

ZERO PREVALENCE OF AVIAN INFLUENZA IN YELKOUAN SHEARWATER, *Puffinus yelkouan*, IN LASTOVO ARCHIPELAGO, CROATIA

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Summary

The paper presents the first systematic study of avian influenza in tubenoses in Europe. Yelkouan Shearwaters were captured in colonies on Lastovo archipelago. Cloacal swabs were collected from all the birds (104) and sera samples were collected from adults only (80). The swabs were tested by virus isolation in chicken embryos and the sera were tested using commercial ELISA kit. During two breeding seasons, all of the samples were negative for the presence of avian influenza. We assume that one of the limiting factors for spreading of the virus among the birds is the relatively small population of these birds in Croatia. Further investigations, on both tubenoses and sea gulls, which could serve as a possible source of avian influenza infection, are needed to understand the ecology of avian influenza in tubenoses in the Adriatic.

Key words: Avian influenza, *Procellariiformes*, tubenoses, *Puffinus yelkouan*, Yelkouan Shearwater, Lastovo

Introduction

Avian influenza (AI) is a viral disease with primarily fecal-oral route of transmission that has been reported in a range of wild and domestic birds. There are 16 H (H1–H16) and 9 N (N1–N9) subtypes of influenza according to hemagglutinin and neuraminidase protein present on the surface of the virion and most of H/N combinations are isolated from wild birds (Munster *et al.*, 2007). Wild birds, species from *Anseriformes* (ducks, geese and swans) and *Charadriiformes* (gulls, terns and waders) orders are considered the main reservoir of AI viruses (Webster *et al.*, 1992). *Procellariiformes* (tubenoses) is a family of birds ecologically very similar to gulls and terns. All of them are mainly marine birds, gregarious during non-breeding season and they nest on small offshore islands.

There are two species of tubenoses breeding in Croatia; Scopoli's Shearwater, *Calonectris diomedea* ssp. *diomedea*, and Yelkouan Shearwater, *Puffinus yelkouan* (Jurinović, 2006). Yelkouan Shearwater is a medium-sized shearwater that breeds in central and eastern Mediterranean. The birds arrive to breeding grounds as early as October and defend their burrows until the start of courtship in January. After the fledging is completed, both adults and juveniles leave the colonies in July/August, congregate in large flocks and

forage away from breeding areas (Borg, 2010). Burrows sometimes share the same entrance, so it increases probability for disease transmission via fecal material as they defecate in front and inside the burrow itself. Croatian population of Yelkouan Shearwater numbers approximately 250 – 300 pairs (BirdLife, 2011) with the stronghold in Lastovo archipelago.

There were only few systematical researches on avian influenza in tubenoses. Two AI subtypes were isolated from shearwaters, both in Australia. H6N5 subtype of avian influenza was isolated in 1972 in (Downie *et al.*, 1973) whereas H15N9 was isolated in 1979 and described as a new H15 subtype two decades later (Rohm *et al.*, 1996). Further, Dasen and Laver (1970) have found antibodies against influenza in sera from shearwaters in Australia.

The aim of this study was to determine if there are any avian influenza viruses circulating in the Croatian colonies of Yelkouan Shearwater.

Material and methods

Sampling

Yelkouan Shearwaters were captured in the colonies on the Lastovo archipelago (Croatian part of the Adriatic) during

the 2009 and 2010 breeding season. Adults were hand captured during the brood shift and pulli were taken from their nesting holes. All the birds were ringed and cloacal swab samples were taken from all captured birds, while serum samples were collected from adults only. A total of 104 birds were sampled. Number of birds in each age category and year are given in Table 1.

