world confirming 30–100% CHV prevalence in urban canine populations (Fulton *et al.*, 1974; Reading and Field, 1998; Rijsewijk *et al.*, 1999; Ronsse, *et al.*, 2002; Larra *et al.*, 2016).

Host range seems to be limited to domestic and wild canids (dogs, wolves, foxes, coyotes) which are known to be susceptible (Davidson *et al.*, 1992; Evermann *et al.*, 1984). Neutralizing antibodies have also been found in North American river otters (Kimber *et al.*, 2000) and in brown bears in Slovakia (Vitásková *et al.*, 2019).

Adult dogs infected with CHV do not usually show any signs. However, infection of susceptible puppies at 1–2 weeks of age can lead to a generalized necrotising, haemorrhagic disease. Clinical signs are more likely to appear in animals that are hypothermic or immunosuppressed (Lust and Carmichael, 1971). After symptomatic or asymptomatic primary infection, dogs become latently infected. CHV in latently infected patients is detectable in the trigeminal ganglia and other sites, such as the lumbosacral ganglia, tonsils and parotid salivary glands. In association with immunosuppression a periodic reactivation of virus may occur (Okuda *et al.*, 1993; Burr *et al.*, 1996).

In Slovakia, the serological detection of antibodies against CHV and virus isolation attempts in dogs (n = 68) were conducted in Slovakia (Smrčo *et al.*, 2008). The blood sera were analyzed by ELISA test. Swab samples collected from dogs were cultivated on the MDCK cell line for the presence of typical cytopathic effect and by necropsy. The HCV infection was confirmed in 37% of examined dogs.

Cyprinid herpesvirus 3

A new herpes-like virus was isolated in 1998 from koi (*Cyprinus carpio koi*) in Israel. The newly described pathogen was initially designated as koi herpesvirus (KHV) (Hedrick *et al.*, 1999). Subsequently, thanks to un-

regulated fish transport, the pathogen quickly spread in the species *Cyprinus carpio*, comprising common carp and its ornamental relative. Recently, on the basis of genetic research, the virus was assigned to the family *Alloherpesviridae*, the genus *Cyprinivirus*, the species cyprinid herpesvirus 3 (CyHV-3) (Michel *et al.*, 2010). The virus or evidence for KHV has now been found in broad geographical area. In Europe the disease has been reported from Austria, Belgium, Denmark, France, Netherlands, Poland, Luxembourg (Haenen *et al.*, 2004), Germany (Bretzinger *et al.*, 1999) and the United Kingdom (Denham, 2003). CyHV-3 has been also reported from USA, Indonesia, Japan (Rukyani, 2002), and Taiwan (Tu *et al.*, 2004).

The koi herpesvirus disease (KHD) occurs predominantly in spring and autumn when water temperatures range from 18 to 26°C. The signs include dark skin, gill necrosis, haemorrhages at fin bases, enophthalmia and internally, adhesions of internal organs (Choi *et al.*, 2004). Therefore, the key environmental factor influencing KHV outbreaks in koi and common carp is water temperature (Bretzinger *et al.*, 1999; Hedrick *et al.*, 2000; Perelberg *et al.*, 2003). Also, the effect of temperature on viral replication suggests that the body temperature of its poikilotherm host could regulate the outcome of the infection (Michel *et al.*, 2010). Transmission of the disease occurs following cohabitation of immunologically naive fish with susceptible fish. Under natural conditions koi carp long 8–67 cm are susceptible (Bretzinger *et al.*, 1999) as are common carp between approximately 500 g to 2 kg (Sano *et al.*, 2004; Terhune *et al.*, 2004).

The first koi herpesvirus disease outbreak in Slovakia

In the state territory of the Slovak Republic, the KHD was documented for the first time in 2019 (OIE, 2019). An outbreak of KHD was localized in the lake Počúvadlo near Banská Štiavnica (county Žiar nad Hronom) on July 22. Clinical disease was recorded in the common carp (*Cyprinus carpio*). The causative agent has been confirmed by the PCR analysis in the Veterinary and Food Institute in Dolný Kubín (National laboratory).

Conclusion

Herpesviruses are able to infect a wide variety of hosts and cause various diseases in humans and animals. They can persist as a subclinical infection without the development of a clinical form of the disease after primary infection and transition to a latent stage. Although their presence in the natural hosts is mild or asymptomatic, their transmission to other species can lead to serious diseases and death.

The aim of the recovery programs targeted on livestock is to eradicate these infectious diseases throughout the Slovak Republic. It will improve the health of animals and eliminate trade barriers in domestic and foreign trade. The recovery is carried out in the whole territory of the Slovak Republic. In consideration of all of the above, it is obvious that constant monitoring of herpesvirus diseases in animal husbandry and in wild animal species is necessary to maintain animal health and prevent economic losses.

Acknowledgments. This work was financially supported by the project KEGA No. 014UVLF-4/2019 granted by The Ministry of Education, Science, Research and Sport of the Slovak Republic.

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