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RESEARCH ARTICLE

Sero-Prevalence of Chicken Anemia Virus in Local Fowls and Japanese Quails

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ABSTRACT

Serum samples were collected from local fowls (indigenous) and Japanese quails from different locations of Diyala province for the period of October, 21st 2017 to June 5th 2018. All the birds (local fowls and Japanese quails) were apparently healthy.These samples were 100 from local fowls of four villages, and 100 samples from four quail commercial farms. The samples were collected according to the age and subjected to ELISA test using IDEXX , ELISA Germany commercial kit. The results showed for the first time in Iraq that 87 serum samples of local fowls out of 100 were positive for chicken anemia virus (CAV) antibodies, and 29 serum samples out of 100 samples from quails were positive to CAV antibodies. The ages of local fowls were 12, 24,27, and 30 weeks and the age of birds do not significantly affected the sero-positivity of the result (P=0.211). In contrast the ages of quails were 2, 3, 4 and 5 weeks. The age of birds was significantly correlated to the results (P<0.004), when 13 samples out of 29 positive samples were from young quails of 2 weeks of age. 42.5% of positive samples collected from local fowls appeared with low S/N ratio (0.001-0.199) and high antibody titer, whereas, 20.5% of positive samples from quails showed the same above mentioned S/N ration. It seems that local fowls are highly susceptible to CAV in comparison to quails and might be a source for the infection of other commercial farms.

Keywords:- Local fowls, Japanese Quails, Chicken anemia virus

INTRODUCTION

Chicken anemia virus disease (CAVD) or chicken infectious anemia virus disease (CIAVD), is one of the important viral disease that affect the immune system of susceptible birds of poultry industry worldwide (1,2,3). The disease was circulated in USA since 1959 (4), but it was firstly isolated from infected chicken in Japan 1970 (5).

Chicken anemia virus (CAV) as a contagious and infectious disease was reported to infect young chicken of 1 to 4 weeks of age, causing severe anemia and lymphoid atrophy (6,7).The resulted immunosuppression due to lymphoid atrophy caused by the virus made the infected birds susceptible to secondary bacterial or viral infections (8,9,2,10).Clinically, infected birds showed depression, pale mucous membranes and lethargic. Hemorrhages might be observed under the skin of different parts of the body, included the wings (Blue wing disease).Mortality rates generally were variable and increased with presence of secondary infections due to immune suppression (11).The economic losses in poultry industry due to CAV might be attributed to poor weight gain of infected birds in comparison to uninfected birds, immunosuppression and susceptibility to secondary infection, and losses from mortality due to active infection (12,13).

The causative virus was found to be transmitted horizontally by fecal-oral route (14) or vertically from infected male and female parents (15,16) regardless their immune status or antibody titer (17,18).Chicken anemia virus is a single stranded circular negative sense or ambisense DNA virus. It is the smallest among DNA viruses and classified within the genus *Gyrovirus*. This genus and the genus *Circovirus* were classified with the family *Circoviridae*. The virus DNA was encoded for three proteins VP1, VP2 and VP3 (19).Many studies or authors suggested that chicken was the only bird species to be infected with CAV, whereas, pigeons, pheasant and duck were negative (20,21,22). In contrast many other reports mentioned the CAV infection in other birds like fancy chicken (23), Japanese quail (24,25), rooks and jackdaws(26).In Iraq no data were available on screening of poultry for the presence of CAV infection in broiler, layers and other birds. Furthermore, virus gens were not detected and/or the virus was not isolated. Accordingly, the present study was designed and aimed to screen, local fowls and Japanese quails of Diyala province for CAV antibodies.

MATERIALS AND METHODS

This survey study was conducted in Diyala province. The study was extended over the period from October twenty first 2017 to June 5th, 2018. The main objective is to point out the presence of chicken anemia virus (CAV) antibodies for the first time in local fowls and Japanesequails.

Serum samples

A total of 200 blood samples were collected from 4 commercial quail farms, 4 villages for local fowls (Table 1) in Diayla provincebyveno-puncture of the wing vein using sterile syringes andvacuum blood collection tubes/gel/clot activator (UNIMEDIC, Iraq). Sera were separated and placed in Eppendorf sterile tube, labelled and centrifuged at 1500 rpm for 5 minutes (Cold Eppendorf centrifuge. THERMO FISHER, USA).Supernatant serum was collected from each sample and transferred to another sterile Eppendorf tube labeled and stored at -20°C until used.