There are several reasons why birds in the Croatian population of Yelkouan Shearwaters were negative for AI. The most important factor could be the number of birds in the population. Guberti et al. (2007) estimated that host density threshold for AI virus persistence in dabbling ducks is 380 susceptible individuals per day, whereas the critical community size needed for maintaining the virus throughout

Table 1. Number of birds sampled in each colony in 2009 and 2010. Cloacal swabs were taken from all the birds and sera samples only from adult birds

Tablica 1. Broj ptica iz pojedine kolonije od kojih su uzeti uzorci u 2009. i 2010. godini. Svim pticama su uzeti kloakalni brisevi, a odraslima i uzorci seruma

Colony	2009		2010		Number of breeding pairs
	Adult	Pulli	Adult	Pulli	
A	6	4	0	0	20
B	14	2	0	0	15-20
C	42	9	18	9	60-80
Σ	62	15	18	9	95-120

Due to huge chick predation by ship rats, *Rattus rattus*, in the colonies during the second research year, many chicks did not survive until fledging. Consequently, their parents had no motivation to visit the colonies and the number of sampled birds was much smaller.

For conservation purposes, exact locality names are coded with letters A, B and C.

Virus isolation

Virus isolation in chicken embryos was carried out according to standard procedures (OIE, 2004).

Serology

All of the sera were tested for presence of antibodies against avian influenza virus using FlockChek* AI MultiS-Screen Antibody Test KitELISA kit (IDEXX) according to the manufacturer's instructions.

Results & Discussion

All swab samples were negative for presence of AI viruses by virus isolation in chicken eggs. Recently, molecular methods are widely used for monitoring of AI viruses in wild birds. Nevertheless, a significantly reduced sensitivity of PCR based detection methods has been observed due to primer and/or probe mismatch, especially in novel influenza viruses (Klungthong et al., 2010; Abdelwhab et al., 2010, Medina et al., 2011). Little is known about influenza viruses in shearwaters, thus we believed that virus isolation in chicken embryos, as a gold standard, has advantage over PCR based method in this case. In accordance with negative result for AI virus detection, there were no specific antibodies against AI viruses found in collected sera using ELISA kit.

the winter has been estimated to be about 1200 individuals. We assume that host density influences persistence and maintaining of AI viruses in other susceptible birds species, including shearwaters. Therefore, a relatively small number of the birds in Croatian population could be a limiting factor for spreading of the virus within the population. Further, it could be expected that shearwaters would contract AI infection from gulls or eventually terns, rather than other wild bird reservoir species i.e. *Anseriformes*. Thus the incidence of AI in gulls and terns could reflect on AI findings in shearwaters. Further studies of AI in shearwaters in Croatia should be therefore conducted in conjunction with AI studies in sea gulls which share the same habitats, similar ecological patterns and are present in large numbers, primarily Yellow-legged Gulls (*Larus michahellis*).

To our knowledge there is no published systematical research conducted on avian influenza in European tubenoses.

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NULTA PREVALENCIJA VIRUSA INFLUENCE PTICA U GREGULAMA, *PUFFINUS YELKOUAN*, NA LASTOVSKOM ARHIPELAGU, HRVATSKA

Sažetak

Rad predstavlja prvo sustavno istraživanje prisutnosti virusa influence ptica među cjevonosnicama u Europi. Gregule su ulovljene na kolonijama u lastovskom arhipelagu. Svim pticama (104) su uzeti kloakalni brisevi, a odraslima (80) i uzorci seruma. Brisevi su pretraženi izdvajanjem u kokošjim embrijima, a serumi komercijalnim ELISA kompletom. Tijekom dvije gnijezdeće sezone, nije izdvojen virus influence ptica ni iz jednog brisa, a svi su serumi bili negativni na prisutnost protutijela za virus influence ptica. Pretpostavljamo da je jedan od ograničavajućih čimbenika za širenje virusa relativno mala veličina populacije gregula u Hrvatskoj. Potrebna su dodatna istraživanja cjevonosnica i morskih galebova, koji su mogući izvor infekcije virusom influence ptica, kako bi se mogla bolje razumjeti ekologija virusa među cjevonosnicama.

Ključne riječi: influence ptica, *Procellariiformes*, cjevonosnice, *Puffinus yelkouan*, gregula, Lastovo