UDC 619:616.98:578.832.1:616-076

DOI: 10.29326/2304-196X-2019-3-30-51-56

SPREAD OF LOW PATHOGENIC AVIAN INFLUENZA A/H9N2 IN THE WORLD AND RUSSIAN FEDERATION. CHALLENGES OF DISEASE ERADICATION

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SUMMARY

The paper demonstrates data on global and national spread of subtype H9 low pathogenic avian influenza in poultry. Due to no need of the mandatory disease notification to the OIE, published data are used for the analysis of the infection spread. Cases of combined low pathogenic avian influenza clinical manifestation in poultry population are described. Challenges of the infection eradication are addressed. Global spread of A/H9 virus in poultry, its rapid evolution and zoonotic potential require close attention. Since the disease signs and clinical course are similar to the ones of other avian infectious diseases (Newcastle disease, infectious laryngotracheitis, infectious bronchitis, metapneumovirus infection, mycoplasmosis, infectious coryza, etc.), the routine laboratory diagnosis should include tests for low pathogenic avian influenza thus facilitating the rapid response to the threat. H9 virus circulation is reflected not only in the physical condition of birds and their productivity, but it also affects the live vaccines' efficacy during scheduled immunizations through the reduction of their protective effect and increase of post-vaccination reactions. In spite of the availability of specific prevention, the opinions of the researchers divided between its opponents and proponents. On the one hand, the vaccination eliminates the economic loses and the virus escape into the environment, but on the other hand, it can aid to "silent" spread of the virus and its established variation that is typical in case of low strength of the poultry population immunity.

Key words: low pathogenic avian influenza (LPAI), poultry industry, respiratory syndrome, combined disease, H9N2.

INTRODUCTION

Although H9N2 virus is in the category of low-pathogenic avian influenza (LPAI) viruses, it poses continuous threat to the poultry industry due to its widespread. Moreover, the pathogen has a zoonotic potential and can be isolated from the human oropharyngeal mucosa and those poultry farm employees, who handle the infected flock, demonstrate A/H9 virus antibodies. H9N2 virus-induced low pathogenic avian influenza is not an OIE notifiable disease, therefore data on its spread is reported only in the research publications. Despite low virulence the virus is the cause of clinically manifested disease due to coinfection and administration of live vaccines. It causes grave economic losses coming from the increased culling, lethality and production loss both in broiler cross-breeds and layers, especially on the commercial farms. In case of H9N2 LPAI occurrence drastic measures are considered economically inappropriate and vaccination can be an effective tool for the disease control. Many countries (Pakistan, Iran, Israel, Korea, PRC, etc.) use preventive H9 influenza immunization for the reduction of economic risks and for the infection control.

MATERIALS AND METHODS

Routine methods of epidemic investigation were used in the work. The data obtained during the investigation were systemized and subjected to the epidemiological analysis. Post-mortem examination, sampling and transportation of pathological materials were performed in compliance with SP 1.3.3118-13 "On safe handling of pathogenicity (hazard) group I–II microorganisms"; SP 1.2.036-95 "On procedure for pathogenicity group I–IV microorganism recording, storage, transfer and transportation"; Veterinary rules of avian influenza A laboratory diagnosis approved by the Order of the Ministry of Agriculture of Russia of 03.04.2006 No. 105 [1, 4, 5].

RESULTS AND DISCUSSION

Global virus spread

Subtype H9N2 viruses are globally spread and are generally subdivided into two genetic groups – of North American and Eurasia origin. The virus of H9N2 antigenic formula was first isolated from wild birds and turkeys in the USA in 1966 [20].

The H9 virus prevailed in the Asian countries, mostly in China, where two genetic lineages predominated: Y280 (clade h 9.4.2) and since 2013 – G1 (h 9.4.1). Until 2013 the prevalent virus genotypes included: BJ94-like H9N2 and SF98-like H9N2 lineages. Herewith, G1 viruses were mostly isolated from quails and BJ94-like (genotype A) and F/98-like (genotype H) viruses – from chickens. In 2000, genotype H substituted genotype A and became prevalent in China [14].

China is considered the epicenter of the influenza virus spread. In this country H9N2 virus was isolated from chickens, ducks, quails, pheasants, partridges, silkies, chuckar partridges and became the prevalent influenza virus in poultry. According to the statistical data, in 1996–2000 the share of subtype H9N2 virus infected chickens amounted to 93.89% being indicative of the virus global spread. Moreover, the virus is a donor of "internal" genes for reassortants' formation [13].

