

Isolation and detection of reovirus from arthritis in chickens

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Abstract

In this study 70 samples were collected from 14-26 weeks old egg laying hens. Clinical signs of infected chickens characterized by lameness, swelling in hock joint. Samples include blood for preparation of serum also hock joints and tendon for virus isolation. Hyperimmune sera was prepared by injection of broiler chickens four times with vaccine strain Reo 1133^R 0.2 ml subcutaneously in the neck. Samples were processed and prepared for virus isolation by using 6 days old embryonated chicken egg which was inoculated in yolk sac four passages. Lesions in egg embryo was recorded for each passage then the isolates were diagnosed by using neutralization test using convalescent and hyperimmune sera. Clinical signs of infected birds characterized by swelling and enlargement and edema of hock joint, postmortem lesions revealed swelling and injury in tendon, ulceration and erosions in cartilage and discoloration in synovial fluid, hemorrhage in the leg and yellow necrotic foci in the liver, the result of virus cultivation in embryonated chicken egg show dwarfism in growth, death of embryo with subcutaneous hemorrhage, initiated in 2nd passage and subsequent passages, this lesion increase in severity with progress of passages and with decrease in death time in hours and increase in titer of virus particles. The virus titer was decreased when neutralized by using neutralization test it gives 22 isolates were positive from 34 isolates.

Keywords: Avian Reo virus, Embryonated chicken eggs, Arthritis, Neutralization test

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العزل والكشف عن فيروس الريو من حالات التهاب المفاصل في الدجاج

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فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تم في هذه الدراسة تم جمع سبعون عينة من دجاج بيض المائدة بعمر 14-26 أسبوعاً، تمثلت العلامات السريرية في الدجاج المصاب بالعرج وتورم مفصل العرقوب وتمثلت العينات بالدم لغرض الحصول على المصل وكذلك مفصل العرقوب والأوتار لغرض عزل فايروس الريو الطيري. تم تحضير المصل عالي التمنيع عن طريق حقن فروج اللحم أسبوعياً ولأربع مرات وبجرعة 0.2 مل تحت الجلد بالعنزة اللقاحية Reo 1133^R تم معاملة العينات وتحضيرها لغرض العزل الفيروسي باستخدام أجنة بيض الدجاج بعمر 6 أيام والتي تم حقنها في كيس المح ولأربع تمريرات متتالية. سجلت الآفات المرضية في أجنة بيض الدجاج ولكل تمريرة، بعدها شخصت العزلات بواسطة اختبار التعادل باستخدام مصول النقاها والمصل عالي التمنيع. العلامات السريرية للطيور المصابة تميزت بورم وتضخم مفصل العرقوب، الآفات المرضية أظهرت وذمة في مفصل العرقوب، تورم وأذى في الأوتار توسف في غضاريف المفصل وكذلك تغير في لون وحجم السائل الزلالي، نزف في الأقدام ويؤثر نخرية في الكبد. أما نتائج تنمية العزلات في أجنة بيض الدجاج أظهرت تقزم في الأجنة ونزف تحت الجلد والتي بدأت في التمريرة الثانية واستمرت إلى نهاية التمريرات. وازدادت شدة هذه الآفات بتقدم التمريرات مع نقصان في وقت موت الأجنة مع ارتفاع في معيار الجزيئات الفايروسية. انخفض المعيار الفيروسي عند استخدام اختبار التعادل المصلي حيث أعطت العزلات نتائج بواقع 22 من أصل 34 عزلة.

