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Serological Evaluation of Fowlpox virus in Turkeys (Meleagris gallopavo) by Using ELISA test in Tikrit city, Iraq

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ABSTRACT

Background and Objective: Fowlpox is a viral disease of considerable health and economic importance that affects a wide range of domestic and wild birds and can lead to substantial production losses. Although the disease has been extensively investigated in chickens and pigeons, information regarding the serological status of fowlpox virus infection in turkeys remains limited, particularly in Iraq. Therefore, this study aimed to evaluate the serological status of fowlpox virus infection in turkeys in Tikrit, Iraq, using an enzyme-linked immunosorbent assay (ELISA) to detect anti-fowlpox virus antibodies.

Methods: A total of 88 turkeys were included in this study and classified into three groups: clinically infected birds (G1), birds vaccinated with a commercial fowlpox vaccine (G2), and healthy unvaccinated birds serving as the control group (G3). Serum samples were collected from all birds and analyzed using an indirect ELISA to detect antibodies against fowlpox virus. Antibody responses were also evaluated according to age and sex.

Results: The results showed high rates of antibody positives in both the infected group (80.56%) and the vaccinated group (87.5%). A trend toward higher immune response was observed in birds older than six months compared to younger birds, and only minor differences were observed between males and females

Conclusions: This study provides baseline serological data on fowlpox virus infection in turkeys in Tikrit, Iraq. The findings demonstrate strong antibody responses in both naturally infected and vaccinated birds and highlight the value of routine serological monitoring for assessing infection status and vaccine-induced immunity, thereby supporting more effective surveillance and control programs for fowlpox in turkey flocks.

Keywords: Fowlpox virus, ELISA, Turkey, Vaccination.

Introduction

Fowlpox is a viral disease of significant veterinary and economic importance affecting various species of domestic and wild Birds and causing considerable health and economic losses in animal production. The disease is caused by the Fowlpox virus (FPV), a member of the genus Avipoxvirus within the family Poxviridae. This disease is known for its ability to cause distinct pathological changes, which clinically take the form the skin lesions (dry) or mucosal lesions (wet) affecting the respiratory and upper digestive tract, according to the clinical pattern of the disease [1].

Although there are verified reports about outbreaks of fowlpox virus in chickens and pigeons in Iraq, clinical and serological on the occurrence of the disease in turkeys remains extremely limited. This deficit indicates a knowledge gap that requires attention and scientific research to closure. The importance of this type of study has become even more apparent in previous years, coinciding with the significant expansion of turkey farming in areas such as Tikrit and its suburbs, where veterinary prevention measures are weak and vector insect prevalence rates are high, which may contribute to increased opportunities for infection and exacerbate the pathological condition in turkey flocks [2] [3]

Diagnosing fowlpox based solely on clinical signs can be challenging, as these signs may be unclear in some cases or overlap with those of other diseases, which can reduce diagnostic accuracy. In view of this, serological tests, particularly enzyme-linked immunosorbent assay (ELISA), have become one of the most reliable diagnostic tools for the detection of virus-specific antibodies. This technique provides high diagnostic value, both for confirming infection and for evaluating the efficacy of vaccines used in the field, which enhances its ability to support control and prevention programs in different management systems [4] [5]. ELISA is one of the preferred methods for seroprevalence studies because of its high specificity and sensitivity in detecting antibodies, as it can process large numbers of samples in a short time compared to traditional methods. This technique is also important in evaluating the level of immunity induced after vaccination, especially in cases where vaccination programs are absent or insufficiently documented, making ELISA a crucial tool in directing diagnostic procedures and supporting prevention and control measures within flocks [6] [7] [8].

Based on the above, this study aims to investigate the serological status of fowl pox virus in turkeys in the city of Tikrit by dividing the birds into three main groups: birds with clinical signs of disease, birds that had been previously vaccinated, and birds that appeared healthy. Samples have been collected and tested using an ELISA assay for the detection of specific antibodies, with the aim of assessing the level of virus infection and immune response in each group. These data will provide a preliminary reference information to support disease surveillance programs and vaccination strategies in turkey flocks.

MATERIALS AND METHODS

Ethical approval

Ethical approval No. (Tu. Vet.156) was obtained from the Committee for the Care and Use of Animals in Scientific Research at the College of Veterinary Medicine, Tikrit University, on Maech 3, 2025.