H9N2 influenza has been considered to be endemic in the Near East and North Africa since 1990 [26]. Mass outbreaks of H9 influenza were reported in Iraq in 2004–2007 and they were characterized by 70% lethality for broilers and 10% lethality for layers [23]. In 2000–2003, in UAE the low-pathogenic influenza H9 virus induced the disease in quails along with 30% egg drop and 36% lethality for layers [12]. In Israel the infection was frequently reported in turkey flocks, where the lethality varied between 0 and 30% [15]. H9 influenza lethality in broilers in Lebanon in 2004–2005 amounted to 35%, but it did not exceed 2% in layers. H9 AIV antigen-specific immunoglobulins were detected in blood sera of pigs fed with poultry by-products [10]. In 2016, in Morocco H9 influenza infection was reported in layers. The lethality amounted to 2–15% and egg drop reached 80% and remained at such level for 10 weeks [18]. The virus that induced serious health concerns in poultry in Burkina Faso (2017) and Ghana (2018) belonged to G1 lineage [9]. The first case of low-pathogenic influenza H9 virus was reported in Korea in 1996. LPAI H9 viruses causing the disease in this country belonged to a separate Korean-like lineage. On the Korean farms the avian-influenza associated lethality amounted to 5–30% of the poultry population [24].

Thus, the analysis of the published data indicates global spread of subtype LPAI H9 virus, mostly in Asian and Near East countries. Herewith, genetic groups G1, Y280 and Y439 are being isolated. The presumptive area of the pathogen's spread may be even wide due to the lack of unbiased OIE data on H9 influenza as the disease is not notifiable to the OIE.

Virus spread in Russia

In February 2012, respiratory disease along with increased mortality were reported on one of the large commercial poultry farms in the Far Eastern region of Russia. Epidemic data analysis and laboratory test results concluded the concurrent low-pathogenic H9N2 avian influenza induced by BJ94-like genotype virus widely spread in Mainland China. During subsequent active monitoring similar virus was isolated from pigeons. Clinical disease in commercial poultry of heavy crosses was manifested as respiratory syndrome (rales, heavy breezing with beak open, crowding) and increased mortality (Fig. 1, 2).

Post-mortem lesions were mostly concentrated in the upper parts of the respiratory system (laryngitis, tracheitis, caseous plugs in the lower part of trachea; Fig. 3, 4).

Studies of the virus biological properties demonstrated its low virulence. In 2012, H9 virus genome was also isolated from the biological materials collected from the wild birds in the Central Russia, Siberia and Far East. In 2012–2017, antibodies to H9 virus antigen were repeatedly detected in the wild birds shot on Uvs-Nuur Lake (Republic of Tyva, border with Mongolia), mostly in waders (*Charadriiformes*) – terns, gulls. In 2017, serological tests of birds in the Kirov Oblast demonstrated specific anti-hemagglutinin antibodies (4–7 $\log_2$) in sera of black grouse (*Lyrurus tetrix*) and mallard (*Anas platyrhynchos*) that are being indicative of the pathogen circulation in the birds of natural ecosystems. In spring 2017, H9N2 pathogen was detected in decoy ducks in one of the hunting entities in the Tula Oblast. During the active monitoring performed by the Rospotrebnadzor in 2018, H9N2 virus was isolated from wild migratory birds in the Tomsk Oblast and Khabarovsk Krai. In 2019, LPAI A/H9 was detected in chickens in the Urals. The virus was assigned to genetic lineage G1 that is typical for the Near East countries. It should be noted that circulation of H9 viruses of all three genetic lineages is reported in Russia (G1, Y280, Y439).

Fig. 1. Heavy breezing with the beak open

Fig. 2. Crowded broiler chicks

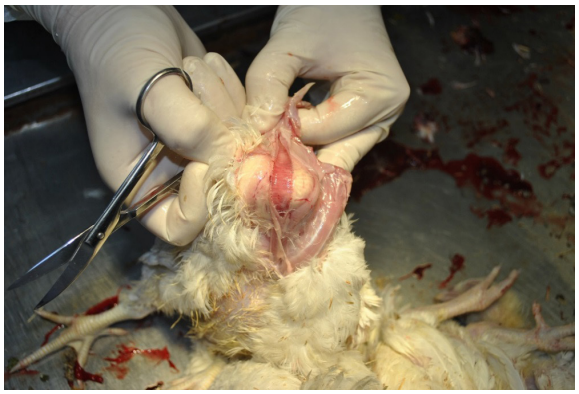

Fig. 3. Tracheitis (hyperemic and swollen tracheal mucosa with viscous secretion)

