



Field Qualitative Detection of Avian Influenza Using Antigen Rapid Test in Different Districts of Nineveh Governorate, Iraq

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Received date: 20 May 2019 Accepted: (453) 17 Jun 2019 page (25-31) Published: 30 Jun 2019

DOI: <https://doi.org/10.36326/kjvs/2019/v10i1319>

Abstract

Sensitive and rapid diagnostic kits for avian influenza virus are required to screen large numbers of samples during a disease outbreak and to prevent the spread of infection. The aim of study is to investigate on avian influenza virus type A serotype H5 among different avian species in different district of Nineveh Governorate, Iraq using Anigen rapid H5 AIV Ag test kit. In this study, we employed One-step avian influenza virus type A and serotype H5 antigen test (Anigen rapid H5 AIV Ag test kit). The test was run out in different districts of Nineveh governorate, including broiler farms local poultry markets, and house poultry breeders (local breeds, broiler, and layers) as well as pigeon houses. Of tested 686 swab from total 241477 poultry samples were been tested, no avian influenza virus positive cases result were noted or confirmed by rapid screening test. No clinical signs were recorded in all examined poultry species. The study conclude that kit used for rapid diagnosis of avian influenza type A serotype H5 was easy, reliable and its interpretation less than thirty minutes.

Keywords; avian influenza, field approach, antigen rapid test, H5 AIV Ag test kit

الكشف النوعي عن إنفلونزا الطيور باستخدام اختبار المستضد السريع في مناطق مختلفة من محافظة نينوى ، العراق

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الخلاصة

لغرض مسح عينات كبيرة في حالة الوباء ولمنع انتشار الإصابة كانت الحاجة الى عدة انفلونزا الطيور سريعة وحساسة. وان الهدف من هذه الدراسة للتقصي عن انفلونزا الطيور نوع (A) النمط المصلي (H5) بين مختلف أنواع الطيور لمناطق مختلفة من محافظة نينوى، العراق باستخدام عدة المستضد السريعة نوع H5 AIV Ag test. في هذه الدراسة وظف اختبار الخطوة الواحدة لاختبار مستضد انفلونزا الطيور نوع A نمط مصلي H5. تم استخدام هذا الاختبار السريع في مناطق مختلفة من محافظة نينوى تضمنت حقول فروج اللحم، أسواق بيع الدجاج المحلي، الدجاج المنزلي فضلا عن الحمام المنزلي. اختبرت 686 مسحة مخرجية من مجموع 241477 عينة دواجن ولم تظهر ولم تسجل اي حالة موجبة لانفلونزا الطيور. كما لم تسجل اية علامات سريرية للمرض في جميع الانواع الطيور المفحوصة. استنتجت الدراسة ان العدة السريعة لتشخيص هذا النوع من الانفلونزا سهل التطبيق ومفيد جدا في التشخيص المبكر وتفسير نتائجه بسيط ويستغرق اقل من 30 دقيقة.

Introduction

Avian influenza (bird flu) is a viral disease caused by Orthomyxoviridae family genus' influenzavirus, which classified into three main types; A, B and C. Type A virus known as pathogenic to the birds (1). The birds are being recorded as susceptible for infection with influenza A virus. For instance; aquatic birds form a major reservoir of these viruses, and the overwhelming majority of isolates have been of low pathogenicity (low virulence) for chickens and turkeys (2). The bird flu virus (type A) was classified into different serotypes depending on their antigenically related nucleocapsid and matrix proteins, which in turn also divided into sub serotypes according to their haemagglutinin (H) and neuraminidase (N) antigens. (3). To date, **more than 18 H subtypes** (H1–H16) and 9 N subtypes (N1–N9) are recognized with proposed new subtypes (H17, H18) for influenza A virus and the naturally occurring highly pathogenic influenza A virus that produces acute clinical disease in chickens, turkeys and other birds of economic importance has been associated only with the H5 and H7 subtypes (4-6). Highest ratios of low pathogenicity for poultry serotype are H5 and H7 has been recorded and defined as (H5/H7 low pathogenicity avian influenza [LPAI]) and develops into highly pathogenic avian influenza (HPAI) by mutation (7).

World organization for animal health was recorded all serotypes of H5/H7 LPAI viruses from poultry as well as all HPAI viruses from poultry and other birds, including wild birds (1). Many

factors play an important role in susceptibility of the host to the influenza infection, for instance; birds' species, type age, as well as strain of virus and environmental conditions in addition to pathogenicity of the disease (8). Characterization and diagnosis of avian flu involve different approaches such as isolation of virus and serological tests for detecting plus differentiation of various immunosuppressive-respiratory diseases in poultry(9-12). However, typical indicative methods are time-consuming and labor intensive. Moreover, non-nonspecific reactions or cross reactions frequently hinder serologic assays (12).

Rapid and sensitive approaches for diagnosing and categorizing pathogenic viruses affecting respiratory tract in poultry are vital for implementing appropriate preventive and control measures to mitigate economic losses (12-15). (16), found that Mosul city clear from avian influenza by using a screening rapid test for identification of avian flu in different species of local birds. The aim of this qualitative study is proof that the Nineveh governorate clear from avian influenza by using of antigen rapid H5 test kit.

Materials and methods

Swab sampling from the cloaca region of randomly picked out broiler from different areas at different ages between 12-40 days from 30 randomly selected broiler farms, in addition to local poultry markets, and house poultry breeds as listed in table 1.