Study Design

The study design included a total of 88 turkeys (Meleagris gallopavo), which were divided into three groups. Sample were collected from these birds between March to July 2025 from various areas of Tikrit city and its surroundings regions in of Salah al-Din province, Iraq. The turkeys were divided into three groups: Group 1 (G1) a clinically infected group from which samples were taken to confirm infection (fig 1); Group 2 (G2), birds vaccinated with a fowlpox vaccine to evaluate the immune response, and Group 3 (G3), healthy control group used as a negative control for the ELISA test, as show in (Table 1). Blood samples were collected from the brachial (wing) vein of each turkey, allowed to clot, and the serum was separated by centrifugation and stored at −20°C until serological analysis using an indirect ELISA [9] [10].

Table 1: Study Design and Bird Grouping

Group with	Criteria	Purpose	Number
(G1) Infected group (fig 1)	Turkeys showing clinical signs suggestive of fowlpox	Detection of fowlpox virus infection using ELISA a	36
(G2) Vaccinated healthy group	Clinically healthy turkeys vaccinated with commercial fowlpox vaccine	Evaluation of humoral immune response using ELISA	40

(G3) Control group	Clinically healthy turkeys	Negative control for ELISA assay	12
			88

Immunization protocol of vaccinated birds

The second group of birds was vaccinated with a live attenuated vaccine against fowlpox (Himmvac FowlPox® Live Vaccine, KBNP Inc., 2025). The vaccine was prepared by dissolving it in 10 ml of the diluent provided and then given to clinically healthy birds using the wing web stab method (Fig 2). After vaccination, the birds were monitored for one week for any local or systemic reactions, and blood samples were collected after five weeks later to evaluate the immune response induced by the vaccine using an ELISA assay.

ELISA-Based Serological Assay Method

In order to detect antibodies to fowl pox virus in turkeys, an indirect ELISA test was used, following the manufacturer's instructions, using a commercial kit (Poultry Fowlpox Virus Antibody ELISA Kit) from) SunLong Biotech, China). The test was used to assess the serological status of infected, vaccinated, and healthy birds.

Figure 1: illustrates typical cutaneous Fowlpox lesions on the head and neck of infected turkey

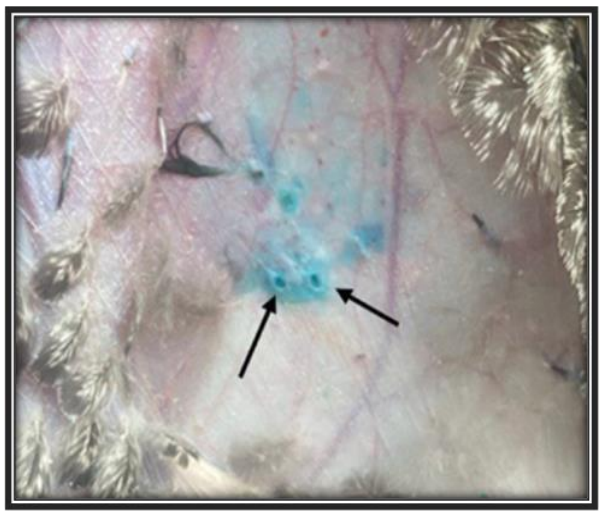

Figure 2: show the vaccination stab for Fowlpox using a double pronged vaccination needle.

Results

The ELISA test results showed high and nearly identical levels of antibodies to fowlpox virus (FPV-Ab) in both clinically infected and vaccinated birds, while all control samples remained negative.

1. Result of the Infected Group

Results of the ELISA test in the group of turkeys showing clinical signs of fowlpox distinct a clear serological response, with 80.56% of samples testing positive (29/36 samples). This high percentage indicates a close relationship between the clinical signs presented and the levels of antibodies revealed in the blood serum. The positive rate increased with age. Birds younger than 6 months had a positive rate of 72% (18/25), while the percentage of positive cases reached 100% (11/11) in birds older than 6 months, shows an increased susceptibility to infection with advancing age, as shown in (Table 2).

Table 2: ELISA result in infected turkey by age

Age	No.	+ sample	rate
less 6 month	25	18	72.0%
More 6 month	11	11	100%

By gender, the infection rate was 77.8% in males (14/18) compared to 83.3% in females (15/18), indicating that both sexes are susceptible to fowlpox virus infection, with only a slight difference in positivity rates, as shown in (Table 3).

