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Monitoring and prevalence of influenza A virus in the population of mallard duck in the Czech Republic between 2008-2010

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Abstract. In the current study 744 cloacal samples were collected from mallards (*Anas platyrhynchos*) in the Czech Republic and tested for the presence of influenza virus between 2008 and 2010. Of the total number of 744 mallards tested nine were positive (prevalence 1.2 %) for influenza virus. All the mallards were up to 1.5 years old and the majority (89 %) were killed by hunters.

Key words: PCR, detection

Introduction

The long-term monitoring of influenza virus in populations of wild waterfowl in North America (Hinshaw et al. 1980, Krauss et al. 2004, Parmley et al. 2008) and Europe (Munster et al. 2006, Olsen et al. 2006) indicates that not all the subtypes of influenza cause serious mortality as these hosts rarely show signs of infection. The influenza viruses replicate in the intestine and respiratory system of the host and is shed with their faeces mainly in and around inland waters. The publications on the occurrence of highly pathogenic influenza viruses in domestic poultry indicate that these viruses originally came from wild birds and were not pathogenic in the original hosts, where they most often cause unapparent infections (Capua & Mutinelli 2001, Hirst et al. 2004). Especially in Eurasia, migrating mallards are infected with ancestral forms of the H5 and H7 influenzas, which are highly pathogenic for poultry (Webster et al. 1992, Munster et al. 2005). The objective of this research was to monitor mainly autumn populations of mallards that were reared in captivity (in the Czech Republic) and then released in the Czech Republic and compare the prevalence of influenza virus in these birds with screening results for Europe and the world.

Material and Methods

During 2008-2010, between April and November, cloacal swabs were collected in the Czech Republic (Fig. 1). The swabs (n = 744) from mallards (*Anas platyrhynchos*) were collected from 12 main areas (ponds, reservoirs), equally spread throughout the Czech Republic. From each bird was collected one swab sample. These samples came mainly from birds captured on their nests (2 %), killed by hunters (89 %) or collected during ringing (9 %). Samples were analyzed using reverse transcription PCR (RT-PCR). RNA was extracted using QIAamp Viral RNA kit (Qiagen) and following the manufacturer's instructions. Reverse transcription (RT-PCR) and primers (M52C, M253R) of segment M of the influenza virus were used as previously described (Fouchier et al. 2000). There were both positive (A/ Puerto Rico 8/43 H1N1 ($EID_{50} = 10^8$)) and negative controls in each test.

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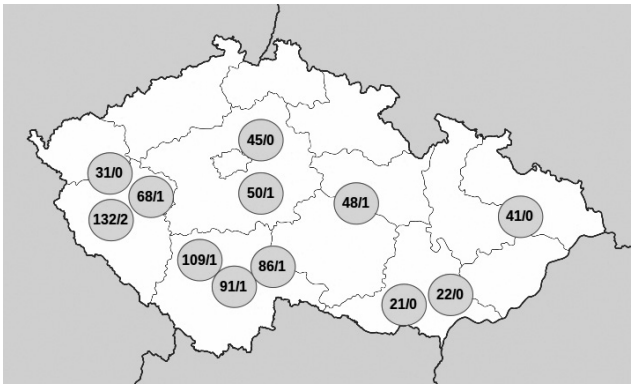

Fig. 1. *The geographical distribution of sampling sites in the Czech Republic between 2008-2010 (grey spots; total number of the tested samples/ incidence of the positive samples in area).*

Results

Of the 744 cloacal swabs from mallards (*Anas platyrhynchos*), nine (1.2 %) tested positive for influenza virus type A. The positive samples all came from individuals that were not older than 1.5 years, of which six were males and three females. The first positive samples were recorded in August 2008 (2 samples), followed by one positive in April 2009, four in the autumn hunting season in 2009 and two in August 2009. In terms of the areas where the positive samples came from, four were in South Bohemia (n = 286), which is equivalent to 1.3 % of the population, three in West Bohemia (n = 231) and again 1.3 % of the population, and one in East Bohemia (2.08 %) and one in Central Bohemia (1.05 %). All the mallards tested were healthy and showed no signs of infection (Fig. 1).

Discussion

Screening for the influenza virus in the U.S.A. and Canada (Krauss et al. 2007, Parmley et al. 2008) between 2001 and 2006 confirmed the presence of the influenza virus in mallards in 16.6 % of the cases (n = 590). Similarly in Alaska between 2006 and 2007 a detailed study (Ip et al. 2008) of the presence of the influenza virus in waterfowl (n = 16767) indicated a prevalence of 1.7 % in mallards. Studies in Sweden and France, which focused on screening for the influenza virus, indicate an incidence of influenza in mallards in Europe of 6.1 % (Munster et al. 2007). The results of the current study indicated an incidence 1.2 %, which is lower than that recorded for mallards in other countries in Europe and North America. It is known that waterfowl are a reservoir of influenza viruses and are important in the epidemiology of influenza in man (Boyce et al. 2009, Munster & Fouchier 2009). Monitoring the influenza virus in free living waterfowl, in areas where free living mallards come in contact with artificially bred mallards intended for hunting, provides information on the possible transfer of influenza viruses between these populations and increases our knowledge of the epidemiology of this pathogen. Epidemiological studies make it possible to assess the risk of mixing populations of mallards that are reared and then released for hunting, and free living wild mallards, resulting in a greater spread of infection and in particular an increase in the incidence of influenza outbreaks in man.

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