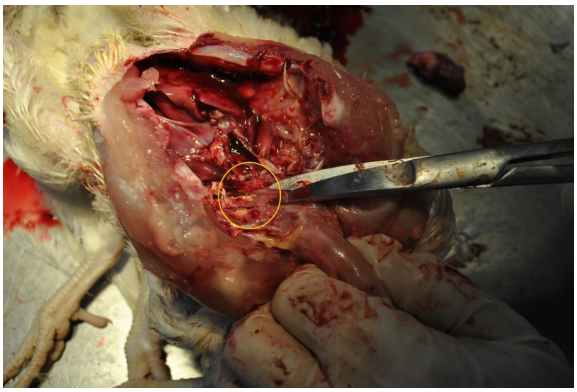

Fig. 4. Caseous plug in the lower part of trachea

Why does low pathogenic A/H9N2 virus cause serious health problems, increased mortality and culling in the commercial poultry population? Indeed, there are no serious clinical changes observed following the infection of the experimental SPF-birds and naive birds not immunized with live vaccines. However, on the commercial poultry farms, under the crowded housing and stress factors, effects of several infections are observed simultaneously. Co-infection triggers growth of H9 virus effect on the poultry body. Administration of the live vaccines against respiratory infections in the presence of the background LPAI infection instigates development of clinical signs and subsequent lethality increase, as the pathogenic effect of several viruses having similar replication mechanisms (same portal of the infection entry, target organs and cell receptors) may grow many-fold. The foreign research publications confirm this hypothesis [7]. Thus, N. Arafat et al. demonstrated that administration of the live attenuated ILT vaccine drastically worsens subtype H9 virus-induced low-pathogenic avian influenza. Bursa and thymus atrophy was reported in experimental chickens infected with H9 virus and immunized with live attenuated ILT vaccine. Immunity suppression is therefore observed that is the cause of secondary infections, increased lethality and failure of ILT vaccination. H9 virus did not however affect the ILT vaccination efficacy: the vaccination of H9 virus-infected chickens against ILT did not result in grave consequences. Thus, the studies demonstrate that chickens' infection with LPAI A/H9N2 virus following immunization with live vaccines, inter alia against ILT, result in clinical manifestation of the respiratory syndrome, increased lethality and bird culling. AI H9 infected chickens additionally demonstrate suppression of both cellular and humoral immunity [16]. On one of the farms in the Primorsk Krai concurrent H9 influenza infection following live ILT vaccine administration was also reported. The clinical signs included depression, swelling of infraorbital sinuses, watery greenish diarrhea and petechial hemorrhages in the oral cavity (Fig. 5, 6).

Post-mortem examinations demonstrated laryngotracheitis and lung edema (Fig. 7).

Increased mortality of poultry, mostly of broilers, is observed in case of concurrent H9 low-pathogenic avian influenza and respiratory mycoplasmosis (*Mycoplasma gallisepticum*), infectious synovitis (*Mycoplasma synoviae*), ornitobacteriosis (*Ornithobacterium rhinotracheale*), infectious laryngotracheitis (*Herpesviridae*), infectious bursal disease (*Birnaviridae*), Newcastle disease (*Paramyxoviridae*).

Virus evolution and its zoonotic potential

Low-pathogenic H9N2 influenza virus can be potentially transmitted from animals to humans and vice versa. Four basic clades from h 9.1 to h 9.4 are identified in order

Fig. 5. Depigmented eggs contaminated with watery green feces

Fig. 6. Petechial hemorrhages in oral cavity

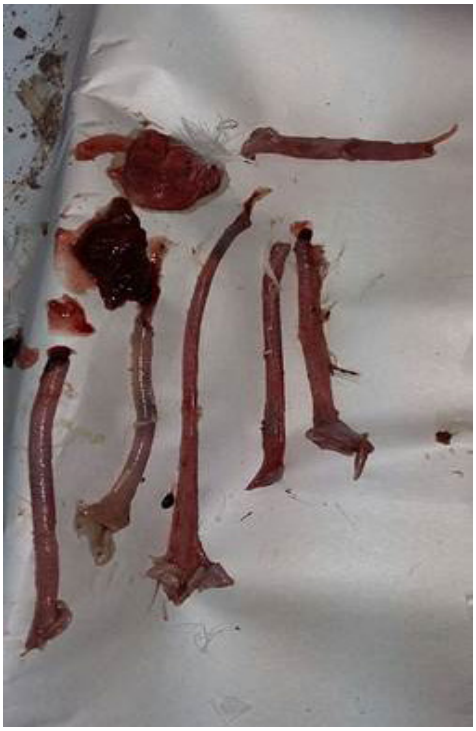

Fig. 7. Laryngotracheitis, edemic and hyperemic lungs

to understand the low-pathogenic virus evolution. To date, genetic clade h 9.4 is the most numerous one. Annual analysis demonstrated that H9 virus is subject to rapid evolution due to reassortation processes and today there are over 70 known virus genotypes. It was additionally established that highly pathogenic avian influenza H5N1 virus that caused human infection in Hong Kong (1997) contained internal genes of G1-like H9N2 virus [17, 25]. Reassortations of the field and vaccine viruses are occurring and they are being demonstrated by the genetic tests [6].