Processing of Samples for ELISA Test

The sera were tested using a commercial ELISA kit (IDEXX Lab,Germany) at a 1:10 dilution and the results were expressed as S/N ratios (sample to negative ratio) according to manufacturer's instructions. Processed ELISA sample plate was washed using ELISA washer automatic system ELX 800™,Optical density value was red at 650 nm wave length on an ELX 800™ microplate reader (BIO-TEK Instruments, USA).

Interpretation of the CAV ELISA Results

The negative control index (NCX) must be calculated for both duplicated wells and the same could be applied for the positive control index (PCX) at 650 nm absorption and according to the following:

$$NCX^{-} = \frac{NC1\ A(650) + NC2\ A(650)}{2} \qquad PCX^{-} = \frac{PC1\ A(650) + PC2\ A(650)}{2}$$

Validity criteria

$NCX^{-} \geq 0.600$ $PCX^{-} / NCX^{-} \leq 0.50$

$$S/N = \frac{SAMPLE\ A(650)}{NCX^{-}}$$

The presence or absence of antibodies (Ab) to CAV was determined by the sample to negative (S/N) ratio for each. Accordingly, the 1/10 diluted serum samples that showed S/N > 0.60 were negative for CAV antibodies, whereas samples showed S/N ≤ 0.60 were positive to CAV antibodies.The results were statistically analyzed using IBMSPSS V21 PC 9IBM (statistical package of social science).

RESULTS

All the flocks of both types of birds were positive for CAV antibodies when were tested by ELISA IDEXX kit (Table-2,figure-1).Sero-prevalence of CAV Ab in local fowls showed that 87 out of 100 serum samples were positive to CAV, whereas 29 serum samples out 100 from Japanese quails were positive to CAV. The correlation in the positivity rate between the two groups of different birds was highly significant (P<0.000).In local (indigenous) fowls the sero-prevalence of CAV antibodies showed that 87 out of 100 serum samples were positive for such antibodies. The correlation of age to positivity rate of each four groups of local fowls was not significant (p=0.211) (Table-3, figure-2).

The sero-prevalence of CAV antibodies showed that 29 out of 100 serum samples were positive for such antibodies. The correlation of age to positivity rate of each four groups of quails was highly significant (P≤0.004) (Table-4, figure-3).Serum samples from both birds that were positive for CAV antibodies showed that were 37 serum samples from local fowls out of 87 with high Ab level and low S/N ratio, 30 with moderate Ab level and medium S/N ration and 20 with low Ab level and high S/N ratio (Table-4).In Japanese quails the result showed that 6 out of 29 serum samples were positive with high level of Ab and low S/N ratio, four samples with moderate level of Ab and medium S/N ratio, and 19 with low Ab level and high S/N ration (Table 5, figure-4).The correlation of Ab level between the two groups of bird was highly significant (P≤0.000).

DISCUSSION

It is well known that chicken anemia virus was worldwide in its distribution and was recorded broilers, layers and breeder farms (21,22). In some poor countries backyard (indigenous) chickens were regarded as one of the main economic incomes for such people like African (27).In Iraq there is no data regarding the infection of local fowls with chicken anemia virus.The present study showed that 87 out of 100 samples that were collected from four different locations were positive to CAV antibodies. Similar findings were reported by Hernandes-Divers *et al*. (28), when they reported 90% positivity rates to CAV antibodies in backyard chicken in Ecuador regardless their ages. Emikpe*et al*. (29) reported for the first time CAV antibodies in apparently healthy indigenous chickens of Nigeria from four communities. The sero-prevalence was 88.9%.The local fowls of present study had an age ranged from 12 to 30 weeks and the high level of antibodies might be caused subclinical infections as all samples were collected from apparently healthy birds (7).These local fowls may be play a role of CAV transmission to healthy commercial chicken (29,27), or CAV might be transmitted from adjacent broiler and layer farms to backyard birds or vice versa (30). Bülow and Schat(31) stated that breeders, broilers, and layers were the source of CAV infections in backyard chickens. The same authors added that, contaminated eggs, cells, and vaccines of live type may be the source of CAV dissemination to backyard chickens. In contrast Barrios *et al*. (32) reported that the source of CAV in backyard chickens was unknown,

and not got a concern for study or detection for longtime and might be the source of CAV infection in poultry industry.

