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Prevalence of Antibodies Against Chicken Infectious Anaemia Virus Among Free-Range Chickens in Northeastern Libya

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ABSTRACT

Chicken Infectious Anaemia Virus (CIAV) has expanded much significance as an immunosuppressive and economically notable emerging aetiology of chickens worldwide. CIAV is the causative agent of Chicken Infectious Anaemia (CIA), an emerging infectious disease mainly noticed in young chicks of 2-4 weeks of age, which usually acquire the infection vertically. The disease is characterised by increased mortality, reduced weight gain, anaemia, aplasia of bone marrow, atrophy of thymus and concomitant marked immunosuppression with enhanced susceptibility to other pathogens and diminished vaccine responses leading to severe economic losses. This research was achieved due to the scarceness of recent facts iabout CIAV in Libya, and it is reported in bordering country near to the study area. For this purpose, in the period of between 3rd and 6th of January 2010, a prevalence study was performed on free-range chickens with collection of 96 serum samples from 15 flocks in northeastern area-Libya, and screened for the presence of CIAV antibodies using indirect enzyme-linked Immunosorbent assay (ELISA) kit. Moreover a structured questionnaire was designed for the purpose of this research and administrated to owners to gather information on demographic and management data. The overall prevalence was 71.87%. The prevalence-within-flock ranged from 60% to 100%. There are age variation in the infection distribution was noted in third breeding interval and significantly lower than other three breeding intervals ($P < 0.05$). The specific antibodies against CIAV in the flocks of Al-Marj suburb were significantly low ($P < 0.05$) compared to the rest suburbs. The findings of the current study show that CIAV is widespread among village chicken populations in the surveyed area. Hence, further studies are required to evaluate the epidemiological effects and yield losses of infection in commercial chickens, and to assess the expenditure profit of prevention procedures.

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INTRODUCTION

Chicken Infectious Anaemia (CIA) is one of emerging viral diseases posing a serious menace to the poultry industry; of which, CIA has assumed significance due to it is immunosuppressive effects. The causative agent was first isolated in Japan by Yuasa and coworkers in 1979. Since then,

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the disease has been demonstrated by serological, virus isolation, and PCR methods in various countries [1], this infectious agent was first named Chicken Anaemia Agent (CAA) and is now known as Chicken Infectious Anaemia Virus (CIAV or CAV). CIAV is thus far the only member of the genus Gyrovirus, which belongs to the family of *Circoviridae* together with viruses of the Circovirus genus [2]. However, the

genome of a second gyrovirus was recently amplified from serum from a diseased chicken in Southern Brazil and The Netherlands. This virus, tentatively named avian gyrovirus 2 (AGV2), has a genome of 2,383 nucleotide with a similar genome organization as CIAV and an nucleotide identity of approximately 40% [3,4]. CIAV is a smallest DNA avian virus (25-26.5 nm), icosahedral, non-enveloped virus having a 2.3 Kbp circular single stranded genome. The genome codes for three viral proteins (VP1, VP2 and VP3) transcribed from single major transcript of 2.0 Kbp size from three overlapping reading frames (ORF1, 2 and 3). It is believed that CIAV genome replicates through rolling circle model [5]. Age resistance develops rapidly during the first week of life and become complete by 3 week or even earlier in immunologically competent birds [6,7]. Furthermore, vertical transmission of the virus is unlikely to occur from antibody-positive hens [1]. CIAV is very hardy, difficult to inactivate thermally or with common disinfectants, which limits the utility of normal sanitization practices.

CIAV is omnipresent and there are serological evidences indicating CIAV presence in most commercial chicken farms, with a high seroprevalence within the chicken flocks [1]. At the same time, the epidemiological situation of CIAV infection in free-range chickens (*Gallus gallus domesticus*) still fundamentally unidentified in different parts globally. Exceptions are small-scale serological surveys like; detecting CIAV-specific antibodies in Nigerian indigenous chickens [8,9], Chinese live-bird markets [10] and in fancy chicken breeds [11]. In addition to serological testing of 115 birds, Oluwayelu and Todd (2008) also demonstrated a presence of CIAV-DNA in sera from Nigerian indigenous chickens by polymerase chain reaction, and showed that they contained a mixed population of CIAV strains with different restriction endonuclease patterns [9].

