Original article

УДК: 635.5.09:[616.98:578.828.3(497.7)"2008"

SURVEILLANCE OF AVIAN INFLUENCA VIRUSES IN FARMED POULTRY IN 2009 IN REPUBLIC OF MACEDONIA

Cvetkovik Iskra, Dodovski Aleksandar, Naletoski Ivanco, Mrenoski Slavco, Mitrov Dine, Krstevski Kiril, Dzadzovski Igor, Cvetkovik Aleksandar

Faculty of Veterinary Medicine in Skopje, R. Macedonia

e-mail: iskra@fvm.ukim.edu.mk

ABSTRACT

The aim of the study was to determine the presence and distribution or to confirm the absence of avian influenca viruses in farmed poultry in the poultry production systems 1 and 2 from the eight statistical regions in R. Macedonia. Total number of 1215 cloacal swabs from poultry were sampled. Each sample was processed and analysed by both molecular (RRT-PCR) and classical virology methods (virus isolation and identification). All samples gave negative result for presence of avian influenza viruses. Commercial poultry production systems have biosecurity measures preventing the entry of pathogens i.e avian influenza viruses, therefore resulting with no circulation of these viruses in the sampled farmed poultry flocks.

Keywords: *avian influenza viruses, farmed poultry, cloacal swabs, RRT-PCR, virus isolation*

INTRODUCTION

Avian influenza (AI) represents one of the greatest concerns for public health that emerged from the animal reservoir in recent times.

AI is a listed disease of the World Organisation for Animal Health (OIE) that has become a disease of great importance both for animal and human health. Until recent times, AI was considered a disease of birds with zoonotic implications of limited significance. The emergence and spread of the Asian lineage highly pathogenic AI (HPAI) H5N1 virus has dramatically changed this perspective; not only has it been responsible of the death or culling of millions of birds, but this virus has also been able to infect a variety of non-avian hosts including human beings. [1]

According OIE, Notifiable AI (NAI) is an infection of poultry caused by any Influenza Type A virus (IAV) of the H5 or H7 subtypes, or by any IAV with an intravenous pathogenicity index (IVPI) greater than 1.2 or killing at least 75 percent of the inoculated chickens. NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) viruses and low pathogenicity notifiable avian influenza (LPNAI) viruses. [2]

IAV are orthomyxoviruses infecting a wide range of domestic birds, wildfowl and shorebirds, but also many other species, including humans, pigs, horses, mink, felids and other mammals [3]. Their genome is comprised of eight (8) negative-sense RNA segments that code for ten distinctive proteins. They are classified based on two surface glycoproteins expressed on virus particles: hemagglutinin (HA) and neuraminidase (NA). In poultry and wild birds, IAV representing 16 HA (H1- H16) and 9 NA (N1-

N9) antigenic subtypes are in circulation in numerous combinations (i.e. HyNx). [4, 3]

Like most zoonotic diseases, the epidemiology of IAV in poultry is defined by interactions between hosts, agents and environments [5]. Among avian species IAV prevalence can vary greatly according to season and location and because individual species - and populations within species exhibit different food, climatic and habitat preferences, migratory behaviours and agro-geographic ranges, individual species within these groups may play radically distinct but important roles in the epidemiology of bird flu [6].

Predominantly water-associated wild birds such as ducks, geese, gulls and shorebirds form the reservoir of influenza A viruses in nature. All sixteen antigenic subtypes of the virus surface glycoprotein haemagglutinin and all nine subtypes of neuraminidase that have been identified to date have been isolated from these bird species [3]. Avian influenza viruses preferentially infect cells lining the intestinal tract of birds and are excreted in high concentrations in their faeces. The transmission of influenza viruses between birds is thought to occur primarily via the faecal-oral route. Whereas avian influenza viruses are generally nonpathogenic in their natural hosts, they may cause significant morbidity and mortality upon transmission to other species. [7]

MATERIAL AND METHODS

The survey included farmed poultry from the poultry production sectors 1 and 2 (FAO Classification) from the eight statistical regions of R. Macedonia. According the Programme for eradication of avian influenza [8] and Commission Decision 2007/268/EC [9], the sampling was representative for the whole state. Cloacal swabs were sampled from total of 1215 poultry from 41 poultry farm in 30 localities. The locality distribution in municipalities is shown in Figure 1.