Table 3: ELISA result in infected turkey by sex

sex	**No.**	**+ sample**	**rate**
Male	**18**	**14**	**77.8%**
Female	**18**	**15**	**83.3%**

2. Result of the Vaccinated Group

The results of the ELISA assay in the vaccinated turkey group show a significant positive immune response against fowl pox virus, with 35 positive samples from 40 samples, a rate of 87.5%. The positive rate in males was 84.2% (16/19), compared to 90.4% (19/21) in females, indicating a good immune response in both sexes with a relatively higher rate in females, as shown in (Table 4).

Table 4: ELISA result in Vaccinated turkey by sex

sex	No.	+ sample	rate
Male	19	16	84.2%
Female	21	19	90.4%

By ages, birds younger than six months had a positive response rate of 81.8% (18/22), while the rate among birds older than six months was 94.4% (17/18), indicating an improvement in immune response with age, as shown in (Table 5).

Table 5: ELISA result in infected turkey by age

Age	No.	+ sample	rate
less 6 months	**22**	**18**	**81.8%**
More6 months	**18**	**17**	**94.4%**

3. Result of the Control Group

The control group included 12 clinically healthy turkeys that exhibited neither any signs of disease nor had been immunized against fowlpox virus. This group was used as a negative control in the ELISA test to confirm the specificity and accuracy of the test in differentiating between infected and uninfected birds. The test results revealed that all samples in this group had negative results, confirming the reliability of the test used to detect antibodies to the virus.

Discussion

The results of this study demonstrated a high positive rate for antibodies against fowl pox virus in clinically infected turkeys (80.56%), indicating a close association between the presence of clinical signs and viral infection. This high seropositivity may be attributed to sample collection during the active stage of infection, which is associated with enhanced immune stimulation and increased antibody production. The findings of the present study are consistent with those of an observational study conducted in Nigeria by Ibrahim et al. (2022), which reported a high incidence of fowlpox virus infection in turkeys, reaching 69.58%. These observations suggest that turkeys may be more susceptible to the fowlpox virus infection than other types of poultry, possibly due to differences in immune response or to environmental and management factors that increase exposure to infection [11]. This higher positive rate in our study is due to the fact that the screening was based on birds showing clear clinical signs corresponding to fowlpox virus infection.

The study also showed differences in the serological response to fowl pox virus by age group, with higher positive antibody rates recorded in older turkeys compared to younger ones. This is likely due to the maturity of the immune system and increased opportunities for cumulative exposure to the antigen with age. From a diagnostic viewpoint, serological tests such as ELISA reflect the level of immune response and antibody accumulation and are not necessarily a direct indicator of infection rates or primary susceptibility to infection. These observations are consistent with those reported by Ibrahim et al. and Mai et al., which showed that young birds are more susceptible to infection during outbreaks, while adult birds exhibit higher levels of relative resistance [12]. Based on this, the higher titer positivity in older birds in the current study can be understood as a reflection of the strength of the immune response developed over time. The rates of infection among males and females did not show significant differences, with infection appearing to be similar between the sexes, indicating that both are susceptible to fowlpox virus to a comparable degree under the studied environmental conditions. This is partly consistent with a previous study, which reported that females were more susceptible to infection, while another study reported the opposite finding, explaining that males may be more susceptible because of their aggressive behaviour, which increases the likelihood of skin injuries and facilitates viral entry [11] [13]. This variation in results suggests that the influence of gender on infection rates may be more related to environmental and behaviours factors and to specific conditions of rearing than to a fixed physiological difference between males and females.

The results of the current study indicate that older turkeys showed a strong serological response against fowl pox virus after vaccination compared to young turkeys, which is likely due to the maturation of the immune system and increased opportunities for previous exposure to antigens. These results are consistent with those reported by Wang et al., who showed that young birds may exhibit suppression of the immune response during the early stages after infection [14]. On the other hand, a higher serological response was recorded in males compared to females, which may be due to physiological differences that affect the efficiency of the immune system, as indicated by Czerny et al. [15].

Recent studies indicate that the immune response generated by vaccination with the fowlpox vaccine is marked by the combination of humoral and cellular immunity. Azizi and Asli have shown that vaccination stimulates the

expression of cytokines such as IFN-γ and IL-2 during the earliest stages after vaccination, indicating the induction of an effective cellular response that contributes to the formation of long-term immunity. They also showed in a previous study that the immune effect of the vaccine is not limited to the production of antibodies detectable by ELISA, but also includes the activation of T-helper and cytotoxic T-cells, which play a critical role in the eradication of infected cells [16].

