

ABSTRACT

To obtain results, in addition to farming methods, care and nutrition which have an important role, poultry diseases prevention, in general, and of infectious diseases, in particular, is a determining factor in how such entities often decimating flocks.

One of these diseases, with great economic and health importance is avian influenza (flu), which in the past century has inflicted great losses of poultry farming, virtually worldwide, but especially in Europe, including România. After 1950, this disease has been less frequently, giving the impression that it is substituted in the territory of another entity (Newcastle disease), but no connection between their etiologic agents.

Today, avian influenza (bird flu) is reported with a relatively low frequency in various parts of the world. Although some outbreaks produce losses through mortality in poultry, the concern the entity produces and extraordinary threat is not due to economic losses, but especially the fact that avian influenza viruses are an inexhaustible source of infection for humans and other mammalian species.

Avian influenza viruses can be isolated from active outbreaks of avian influenza, of greater or lesser severity, but also from birds with discrete forms of the disease or even apparently healthy. Among poultry, avian influenza has been reported most commonly in curcan, duck, goose, partridge, pheasant, găină and various psitacide and from the many species of wild birds, influenza viruses were isolated mainly from aquatic birds aquatic birds that inhabit the shores (gulls, sea swallows), migratory birds, especially web-footed (ducks and geese). Moreover, it has been shown that avian influenza can be maintained for a long time in a population of wild birds, especially migratory and aquatic, from which it may cross from time to time, but not frequently in domestic animals.

It has also been proven influenza viruses traffic in both directions, between wild birds (especially web-footed) or domestic birds (especially curcans and pheasants) on the one hand, and various species of mammals such as pigs, mink, seal, dolphin, human and probably other birds.

Given on the one hand, the presence of favorable climatic and environmental conditions, aquatic and migratory birds, and on the other hand, the possibilities for contact between domestic and wild migratory web-footed, favored by the breeding system, for the reunification of knowledge about movement of these influenza viruses, doctotat thesis has proposed a series of objectives.

The research followed, by serological investigation, the presence and circulation of influenza viruses from different species of wild and domestic birds in the county of Galati, epidemiological, clinical and pathological aspects in an episode of avian influenza, the significance of serological and virological tests in the diagnosis of avian influenza and the surveillance and prevention strategy of avian influenza in the county of Galati.

The thesis includes 157 pages, is written in IX chapters and is structured in two parts. The first part (chapters I, II, III), developed on 39 pages, representing 24,84 %, summarizes the main bibliographic data in the literature regarding avian influenza and represent *State of knowledge*. The second part (chapters IV, V, VI, VII), the most important, extended on 118 pages, meaning 75,16%, presents results of research and represent *Own research*. Each chapter of the second part has included material and working methods, results and discussions with partial conclusions.

In Chapter VIII are summarized in the 16 formulations, the main issues drawn from research and represent *Final conclusions*.

The paper presented 19 tables and 66 figures and bibliography includes 116 titles.

The first part (Chapters I, II and III) is a synthesis of the literature on animal influences, with particular emphasis on the types and subtypes of influenzavirus and their movements, according to the natural reservoir hosts and to the world stage of the research undertaken, being *State of knowledge*.

In Chapter I, entitled *Bibliographic data on history, distribution and importance of avian influenza*, are presented available information about the history, distribution, economic and health importance of infection with influenza viruses.

The first major pandemic human influenza it seems that took place in 1850, then in 1918 (swine source). Other outbreaks have occurred in 1957 and 1968 (bird suspected sources) and 1976 (porcine origin). Since then and until now, in the international literature appeared a huge number of scientific papers, which were proposed to clarify, based on deep knowledge of the nature and properties of mammalian flu viruses, including humans, interrelations which long noticed, have not been fully clarified until today.

Chapter II, entitled *Bibliographic data on etiology, epidemiology and pathogenesis of avian influenza* deals with etiological and epidemiological characteristics of influenza virus infections (flu).

There were reviewed the taxonomy, morphology, replication, resistance, virus antigenic structure and sensitivity, sources of infection, ways of transmission in bird flu.

Chapter III, entitled *Data on symptoms, pathology, diagnosis, prevention and control in avian influenza*, presents clinical symptoms and lesion depending on disease progression. There are also presented methods for diagnosis, prevention and immunoprophylaxis. Virological and serological methods are used for the detection of specific antibodies.