Extensive farming of free-range chickens, even without significant economical importance is a common practice in a lot of countries. Due to the low levels of biosecurity, however, these birds are commonly exposed to infectious viral, bacterial and fungal organisms through the environment, people and food. Moreover, the close contact between free-range reared chickens with waterfowl and wild bird's populations allows intensive circulation of different avian pathogens, some of which having the potential to overwhelm the host defences and to evolve into highly pathogenic forms; the AI viruses being those of biggest epidemiological and economical importance [12]. This process would be facilitated in immunocompromised birds, in which viruses would replicate more efficiently and shed in large quantities in faeces and respiratory secretions. Thus, the presence of free-range flocks of birds with weakened immune defences may promote the emergence and introduction into the industrially managed poultry farms of new infectious agents or strains with enhanced virulence. Assuming that CIAV seropositivity relates to immunosuppression and that the available data on the occurrence of CIAV in free-range fowl flocks are quite limited.

We designed this serological study to determine the prevalence of chicken infectious anaemia virus in free-range chickens in northeastern Libya, and consider it as an pilot study to initiate further more comprehensive researches to establishing and investigate the epidemiology of CIAV among the commercial breeding chicken populations in countrywide.

MATERIALS AND METHODS

Data and samples collection

From 3 to 6 January 2010, a total of 96 serum samples were gathered from 15 free-range chicken flocks tested for antibodies against CIAV in northeastern area of Libya (located in four suburbs; *Al-Rajma*, *Tukrah*, *Al-Marj* and *Al-Abiar*), with five to seven birds per randomly selected farm. The data which regarding the number of birds, age, previous history of illness, morbidity and mortality from all flocks during this study were recorded. The questionnaire developed for this study to express -as much as possible- the field conditions. The size of flocks varied from 25 to 54 birds. The range of birds age was 1-17 months old. The breeds were mixed (indigenous/ native, exotic breeds, offspring's of commercial fowl strains kept as backyard birds and hybrid- cross varieties of them). All participating flocks were raised for egg production.

Blood samples were collected by venopuncture of the wing vein. The sera separated in a labeled 1.5 ml snap - on lid microtubes, and submitted to Virology & Serology Lab/ The National Centre of Animal Health and Breeders Improvement. The serum samples were frozen at −20 °C until use.

ELISA

A commercial test kit was used to detect specific antibodies against CIAV (BioChek CAV), based on indirect enzyme linked immunosorbent assay, following the instructions of the manufacturer. A serum dilution of 1:500 was used. Optical density (OD) values were read at 405 nm using a Tecan Sunrise ELISA reader. S/P was the ratio of OD of the sample (S) divided by the OD of the positive control (P). Samples with S/P values 0.349 or less were considered negative, whereas these with S/P higher or equal to 0.350 were considered positive.

Data management and statistical analysis

The collected data and the laboratory results were stored in Excel 2007 file (Microsoft) and were used to calculate the mean titer and standard deviation. The SPSS (SPSS for Windows, Version 17.0, Rainbow Technologies) was used for statistical analysis. The one-way ANOVA or Chi-square tests were used to identify a significant difference ($P < 0.05$) in the prevalence rates between the different breeding suburbs, and age intervals.

RESULTS

The clinical observation of the examined flocks not revealed manifest signs suggestive for CIA, although there are history of a few noticeable of particular significant disorders in the performance and the yield of some flocks during their rearing period.