In Japanese quails of present study 100 samples were collected from four farms. Seroprevalence of CAV antibodies in such birds showed that 29 (29%) of the serum samples were positive to CAV antibodies (Table 4). Six of them were with low S/N ratio and high Ab level, four with medium S.N and moderate Ab level and 19 of them with high S/N ratio and low Ab level (Table 5). The seropostivity of Japanese quails of present study came in agreement with many other studies. Farkas*et al. (24)* reported the sero-positivity of 103 serum samples of Japanese quails out of 168samples tested for CAV. Furthermore, the same authors added that the titer of CAV Ab in serum samples collected from quails in 1992 was lower than that collected from quails in 1995. The positivity rate of infection was estimated 83.3% when 10 flocks were positive to CAV Ab out of 12. Zia-Jahromi and Gholami-Ahangaran (25) detected CAV infection in 50 flocks of Japanese quails and suggested that quails could be a host for CAV infection. CAV virus was also detected in the thymus samples of 38 quails (15%) out of 250 samples (33).

In the present study all the four farms were positive to CAV Ab (Table 3).In comparison to seropositivity in local fowls, high numbers of indigenous birds were positive to CAV Ab (87%) when compared to 29 (29%) positive of quail samples. It seems that local fowls were highly susceptible to the CAV infection (7).In a final conclusion this study showed for the first times in Iraq that local fowls (indigenous) and Japanese quails were susceptible to CAV infection. As they were apparently healthy birds they might be act as a source of infection to chicken industry (broilers and layers). CAV was reported as an immunosuppressive and its infection might predispose the poultry farms to the complicated infections with other bacterial and viral agents, or causing vaccine failures.

It is recommended firstly to isolate the virus from clinically infected birds and subjected the isolated virus to molecular study to compare its genomic structure from different isolates to point out the possibility of strain variation, secondly to subjects all the Iraqi poultry farms and local fowls to wide scale of vaccination program with local isolate of CAV.

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Table 1.Serum samples collected from birds according to kind of birds and locality of farms

No.	Number of samples	Age	Location	Kind of bird
1	25	12 weeks	C1/Mindily	Local hens
2	25	24 weeks	C2/Al-Huayder	Local hens
3	25	27 weeks	C3/Al-Mokdadia	Local hens
4	25	30 weeks	C4/Al-Shaab	Local hens
5	25	2 Weeks	Q1/ Al-Khalis	Quails
6	25	3 weeks	Q2/ Buhris	Quails
7	25	4 weeks	Q3/ Al-Mokdadia	Quails
8	25	5 weeks	Q4/Baquba	Quails
Total	200			

Table 2. Sero-prevalence of CAV antibodies in Japanese quails and local fowls

Type of Birds	Antibody positivity to CAV		Total
	CAV-Ab +ve	CAV-Ab-ve	
Local Fowls	87 (87%) 75%	13(13%) 15.5%	100
Japanese Quails	29(29%)* 25%**	71(71%) 84.5%	100
Total	116(52%)	84(42%)	200