ELISA was employed in this study as a tool for serological diagnosis of fowl pox virus, in addition to its role in assessing the immune response resulting from vaccination, due to its sensitivity and the ability to detect specific antibodies in bird serum. This technique provides diagnostic indicators that help to understand the immune status of birds, especially in areas where there are no clear or documented vaccination programs. However, the interpretation of serological results requires a degree of caution, as the presence of antibodies may reflect previous exposure to the virus as a result of natural infection or undocumented vaccination, and does not necessarily indicate a protective or effective immune response to the vaccine used. Therefore, serological test results should be interpreted within the clinical and study methodology, taking into account the history and health status of the birds and the time of sampling, to ensure a more accurate diagnosis and a more realistic understanding of the immune response dynamics.

Conclusion

The findings of this study indicate that fowl pox virus remains an important health and production concern in turkeys, particularly in the absence of organized and organized vaccination programs. The observed seropositivity patterns reflect variations in immune response among birds; however, differences related to age and sex were descriptive and not statistically significant. The results, based on serological detection using ELISA, provide baseline information on the immune status of turkeys and contribute to a better understanding of fowlpox infection under field conditions. Accordingly, these findings support the use of serological data in combination with clinical observations, to improve disease monitoring and vaccination planning in turkey flocks.

Limitations of the Study

This study was based on descriptive analysis without statistical testing. The relatively limited sample size and the absence of statistical comparisons may restrict the generalization of the findings; therefore, further studies using larger sample sizes and appropriate statistical analyses are recommended.

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Declaration of interests

The authors declare that there are no conflicts of interest**.**

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Publication Consent

All authors have read and approved the final version of the manuscript and consent to its publication.

Data and material availability

All data analysed and generated in this study are included in this published research.

Author contribution

Both authors contributed to the conception and design of the study, data collection, data analysis, interpretation of the results, manuscript preparation, and approved the final version of the manuscript.

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التقييم المصلي لفيروس جدري الطيور في ديوك الرومي باستخدام اختبار ELISA في مدينة تكريت، العراق

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الملخص

الخلفية والهدف :يُعد جدري الطيور مرضًا فيروسيًا ذا أهمية صحية واقتصادية كبيرة، يصيب طيفًا واسعًا من الطيور الداجنة والبرية، ويسبب خسائر ملحوظة في الإنتاج. وعلى الرغم من وجود العديد من الدراسات التي تناولت المرض في الدجاج والحمام، فإن المعلومات المتعلقة بالحالة المصلية للإصابة بفيروس جدري الطيور في الديك الرومي، ولا سيما في العراق، لا تزال محدودة. لذا هدفت هذه الدراسة إلى تقييم الحالة المصلية للإصابة بفيروس جدري الطيور في الديك الرومي في مدينة تكريت، العراق، باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA) للكشف عن الأجسام المضادة للفيروس.

المواد وطرائق العمل :شملت الدراسة (88) ديكًا روميًا، قُسمت إلى ثلاث مجموعات: مجموعة الطيور المصابة سريريًا (G1)، ومجموعة الطيور الملقحة بلقاح تجاري ضد جدري الطيور(G2) ، ومجموعة الطيور السليمة غير الملقحة التي استُخدمت مجموعة سيطرة .(G3) جُمعت عينات المصل من جميع الطيور، وأُجري لها اختبار ELISA غير المباشر للكشف عن الأجسام المضادة لفيروس جدري الطيور، كما جرى تقييم الاستجابة المناعية تبعًا للعمر والجنس.

النتائج :أظهرت النتائج ارتفاع معدلات الإيجابية المصلية في كل من مجموعة الطيور المصابة (80.56%) ومجموعة الطيور الملقحة (87.5%). كما لوحظ اتجاه نحو استجابة مناعية أعلى في الطيور التي يزيد عمرها على ستة أشهر مقارنة بالطيور الأصغر عمرًا، في حين كانت الفروق بين الذكور والإناث محدودة.

الاستنتاجات :توفر هذه الدراسة بيانات مصلية أساسية عن الإصابة بفيروس جدري الطيور في الديك الرومي في مدينة تكريت، العراق. وأظهرت النتائج استجابة قوية للأجسام المضادة في كل من الطيور المصابة طبيعيًا والطيور الملقحة، مما يؤكد أهمية الرصد المصلي الدوري في تقييم حالة الإصابة والاستجابة المناعية الناتجة عن التلقيح، بما يدعم برامج الترصد والسيطرة على المرض في قطعان الديك الرومي.

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الكلمات المفتاحية: فايروس جدري الطيور، ELISA، الديوك الرومية، التحصين.