Second Part (Chapters IV, V, VI, VII and VIII), refers to **Own research**.

The start premises in making the research were to highlight, based on serological tests, the presence and distribution of some subtypes of type A Influenzavirus in poultry, but also in some wild water birds, from the south - eastern Romania (Galati), and on the other side for a better understanding of movement and opportunities or developing and spreading the bird flu.

Serological investigations were based on the fact that specific antibodies in blood serum of wild and domestic birds, are a proof of passing the organism by infection with influenza virus, as well as long persistence of specific antibodies in the serum passed through infected birds .

In **Chapter IV**, entitled *Purpose and objectives*, are presented, as a result of influenza infections, the importance for animal and human population, the need for serological research regarding the presence and movement of different subtypes of type A Influenzavirus in poultry, but also to the water birds (web-footed) in the environmental conditions existing in the county of Galati, and, on the other hand, deepen knowledge on the role of the water wild birds as the main reservoirs of infection.

To this aim, research pursued the following objectives:

1. The presence and prevalence of positive serological reactions in different species of poultry.
2. Isolation and identification of influenza virus.
3. Epidemiological, clinical and lesion aspects in bird influenza (flu).
4. Monitoring and control strategy of bird influenza in the county of Galati.

In **Chapter V**, entitled *Serological investigations on the presence of infection with influenza viruses in various domestic birds* are presented the results of investigations performed by haemagglutination inhibition reaction on 2812 serum samples collected from fowls (hens, curcans) and web-footed (ducks, geese) to detect specific anti-flu virus antibodies. Blood serum samples collected during 2008 - 2009, from fowls (1040 from hens and 560 from curcans) reacted serologically negative (100%) at influentavirus antigen.

Lack of positive serological reactions of the sera collected from birds both extensive growth sector (hens and curcans) and those in intensive systems (hen), shows that birds did not come in contact with a source of infection.

Instead, of the 475 sera collected from ducks in 2008, a number of 5 reacted positively to H₅ antigen (1,03%) and from 395 sera collected from geese, a number of 3 reacted serologically positive to H₅ antigen used in the haemagglutination inhibition reaction, which is 0,70%.

In 2009, of the 174 samples, a total of 3 (1,14%) reacted positively, and 171 (98,86%) sera were negative. Also, from the 168 sera collected from geese, 3 (1,78%) reacted serologically positive to H₅ antigen and 165 (98,22%) were negative.

Positive serological reactions to web-footed shows that H₅ virus subtype circulating among domestic waterfowl in some areas of the county. However, it can say that negative serological reactions in fowls and the existence of positive domestic web-footed, and no clinical signs show that H₅ haemagglutinin circulating subtype is low pathogenic.

Given the ecological conditions of water wild birds, contact opportunities with domestic web-footed breed freely, and the presence of positive serological reactions in some water poultry (web-footed), investigations have pursued the isolation and identification of influenza virus (**Chapter VI**).

Virus isolation was based on the inoculation of 0,2 ml of suspension in antibiotic solution of pathological material represented by tracheal and cloacal swabs taken from sentinel web-footed (geese), in the allantoic cavity of 9-11 days old embryonated gâină eggs.

The presence of type A influenza virus family *Orthomyxoviridae* in amniotic fluid taken from embryos killed after 72 hours of the second passage was confirmed by typing with mono-specific positive serum against the 16 known haemagglutinin using haemagglutination reaction.

Identification was performed with high specificity positive sera for H and N subtypes of avian influenza virus by haemagglutination inhibition reaction.

Viral isolates tested by the reaction of haemagglutination had titers of 6 log₂ (1/64). Allanto-amniotic fluid with haemagglutinating activity were tested by haemagglutination reaction and haemagglutination inhibition for influenza virus type A, with positive serum specific to H₅ haemagglutinin subtype. The haemagglutination inhibition reaction with reference positive sera identified bird flu virus, haemagglutinin H₅ subtype, with elevated titers of 1/64 and 1/128.

Viral isolates belonging to subtype H₅ haemagglutinin were tested by neuraminidase inhibition reaction and was determined to belong to the neuraminidase subtype N₃.

Neuraminidase subtype identification in combination with epidemiological situation (contact with wild waterfowl, no morbidity and mortality), virological (two passages) led to the diagnosis of infection with low pathogenic (LPAI), H₅N₃ subtype.