From each farm a total number of 30 cloacal swabs were sampled aside from two farms were due to the technical circumstances 24 i.e 21 cloacal swabs were sampled. According FAO classification [15], 34 farms belong to sector 2 and 7 farms to sector 1.

Sterile, individualy wraped swabs were used for sampling. Immediatelly after sampling, they were submerged in 2,5 ml isotonic PBS (Phosphate buffered saline), *pH* 7,0-7,4 with antibiothics and bovine serum albumine for stabilization of the virus.

Processing of the samples was conducted according Comission decision 2006/437/EC [10] and OIE [2].

The extraction of RNA was performed with RNEasy Mini kit according the user's manual of the producer[11]. Inactivated H4, H5 and H7 viruses were used as positive controls.

The detection of AIV M-gene was performed with Bio-Rad IQ5 RRT-PCR (Figure 2). The method was performed according the protocol of the AI international reference laboratory [12].

In this method the folowing reagents were used

1. Primers and probes:
   - Sep 1 AGA TGA GTC TTC TAA CCG AGG TCG (Operon)
   - Sep2 TGC AAA AAC ATC TTC AAG TCT CTG (Operon)
   - SePRO FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA (Operon)
2. Real time PCR master mix: Qiagen Onestep RT-PCR kit

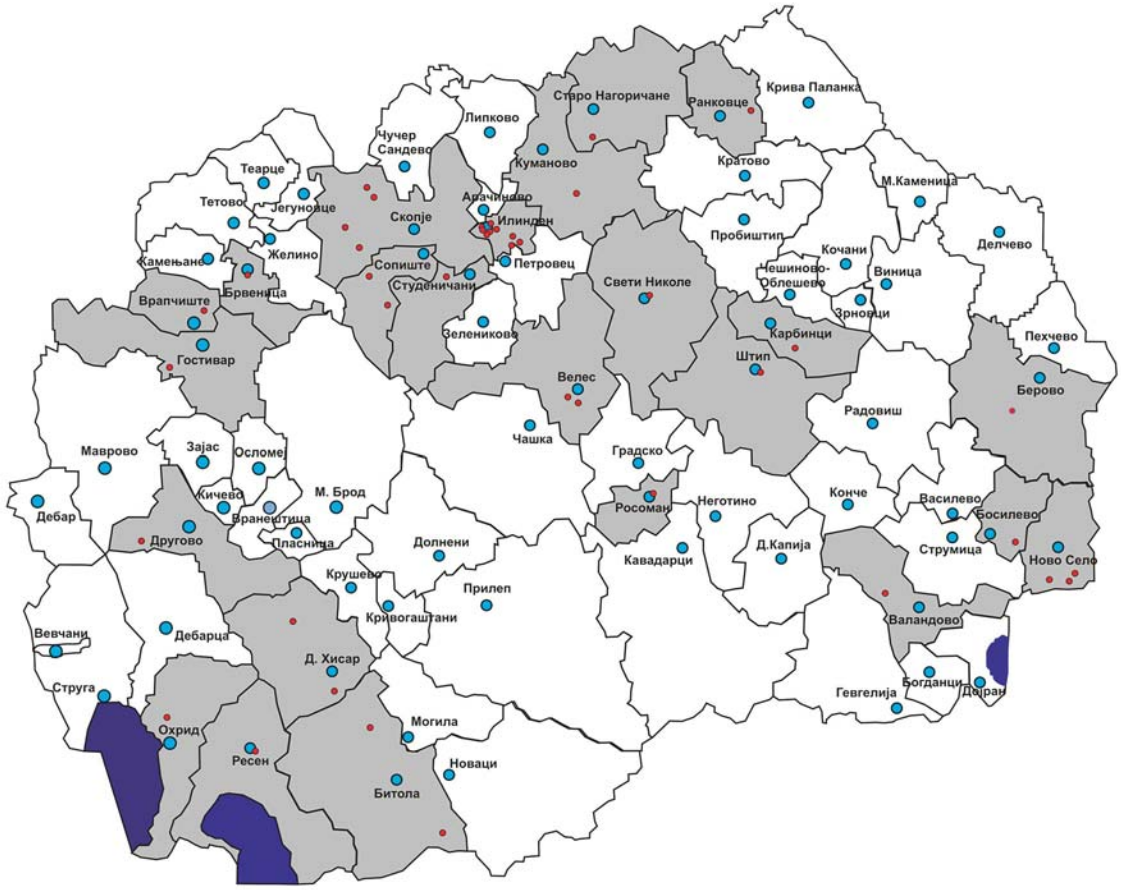

Figure 1. Municipalities with sampled farmed poultry

Figure 2. Bio-Rad IQ5 RRT-PCR