Specific antibodies against CIAV were found positive in 69 (71.87%) of 96 serum samples, with S/P values ranging from 0.356 to 0.604. Evidently, the occurrence of antibodies against CIAV were detected in the all investigated flocks, with seroprevalence rates from 60 to 100%. The prevalence of the specific antibodies against CIAV in free-rang chicken flocks of; *Al-Rajma* suburb was 69.89%, while in *Tukrah* suburb was 80.94%, in *Al-Marj* suburb was 63.68%, and in *Al-Abiar* suburb was 73.09%. ELISA mean titers in the examined flocks of; *Al-Rajma* suburb was 5404.56 ± 3718.51, while in *Tukrah* suburb was 6584.48 ± 4124.17, in *Al-Marj* suburb was 5528.35 ± 4581.54, and in *Al-Abiar* suburb was 6368.28 ± 4057.32 (Table: 1).

Table 1: Prevalence of specific antibodies against CIAV among investigated free-range chickens (*n*=96) in Northeastern Libya

Suburb	Flock ID	Flock size	No. Positive/ total tested serum samples	Positive (%)	Mean Titers	SD
Al-Rajma	FR1	25	4/5	80	5847.66	1988.11
	FR2	46	5/7	71.42	5178.51	1440.63
	FR3	51	5/8	62.5	5446.95	1745.55
	FR4	37	4/6	66.66	5145.14	860.32
	Total	**159**	**18/26**	**69.89**	**5404.56**	**1508.65**
Tukrah	FT1	48	4/6	66.66	7845	2589.44
	FT2	50	5/7	71.42	5788.51	1658.12
	FT3	29	5/5	100	5588.18	1156.75
	FT4	53	6/7	85.71	4556.19	2578.32
	Total	**180**	**20/25**	**80.94**	**5944.47**	**1995.65**
Al-Marj	FM1	31	4/7	57.14	5475.28	3154.19
	FM2	49	5/7	71.42	2451.87	1544.47
	FM3	54	5/8	62.5	3589.25	1179.11
	Total	**134**	**14/22**	**63.68**	**3838.8**	**1959.25**
Al-Abiar	FA1	50	4/6	66.66	1056.85	5481.63
	FA2	29	3/5	60	9855.74	4874.95
	FA3	36	4/5	80	5114.89	2545.09
	FA4	49	6/7	85.71	9459.78	3894.27
	Total	**164**	**17/23**	**73.09**	**6371.81**	**4198.98**
Overall / **18**	**624**	**69/96**	**71.87**	**5389.91**	**2415.63**	

Using one-way ANOVA analysis, the specific antibodies against CIAV in the free-range chicken flocks of *Al-Marj* suburb were significantly low compared to the flocks in other three suburbs.

Prevalence of specific antibodies against CIAV was recorded according to the age of the birds in various intervals (Table: 2).

Table 2: Prevalence of specific antibodies against CIAV in free-range chicken sera according to age intervals

Age (in months)	No. of sera	Positive (%)	Negative (%)
1-4 (*rearing breeding period*)	21	16 (76.19)	5 (23.81)
5-9 (*first breeding interval*)	28	22 (78.57)	13 (21.42)
10-13 (*second breeding interval*)	23	18 (78.26)	4 (21.73)
14-17 (*third breeding interval*)	24	13 (54.16)	11 (45.83)

Chi-square test showed that there was no significant difference between the rearing period, the first and second breeding intervals. In contrast, the prevalence of specific antibodies against CIAV was decreased from 78.26% in the second interval to 54.16% in the third breeding interval.