Introduction

Avian Reovirus (ARVs) belongs to the family Reoviridae, these viruses possess nucleic acid (Double strand RNA) ARVs particle can be range between 70-80 nanometer, the virus infect many types of birds (1), causing variant degrees of clinical signs represented in tenosynovitis, with post mortem changes characterized by edema and swelling in hock joint with lameness, sometimes the lesion developed causing rupture in gastrocnemius tendon with ulceration cartilage this signs combined with other signs including bleeding in legs which showed bluish to green in color, the virus infect chicks 4 weeks old and the morbidity rate reach 10%, while mortality rate is lesser (2), the ARV cause hepatitis, myocarditis, pericarditis, respiratory and gastrointestinal tracts infection in addition the virus cause malabsorption syndrome with immunosuppression and autoimmunity (3). The virus replicates initially in small intestine and bursa of Fabricius then spread after 24-48 hours to another organs. subclinical infection is the main characteristics of reovirus. This character gives the secondary infection to stimulate the latent infection to reactivate infection and interact with other bacterial and viral etiological agents, in addition reovirus like other immune suppressor viruses as avian adenovirus type 4 which cause immune suppression and failure of vaccination program (4,5). The virus spread horizontally and vertically, newly hatched chicks exposed to infection if our breeders is not vaccinated, chick shed the virus through oral and cloacal, through the contaminated foot pad injuries in addition through respiratory and gastrointestinal tracts. The vertical transmission is characterized by death in embryonated chicken eggs and decrease the percentage of hatchability (6). The ARV is able to grow in many types of cell culture lines as Vero cell, chick embryo fibroblast, chick embryo lung. In addition, the virus is capable of growing in 6 days old embryonated chicken eggs if inoculated in yolk sac causing embryo death after 72-96 hours of inoculation the embryo shows yellow necrotic lesions in the liver (7). The virus was also diagnosed through the detection of virus antigens by using agar gel diffusion, direct fluorescent with presence of specific antibodies. moreover, the virus can be diagnosed by using molecular technique as RT PCR (8).

Due to the importance of this virus and its disease and the effects in the poultry industry, economic losses and no previous study were conducted in Iraq to isolate the virus, so this study was performed.

Materials and methods

Sample collection

Total of seventy samples (hock joints, tendon and blood) were collected from egg laying hens at 14-28 weeks

old in Nineveh province. These chickens suffered from lameness, swelling of hock joints. The blood samples were collected, then serum was separated and stored in -20 °C (9,10).

Preparation of hyperimmune serum

Non vaccinated broiler chicks breeds were vaccinated at 4 weeks age with reovirus vaccine (Intervet vaccine Nobilis Reo 1133^R, Log₁₀^{3.1}TCID₅₀) weekly for four weeks intervals, the dose of vaccine was 0.2 ml subcutaneous. Blood collected from vaccinated broilers and the serum separated and stored -20 °C (10).

Preparations of samples

Samples was taken 1 gram of cartilage and tendon cut and grinded it in sterile mortar using sterile pestle and sterile sand then added phosphate buffer saline (3). The granted organ put it in test tube, centrifuged 2500 rpm 4 °C for 10 minutes, the sediment discard and the supernatant added to penicillin 10000 IU per 1 ml and streptomycin 10mg/1 ml then stored -20 °C.

Isolation of ARVs

Virus was isolated by using 6 days chicken embryonated eggs (CEE) with no history of using reovirus vaccine were inoculated with 0.2 ml of supernatant in yolk sac, the eggs were incubated in 37 °C and daily candling and recorded the death time of embryo, lesions and virus titrations then after 5 days of inoculation the yolk was harvested and returned inoculation 4 passages (11).

Diagnosis of virus isolates using neutralization test

For diagnosis, the virus isolates were six double diluted, then equal volume of convalescent serum and hyperimmune sera separately were added to each dilution, incubated 37 °C for 1 hour, the vaccine strain considered as positive control, then inoculated for each dilution in yolk sac of embryonated chicken eggs 6 days old (10 embryo for each dilution) then examined daily, the virus titer measure according to Reed and Munech (13).

Results

Result of clinical signs and post mortem examination

The result of clinical signs of infected chicken show lameness and swelling in hock joints, edema, sometimes rupture in gastrocnemius tendon, post mortem examination lesion revealed hemorrhage in legs with bluish to green in color, erosions in cartilage with change in the color of synovial fluid (Figure 1-3).

Result of virus isolation and propagation in chicken embryo

The result shows no lesions was recorded in 1st passage, then 2nd and subsequent passage revealed lesions which include petechial hemorrhage under the skin, dead of embryo with dwarfism, these lesions increase in severity in the later passages with decrease in embryo death time and increase of virus titer (Figures 4-6) (Table 1).



Figure 1: A 20 weeks old egg laying hens showing edema and hemorrhage in the leg.



Figure 2: A 26 weeks old egg laying hens showing swelling and edema in hock joint.



Figure 3: A 33 weeks old egg laying hens showing increase the volume and change in color of synovial fluid of hock joint.