The virus isolation and identification methods were performed according Comission decision 2006/437/EC [10] and OIE [2].

The samples were inoculated in allantoic cavity of 8-10 days old embryonated chicken eggs (ECE) free from antibodies for influenza (Speciffic antibody negative – SAN eggs) . For each sample 4 ECE were used. For every batch of inoculated ECE, 4 inoculated ECE with LPAIV H4, 2 uninoculated ECE (egg control) and 2 inoculated ECE with PBS i.e negative control (passage control) were used.

The ECE were frst candeled (Figure 3.) to determine the viabillity of the embryo and mark the air sac. After that the samples were inoculated in the AC (Figure 4.), sealed and incubated on 37°C and 50-60% humidity. Following 6-day incubation the ECE were candeled daily. If dead embryos were observed, from those ECE, the harvested allantoic fluid (AF), (Figure 5.) was tested for hemaglutinating activity (Figure 6.). If the embryos were alive during the 6-day incubation, on the sixth day AF was harvested.

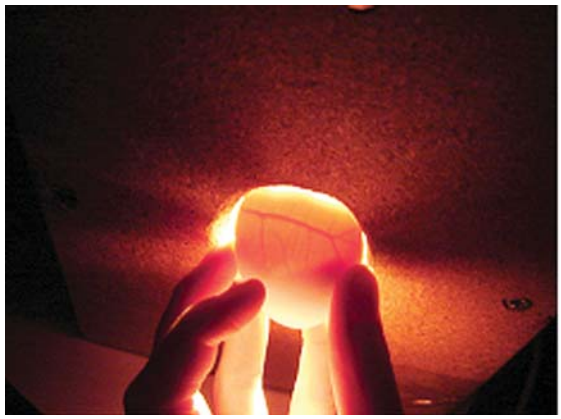

Figure 3. Candeling ECE

Figure 4. Inoculation

Figure 5. Harvesting AF

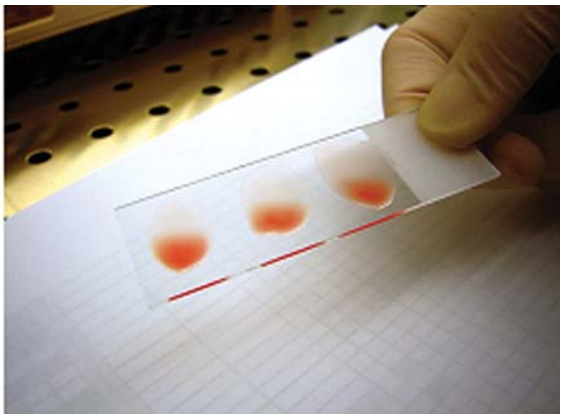

Figure 6. Hemaglutination

RESULTS AND DISCUSSION

This survey of AIV in 2009 was conducted in order to to determine the presence and distribution or to confirm the absence AIV in famed poulty.