DISCUSSION

This present study demonstrates occurrence of natural CIAV infection in free-range chicken flocks in study area. Serological surveys have shown that CIAV infection is widespread in industrial chickens [1]. The findings of the present work suggest that this is the case also for the village chickens flocks. Ninety six free-range birds originating from 15 village flocks in northeastern Libya, were screened and 71.78% of them were detected positive to specific antibodies against CIAV. Also, specific antibodies against CIAV were found in birds from 100% of the surveyed flocks with prevalence rates ranging from 60% to 100%**.** The age of birds were intended to exceed 3 weeks old to exclude maternally derived immunity. Consequently, The titres obtained in the present study could only have been acquired from natural horizontal infection. The small differences between the seropositivity rates observed in Nigeria were 88.9% [8] and 66.2% [9], and actually exhibited in Libya may reflect the differences in the environment conditions, management practices and samples size. The high seroprevalence of CIAV among the village chickens was not unexpected, in view of ubiquitous nature of the virus, the high resistance in the environment, origination of birds from different sources and generations in the same flock, a common practice for the most of the free-range farms and popularity of the live bird markets, additionally promote intensive virus circulation and facilitate horizontal transmission of CIAV. Moreover, vertical transmission, which is an important way of viral spread at the conditions of industrial poultry farming due to the ability of CIAV to persist in the ovaries of laying hens even at a presence of circulating antibodies [13,14], also may play a role in CIAV epidemiology in the village fowls in the cases, where chickens are hatched by brood hens or in hatchery from eggs, collected from infected breeders.

Outbreaks of clinically manifested CIAV infection in the free-range chickens have not been reported so far. Seemingly, single bird with low levels of maternal antibodies may develop clinical disease, but it is not recognized as such. Neither detectable clinical manifestations of infectious anaemia, nor other associated clinical signs, such as gangrenous dermatitis [15] were observed in the households investigated. Thus, the high percentage of seropositivity indicates mostly subclinical infection in sampled birds, since vaccination against CIAV is not embarked in Libyan chicken populations.

Serological surveys in broiler breeder flocks have shown an age dependence of seroprevalence, which increase gradually and reach levels of almost 100% at or near sexual maturity [16]. Whereas, seropositivity to CIAV does not essentially relate to obvious signs, it shows that birds have undergone at least subclinical infection with possible subsequent immunosuppressive effects such as increased tendency to other microbial infections, or suboptimal response to vaccination.

When the poultry owner have well knowledge with awareness in sanitary rules, as improved hygiene practices and the attention paid to the management that could reduce seroconversion rates, and may possibly lead to prevent immunosuppression which can caused by environmental factors or other causative agents and capable to diminish exposure to CIAV infection. This may interpret the lower prevalence of specific CIAV-antibodies among examined flocks in the *Al-Marj* suburb comparing to the flocks in other three breeding suburbs.

Since, it is a regular practice the new free-range flocks to be replaced/ spiking, or present ones completed with new birds through months of March- April, and then the majority of birds at the time of sampling should be at least 8-9 months old. In the current study, the prevalence of antibodies in different age intervals of birds was evaluated. We found that flocks in the rearing period (1-4 months) had a prevalence of antibody positive chickens not significantly different from the first and second age intervals, what indicates they were naturally infected. Moreover, in the third age interval there was a significantly higher percentage of birds (45.83%) that were negative for specific antibodies against CIAV. It may be

that some of the anti-CIAV antibody positive birds became anti-CIAV antibody negative during the last breeding period due to the long elapsed time since the last antigenic stimulation [13,17,18]. Free-range poultry are important for countryside communities in Libya, in aspect of their socioeconomic value. As well as their stamina to tolerate drastic ecological circumstances. One of the marginal principles of the existing study is to provide particular knowledge about the pathogens that infects the village chickens in Libya. A better understanding of the epidemiology of CIAV infection will allow veterinary authorities to planning and devising suitable prevention strategies.

CONCLUSION

This research implies that extensive prevalence of CIAV antibodies in examined free-range chicken flocks. Therefore, in view of it is widespread occurrence, and apparently circulates CIAV infection must be considering as a hazard element linked to the epizootiological picture of village chicken flocks in Libya. Furthermore, there are numerous industrial chicken herds adjacent to the investigated free-range flocks. Infected village fowls represent a latent threat in spread of the virus to their analogous commercial flocks resulting in profitable influences for chickens industry. The current study can be take into account as an fundamental, provide an amount of information and basis for future studies to genetic characterize of the virus from Libyan chicken populations are therefore required. We also advise to constant monitoring of this virus in the field for emergence of any new variants and consequent change in pathogenicity.

DISCLOSURE STATEMENT

The authors declare that there is no conflict of interest.

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