H9N2 virus widely spread in Pakistan contained genes (PB2, PB1, PA, NS) identical to highly pathogenic influenza H7N3 virus. M. Iqbal et al. demonstrated that in the experimentally infected quails the disease is more severe as compared to other bird species. Herewith, crows turned out to be resistant to the clinical disease following H9 virus infection. Cross-species virus transmission between synanthropic birds and chickens was also proved that is indicative of the potential wide spread of the virus in the field [21, 22].

Many mammals are susceptible to influenza H9N2 virus infection and the disease is easily reproduced in ferrets and minks. Serological tests performed on the mink farms in China demonstrated H9N2 virus antibodies in over 40% of sera of animals daily fed with poultry slaughter products. The virus was isolated from dogs, horses and humans [10, 13]. 17% of the personnel occupied on the Iranian poultry farms, where H9N2 influenza was reported, demonstrated virus subtype-specific antibodies in their blood sera [8, 27]. The experimental data indicated that the virus replicated in higher concentrations in the respiratory organs as compared to the intestines [19].

Starting from 1999, H9N2 virus infections were reported in humans in China, Bangladesh and Egypt, and they did not result in severe conditions. H9N2 virus is related to the human receptors. Nevertheless, no confirmed cases of the virus transmission between humans have been reported so far.

Challenges of the disease eradication

Rules of Avian Influenza Control (2006) are the basic instrument regulating the procedure to be used in case avian influenza virus circulation is confirmed in the Russian Federation. According to paragraph 16 of the Rules, any suspicion of the poultry infection with subtype H9 influenza virus without any clinical signs of the disease shall be subject to repeated tests.

Pursuant to paragraph 33.2 "in case of confirmation of commercial poultry infection with low pathogenic avian influenza viruses of subtype H4, H6 and H9 marketing of live birds and hatching eggs shall be prohibited. Poultry meat may be moved from the containment zone under the control of the official veterinary inspector in charge of the designated area for its further processing using processing methods that ensure the virus inactivation. Program for the infection eradication shall be developed and implemented in the organizations; such program involves stamping out of the infected population. Slaughter of poultry shall be permitted after the termination of the poultry growing cycle" [3].

The program for the infection eradication may involve specific prevention immunization as agreed by the veterinary authorities. However, such vaccination is not the basic tool for the disease prevention and it shall not be of mass nature.

Routine mass vaccination of poultry is definitely applied in those countries, where the disease causes grave economic losses for the poultry industry thus inducing negative consequences involving food security threats. The whole-virion inactivated vaccines turned out to be the most safe and effective. Recombinant and vector vaccines are clinically tested but their use is limited. China holds the leading position in LPAI A/H9 prevention, as since 1998 over 11 bln chickens have been vaccinated there. Humoral post-vaccinal antibodies protect the poultry from the clinical infection and reduce/prevent the virus excretion. Insufficient strength and homogeneity of the flock immunity does not however exclude the virus circulation in the vaccinated population that can precondition the virus variability under the antibody "pressure". The program of the vaccine prevention should be accompanied not only by its efficacy control but also with the continuous monitoring of the virus circulation in the immunized population. It should be noted that the vaccination affects the animal health status of the region (subject to regionalization) that can have an impact on the export potential of the Subject.

Currently the basic strategy for the prevention of LPAI/H9 virus introduction to the closed-type farms involves strict compliance with the biosecurity and biocontainment measures [2].

CONCLUSIONS

Low-pathogenic avian influenza A/H9N2 virus is widely spread in the world and circulates in the Russian Federation.

The concurrent disease can result in over 60% lethality of the poultry population, mostly of poultry of heavy crosses. Use of live vaccines in the infected flock leads to both cellular and humoral immunity depression.

Rapid evolution (reassortation) of the virus and its zoonotic potential require close attention to H9 virus spread in poultry and wild bird populations.

Programs of the disease control and eradication can include prevention strategy involving use of inactivated

vaccines. The vaccination, however, should not have the mass nature and biosecurity measures should prevail in the disease prevention on the large commercial farms.

Low vaccination efficacy (low population immunity) can contribute to "silent" spread of the infection and virus variability.

Conflict of interest. The authors declare no conflict of interest.

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Submitted on 26.07.19
Approved for publication on 22.08.19