All analysed samples gave negative results on PCR and VI for the presence of AIV.

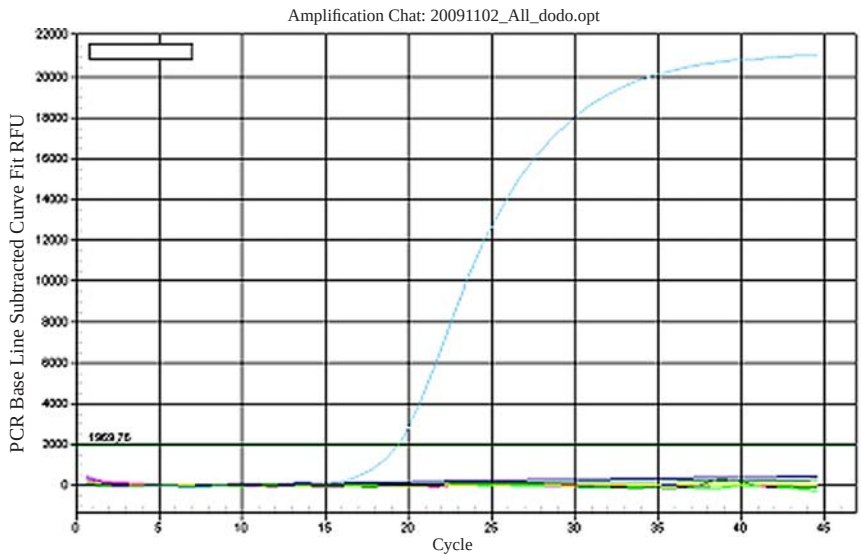

Figure 7. PCR amplification chart

In general, surveillance is aimed at demonstrating the absence of disease or infection, determining the occurrence or distribution of disease or infection, while also detecting as early as possible exotic or emerging diseases. [13]

As far as AI is concerned, at the level of a country, zone or compartment, surveillance may have two objectives: to determine the presence or absence of notifiable AIV (NAIV) infection and to determine the prevalence of NAIV [14]

In that manner, this survey was conducted in order to to determine the presence and distribution or to confirm the absence of avian influenca viruses in farmed poultry in R. Macedonia.

Untill 2009, no molecular or virological (M/V) surveillance was conducted in the poultry comercial sector in Macedonia. The research of Dodovski et al. [15] from September 2008 till March 2009, indicated seropositive flocks by ELISA but negative on hemagglutination inhibition test for H5/H7, wich may be due circulation of non H5/H7 viruses.

Following the fact that seropositive floks are present and no M/V surveillance has ever been done, this research was exactly aimed to give an answer on the epidemiological situation of AIV in R.Macedonia and at the same time gave an answer on the question - If seropositive floks are present - is there a circulation of the virus?

According the aquired results from the M/V surveillance in 2009, the presence of AIV in the poultry comercial sector was excluded, wich of course does not represent a constant result and on the other hand indicates the need of constant ongoing surveillance.

The reason for the negative result in the farmed poultry is comprised in the characteristics of the poultry production systems given in Table 1 (e.g. biosecurity, contact with other poultry and wild birds, keeping of birds etc.)

According to FAO [16] classification sector 1 is represented by industrial integrated system with high level biosecurity and birds/products are marketed commercially (e.g. farms that are part of integral broiler production enterprise with clearly defined and implemented standard operating procedures for biosecurity) and sector 2 as commercial poultry production system with moderate to high biosecurity and birds/products usually marketed commercially (e.g. farms with birds kept indoors continuously; stricly preventing contact with other poultry or wildlife)