***Percent among the total group of samples for each type of birds

**Percent among the total samples of positive/negative samples for CAV antibodies birds.

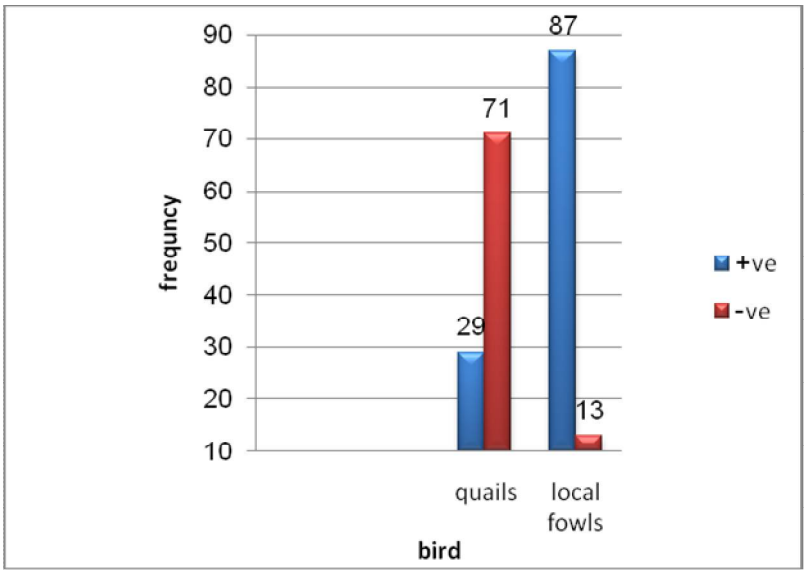


Figure 1. Descriptions of CAV serum positive and negative samples according to bird species

Table 3. Sero-prevalence of CAV antibodies in local fowls

Age	Antibody positivity to CAV		Total
	CAV-Ab +ve	CAV-Ab-ve	
12 weeks	24 (96.0%)	1 (4.0%)	25 (100.0%)
24 weeks	20 (80.0%)	5 (20.0%)	25 (100.0%)
27 weeks	20 (80.0%)	5 (20.0%)	25 (100.0%)
30 weeks	23 (92.0%)	2 (8.0%)	25 (100.0%)

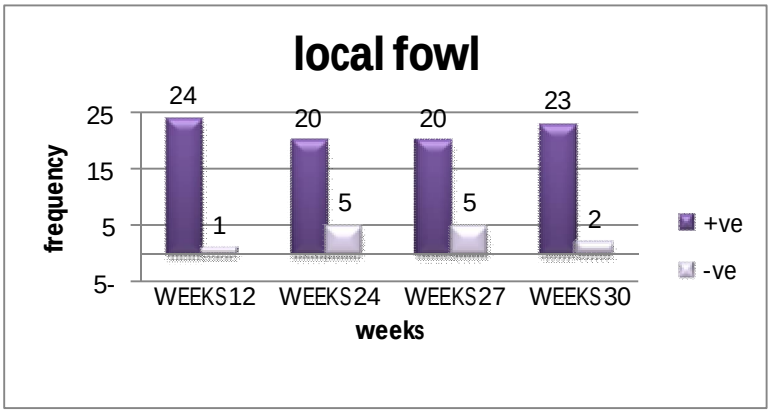


Figure 2. Descriptions of CAV serum positive and negative samples of local fowls according to age groups

Table 4.Sero-prevalence of CAV antibodies in Quails

Age	Antibody positivity to CAV		Total
	CAV-Ab +ve	CAV-Ab-ve	
Two weeks	13(52.0%)	12(48.0%)	25(100.0%)
Three weeks	9 (36.0%)	16(64.0%)	25(100.0%)
Four weeks	5 (20.0%)	20(80.0%)	25(100.0%)
Five weeks	2 (08.0%)	23(92.0%)	25(100.0%)