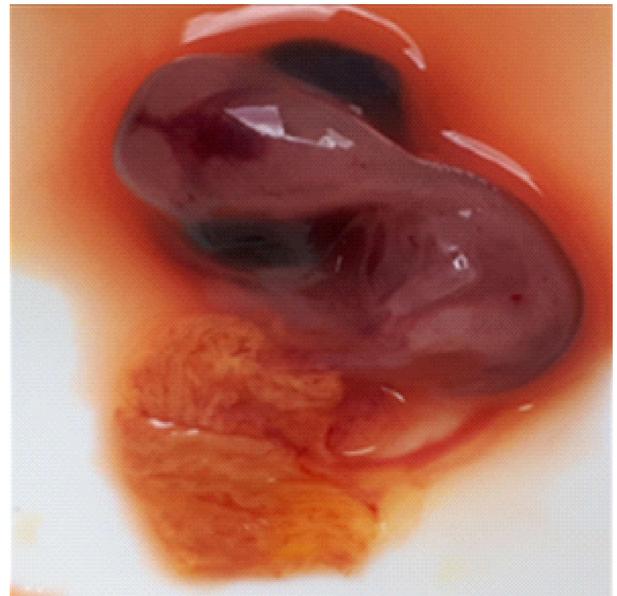


Figure 4: Chicken embryo in 4th passage showing hemorrhage in head.



Figure 5: Chicken embryo in 4th passage showing dwarfism retardation of growth.



Figure 6: Chicken embryo from control group.

Table 1: Result of virus isolation and propagation in chicken embryo

Passages	P4	P3	P2	P1
Mean titer of isolates EID ₅₀ /0.1ml	10 ^{3.2}	10 ^{2.4}	10 ^{1.9}	10 ^{1.2}
Time of death (hours)	72	72	96	-
Dwarfism	+++	+++	++	-
Hemorrhage	+++	+++	++	-
Edema	+++	+++	++	+

This score is representing the severity of lesions, (-) no lesions, (+) mild lesions, (++) moderate lesions, (+++) sever lesions

Diagnosis of isolates using Neutralization test

Result of neutralization test show that the titer of virus isolates decreases when processed with convalescent serum and hyper immune serum. It gives positive result 22 from 34 isolates which can be perversely propagated in embryonated chicken eggs (Table 2).

Discussion

ARVs is one of the effective viruses of poultry industry which cause severe economic losses because it infects respiratory and gastrointestinal tracts in addition to arthritis, this virus is difficult to diagnose clinically or by post mortem examination because these signs interact with some others viral and bacterial infection (14). This virus cause arthritis in chicken which is the most common, so this study focused on these signs and collected the samples from it never than other organs. The samples were collected after post mortem done which is suspected from reovirus infection. The chickens were suffering from arthritis in addition to lesions in the liver, when comparison those signs with standard experimental infection with Reo virus which can match the result of Ballal *et al* (15) from the experimental study in chicken by virus isolates.

Table 2: Result of neutralization test

Type of virus treatment	N ^o of positive isolates	Titer
Mean of virus isolate titer before process EID ₅₀ /0.1ml	34 isolates	10 ^{3.2}
Mean of virus isolate titer after process with convalescent serum EID ₅₀ /0.1ml	22/34 Positive	10 ^{1.1}
Mean of virus isolate titer after process with hyperimmune serum EID ₅₀ /0.1ml	22/34 positive	10 ^{2.4}

The result of propagation of samples in embryonated chicken eggs show cytopathic Effects in embryo which manifested by hemorrhage and edema in embryo those result similar to Ballal *et al* (15) Natalia and Hanna (16) in severity, the result in this study show hemorrhage in visceral of embryo with necrotic lesions in liver, the reason of differences in severity of lesion to the fact that differences in virulence between the viral strains which

causes severe lesion in both chickens and embryos. The severity of lesions in embryo increase with progress of passages accompanying death in embryo with dwarfism, which seriously with increase the virus particles. Those lesions are not recognized in 1st passage, the reason of that is due to titer of the virus particles which increase with progresses of passages (18).

The virus isolates were diagnosed by neutralization test which give real picture to viability of virus in cultivation host. The result show decrease in the titer of virus isolates when processed with convalescent serum and hyperimmune sera. The reason of that is to be accustomed to adduce by Wickramasinghe *et al* (19) the reason of variation due to several reasons firstly the variation in the antigenicity of viral strain which can be used in neutralization test and the strains which induce the infection in field. This reason causes decrease in positive samples in neutralization test, although this negative strain induces CPE in chicken embryo and pathognomic clinical signs in infected chickens, this corresponds with mentioned Rafael (2) the time of collection of samples attempted in the acute phase of infection and appearance of clinical signs. This induce increase of virus particles in samples, Lobani *et al* (20) said that the severity of infection in adult chickens is more than the young ones.

Conclusion

This study concluded that these viruses are severely affected poultry industry in Iraq.

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