Table 1. FAO poultry production systems

Sectors FAO Definition	Industrial and integrated	Commercial poultry production
	SECTOR 1	SECTOR 2
Biosecurity	High	Mod-High
Market outputs	Export and urban	Urban/rural
Dependence on market for inputs	High	High
Dependance on goods roads	High	High
Location	Near capital and major cities	Near capital and major cities
Birds kept	Indoors	Indoors
Shed	Closed	Closed
Contact with other chicken	None	None
Contact with ducks	None	None
Contact with other domestic birds	None	None
Contact with wildlife	None	None
Veterinary service	Own Veterinarian	Pays for veterinary service
Source of medicine and vaccine	Market	Market
Source of technical information	Company and associates	Sellers of inputs
Breed of poultry	Commercial	Commercial
Food security of owner	High	Ok

The EU legislation claims that every Member state must implement surveillance porograms for avian influenza in poultry complying with the guidelines given in the Commission decision 2007/268/EC [9]. Also the annual programme for AI surveillance in R. Macedonia comprises the surveillance programmes for poultry and wild birds, but unfortunatelly untill now no surveys are conducted. This on the other hand is a big gap in the assessment of the epidemiological situation of AI in R. Macedonia because of the lack of information for the previous years.

In that contex the implementation of the surveillance programmes for AI must be an imperative for better preparedness and response in case of AI outbreak and on the other hand to fulfill the claims of EU as an EU candate country.

REFERENCES

1. Capua, I. and Alexander, D.J. (2008). Ecology, Epidemiology and Human Health Implications of Avian Influenza Viruses: Why do We Need to Share Genetic Data? Zoonozes and Public Health 55, 2-15.

2. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2009)

3. Webster, R.G., Bean, W.J., Gorman, O.T., Chambers, T.M. and Kawaoka, Y. (1992). Evolution and ecology of influenza A viruses. Microbial Review 56, 152-79.

4. Fouchier, R., Munster, V., Wallensten, A., Bestebroer, T.M., Herfst, S., Smith, D.J., Rimmelzwaan, G.F., Olsen, B. and Osterhaus, A. (2005). Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J. Virol., 79: 2814-2822.

5. Stallknecht, D. and J.D. Brown, 2007. Wild birds and the epidemiology of avian influenza. J. Wildlife Dis., 43: S15-S20

6. Webster, R., S. Krauss, D. Hulse-Post and K. Sturm-Ramirez, 2007. Evolution of influenza A viruses in wild birds J. Wildlife Dis., 43: S1-S6.

7. Munster Vincent Jacobus (2006) Ecology, Evolution and Pathogenesis of Avian Influenza Viruses, chapter 13, ISBN-10: 90-9021242-6

8. Annual programme for AI surveillance (Public Enterprise Official Gazette of the Republic of Macedonia. No 82, June,2007).

9. Commission decision 2007/268/EC

10. Comission decision 2006/437/EC

11. Qiagen. (2006). RNeasy® Mini Handbook, Fourth edition, pp. 88.

12. Avian Influenza Community Reference Laboratory. (2007). Detection of influenza A matrix gene by realtime Taqman® RT-PCR, pp. 10.

13. OIE, Terrestrial Animal Health Code 2009, Chapter 10.4

14. Zepeda C. (2006) Surveillance and compartmentalisation as a tool to control avian influenza. OIE/FAO International Scientific Conference on Avian influenza.Dev.Biol (Basel), Karger, 2006, vol 124, pp 163-169

15. Dodovski A, Naletoski I. Mitrov D. (2009) Serological surveillance of avian influenza in commercial flocks in Macedonia. Book of abstracts:XVIth World Veterinary Poultry Association Congress, Marrakesh, Morocco, p. 268

16. FAO Poultry production systems Characteristics, http://www.fao.org/docs/eims/upload/214190/ProductionSystemsCharacteristics.pdf

**The survey was conducted as a part of the FP6 Project" Upgrading and introduction of sustainable diagnostic tools for the major transboundary diseases of animals in F.Y.R. of Macedonia" (TRANSB_DIS_ANIM_MK) – project coordinator: Ass. Prof. Dr. Ivanco Naletoski*