Table 1: sampling for avian influenza rapid test			
	Total number	Total population	Tested samples
Broiler farms	30	240000	450
Local poultry markets	5	At day of test 360	43
House Poultry farming	110	637	58
Pigeon houses	40	480	76

One step avian influenza virus type A and serotype H5 antigen test (Anigen rapid H5 AIV Ag test kit, BIONOTE, Korea) was employed in this study which contains:

1. Rapid AIV antigen test devices.
2. Sample collection tubes containing 1 ml of assay diluents.
3. Sample collection swabs.
4. Disposable droppers.

The test was done according to kit manufacturing instruction by Anigen rapid H5 AIV Ag test kit. Swab sampling from the cloaca was involved via inserting sample swabs 3-4 times inside the cloaca to ensure that the head of the swab is

covered with mucus secretions of the tested cloacae broiler and chickens. The swabs were soaked in sample collection tubes containing assay diluents and mixed gently until dissolved in the diluent solution. The tubes were left for a while (1-2 minutes) to give chance to large particles to settle down at the bottom of the tube. Using the disposable dropper four drops from the supernatant were added to the sample hole on the test device. A purple colour was developed as the test begins to work moving forward across the window in the center of the test device. The results were interpreted within 30 minutes. Figure 1 shows the steps used for the rapid test.



Figure 1: Steps were used for the Anigen rapid H5 AIV Ag test kit

Results

Field examination of broiler farms, house poultry farming and local poultry marketing in different districts of Nineveh governorate did not reveal any positive results by using rapid antigen test of avian influenza A virus serotype H5. Healthy

avian species, including broiler, chickens, hens, pigeon, and ducks were found at the time of checking for avian influenza. No clinical signs or mortality rates were reported at these districts. Table 2 shows the distribution of samples collected during the survey and their results.

Table 2: Sample distribution collected and examined from different broiler farms, local poultry markets, house poultry farming and pigeon houses.

Regions		Total No. of farms	Total population	Examined No. by AIV Ag test kit	Results
Broiler farms	Al-hamdanya	12	90000	200	-ve
	Gogjali	8	47000	105	-ve
	Waterfalls area	4	38000	85	-ve
	Ba'asheqa	6	65000	60	-ve
Local poultry markets	Shaquille	3	237	24	-ve
	Mosul-Erbil highway	2	123	19	-ve
House Poultry farming	Qaraqoosh	34	161	21	-ve
	Kaberlie	26	182	17	-ve
	Karamlees	18	125	8	-ve
	kaznah	32	169	12	-ve
Pigeon houses	Different areas	40	480	135	-ve
Total			241477	686	-ve

Discussion

The outpatient visits for influenza-like-illness were increased in highly percentage during last few years in Iraq because of a wide trading plan of poultry products in Iraq from deferent neighboring countries suggests an increased risk for global human public health. The zoonotic influenza viruses, such as avian influenza (AI) virus subtypes, A/H7 subtype have been reported in our neighboring countries, push the Veterinary Hospital of

Nineveh Governorate to take precautionary and preventive measures to reduce and prevent the spread of the disease within the city of Mosul. Most of the zoonotic strains of avian influenza had been narrow within the H5, H7, and H9 subtypes. Highly pathogenic avian influenza (HPAI) virus strains of the H5 subtype continue to pose a significant threat to animal health (17). Therefore, we conducted the field survey in the different areas of Nineveh province as a border city

with Turkey, Syria and Jordan, in addition to the Kurdistan border region with Iran to monitor the movement of disease in the area using of Anigen rapid H5 AIV Ag test kit.

Of 686 swab, wash, from 241477 poultry samples, no avian influenza virus positive result was registered. This study has been focused on running rapid diagnostic methods for the identification and diagnosis of avian influenza type A virus serotype H5 via using of antigen rapid test kit. The study data revealing negative results, which indicate that different districts are free from avian influenza serotype H5 of type A virus. This rapid immunochromatographic immunoassay designed to aid in the qualitative detection of Influenza Type A virus in tracheal and cloacal samples from symptomatic birds or flocks. This assay detects all 16 subtypes of Influenza Type A virus (15). It was found that H5 serotype of avian influenza A virus more virulent to both birds and human (1). Human infections with influenza viruses derived directly from wild birds or poultries are rare, but, recently, over 600 human infections by influenza A (H5N1) viruses have been detected as fatal cases (16). H5N1 infection rapidly leads to severe pneumonia and acute respiratory distress syndrome (ARDS), which are pathologically characterized as diffuse alveolar damage (18, 19). Thus, it should be checked periodically to monitor the disease or virus movement in Iraq and especially Nineveh governorate, which has characterized by heaviest poultry industry on the north of Iraq. This finding was supported by (20), who confirm that Mosul city was free from avian influenza type A virus serotype H5. H5-subtype of AI viruses has long caused serious health problems both locally and globally. Since AI viruses could mutate and gain the ability to spread easily between humans, veterinarians and doctors considered monitoring of birds and human AI infections to control the disease in early

stages and to avoid the outbreak in poultry farm, which leads to heavy economic loss.

Conclusion

Different districts of Nineveh Governorate are clear from avian influenza type A serotype H5. In this study, the kit used for rapid diagnosis has antibodies selected for avian influenza type A serotype H5 was easy, reliable and its interpretation needs not more than thirty minutes when the reaction colour change to be purple in positive result and remain no change in negative result according to the colored positive and negative control provided by the kits' information which can be used as screening tests.

Acknowledgments

This study was supported by the veterinary hospital of Nineveh governorate. Authors would like to thank all farmers and owners for their help and endless support. Finally, authors would express their gratitude to the Iraqi government represented by Iraqi ministry of agriculture / General Authority of Veterinary Medicine.

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