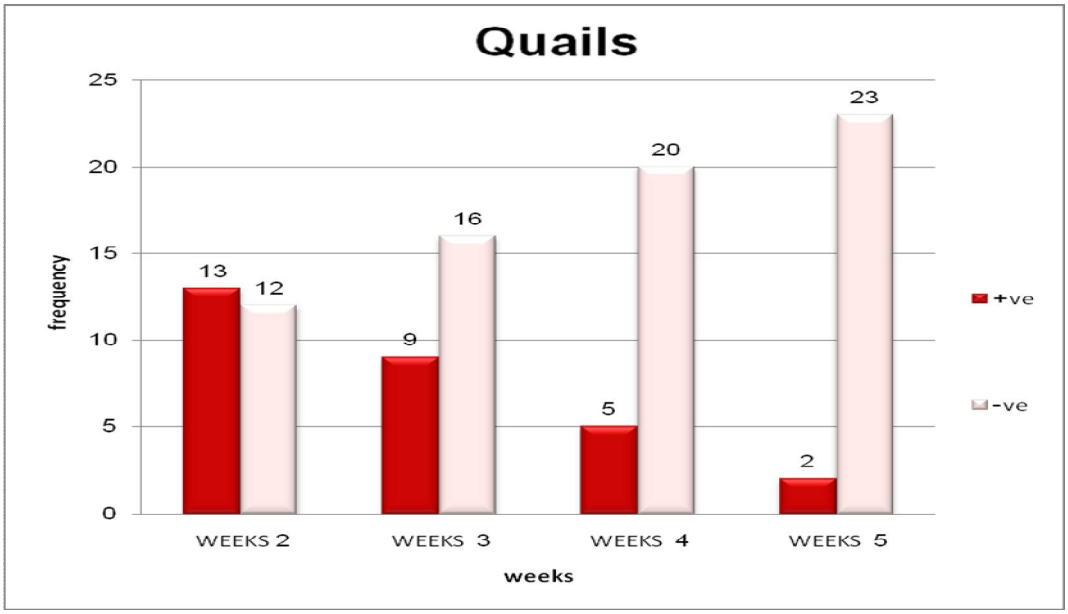


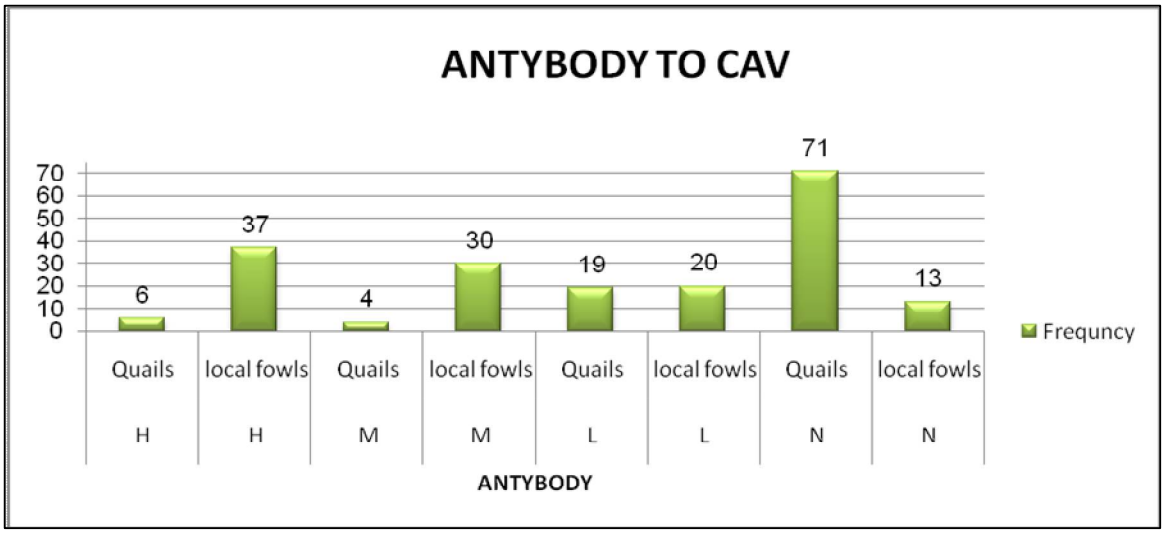
Figure 3. Descriptions of CAV serum positive and negative samples of Japanese quails according to age groups

Table 5.Antibody levels according to S/N ratio in local fowls and Japanese quails

S/N ratio *	Antibody level	Type of Birds		Total
		Local Fowls	Japanese Quails	
Low (0.001 to 0.199)	High	37(86%) 42.5%	6 (14.0%)** 20.7%***	43(100%) 37.1%
Medium (0.200 to 0.399	Moderate	30(88.0%) 34.5%	4 (12.0%) 13.8%	34(100%) 29.3%
High (0.400 to 0.599)	Low	20(51.3%) 23%	19(48.7%) 65.5%	39(100%) 33.6%
Total		87(81%) 100%	29(25%) 100%	116(100%)

*Serum samples with S/N > 0.60 were negative for CAV antibodies, whereas samples showed S/N ≤ 0.60 were positive to CAV antibodies

**Percent among total group of each S/N ration



***Percent among the total group of positive samples

Figure 4.Shows the descriptions of CAV serum positive and negative samples according to bird species. Bars are the number of samples, H, M and L (high, medium and low) levels of CAV antibodies respectively, N (negative) for CAV antibodies.