Chapter VII, entitled *Epidemiological, clinical and lesion research in avian influenza* revealed the results of investigations in an episode of avian influenza. Thus, following epidemiological investigations, households in the village L where there have been cases of illness and sudden death in găinăș, are located close to ponds, surrounded by cereal areas. Birds bred in extensive system, free, had access to water, particularly ducks and geese, sometimes with wild birds, many of them migratory. These flocks bred in semi-wild, and those who are allowed to leave daily the household have permanent or regular contact with wild birds, implicitly the species incriminated in the epidemiology of the disease (avian influenza).

The source of infection was represented by the infected wild web-footed (ducks, geese), who came into contact directly or indirectly with flocks of water birds.

Analyzing the occurrence of disease in terms of climatic conditions is found that it occurred during the time that there were often positive temperatures over 10°C in March 2010. This suggests that during the winter flu virus remained in the region, with the birds that brought it, which means that the risk of transmission from birds that not migrate is very high. Infected birds excrete virus in high concentrations via the faeces, as well as nasal and ocular excretions. The absence of other diseases in the area, but no further development of outbreaks of avian influenza, suggests that the local water wild birds were not contaminated and therefore bird flu is endemic.

Clinically, of the 269 birds, symptoms of disease were recorded in seven birds, which is a morbidity of 2,60%. Also, analysis of results showed, however, the morbidity rate differently depending on the household. Thus, in the household number 1, of the 154 existing poultry were ill four birds, representing 2,60% and in the household number 2, of the 115 poultry, three birds were sick, which represented 2,61%.

Clinically, the examined birds showed strong adynamy and depression associated with loss of appetite and excessive thirst, dyspneic and noisy breathing, increased lacrimation, beard and ridge cyanosis, abrupt cessation of laying, subcutaneous hemorrhage, diarrhea, occasionally nervous symptoms and rapid death.

As the disease progressed, the depression state was accentuated, bird hardly walked, sat crouched with wings left down, with half closed, swollen, eyelids, and supported their head in the ground. If evolution took longer than 1-2 days, nerve disorders were observed, represented by curious attitudes, convulsions, amaurosis, clonic contractions of different muscles, paralysis, etc.

Necropsy examination conducted at the seven bodies (laying) showed septicemia lesions, represented by congestivo-hemorrhagic appearance.

Analyzing the frequency of macroscopic lesions it can be observed that most often has been the congestivo-hemorrhagic appearance of organs (2,60%) and crest and beard cyanosis (2,23%), which shows an acute septicemia evolution, and the least common injuries to the stomach wall (thickening and the presence of mucus) (1,49%).

Virological examination conducted by inoculating pathological material represented by triturates of organs with lesions at a dose of 0,2 ml in the alanto-amniotic cavity of 9-10 days old găină embryos, allowed the isolation of a virus with haemagglutinating activity in titer of $6 \log 2$ (1/64). The haemagglutination inhibition reaction with reference positive sera identified avian influenza virus, highly pathogenic (HPAI), subtype H₅N₁.

In **Chapter VIII** entitled *Surveillance and control policy measures in bird flu* is mentioned in a *Program - Framework for prevention and control of avian influenza*, developed in the following official documents:

- Ordinance No. 42 of 29 January 2004 on the organization of veterinary activity;
- Law no. 215 of 27 May 2004 approving Government Ordinance no. 42/2004 regarding the organization of veterinary activity;
- Law no. 127 of 17 May 2005 approving Government Emergency Ordinance no. 88/2004 amending and supplementing Government Ordinance no. 42/2004 on the organization of veterinary activity and food safety;
- Order No. 98 of 7 October 2005 on the approval of the quota (of necessity) of Romania for avian influenza;
- ANSVSA Order no.54 of 28 February 2007 control measures for avian influenza.

Framework Programme shows that maintenance of herds as free of avian influenza viruses requires continuous application of more effective surveillance and control measures, including: biosecurity measures, measures in the veterinary protection area, measures in the veterinary surveillance area, active surveillance measures, control measures, development and respect for contingency planning and the establishment of the crisis.

In **Chapter IX** entitled *Final conclusions* in the 16 formulation are summarized main issues drawn from research on avian influenza.

The paper presented a number of 19 tables and 66 figures, and bibliography includes 116 titles.