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ORIGINAL ARTICLE

Efficacy of chicken anemia vaccine in broiler parent stock

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Abstract

Background: Chicken anemia virus (CAV) is an important poultry pathogen, which causes immunosuppression and varying levels of mortality. Poultry production is a major livelihood for the people in Bangladesh. The broiler parent stock of Bangladesh using vaccine against CAV but the efficacy of this vaccine against CAV is not well understood. The present study highlights the vaccine efficacy of CAV and maternal transfer of antibodies to the hatched chicks.

Methods: Total 7 broiler parent stock (Cobb 500) farms were selected from 7 districts of Bangladesh. Vaccines against CAV administer single time at 80 days of age by live Nobilis® CAV P4 vaccine. Total 516 blood samples were collected in 6 times (at 0, 17, 25, 33, 41 and 49 weeks) from each farm. Then again 143 blood samples were collected from next generation broiler chicks of corresponding broiler parent stock farms at 0, 15 and 30 days. There was no CAV vaccine used in this broiler. Test methods were indirect ELISA test for the detection of blood antibody level against CAV by commercially available kits.

Results: No adverse reactions were observed in any of the birds during the course of the study. Our results suggest that the CAV antibody starts decreasing 10 weeks post vaccination. Moreover, a substantial maternal antibody titer has been observed in all groups of chicken hatched out from the earlier vaccinated birds which is sufficient to protect up to first 30 days of life.

Conclusions: The antibody titer against CAV become declined after 10 weeks of post vaccination to broiler parent stock and maternally derived antibody can protect chicks until 30 days of live. The study reports the efficacy of vaccination against CAV in Bangladesh and its possible implications in further optimizing the strategy for its vaccination.

Key words: Chicken anemia virus; Vaccine; Pathogenicity; Immunosuppression; Antibody

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Introduction

Chicken anemia virus (CAV) is an economically important poultry pathogen. It was initially classified in the family *Circoviridae* but due to its sequence divergence it has now been classified in family *Anelloviridae* and genus *Gyrovirus* (Rosario *et al*., 2017). The virion contains a small single-stranded, negative sense, circular DNA of approximate size 2.3kb. The virus produces three major proteins VP1, VP2 and VP3. VP1 (51.6 kDa) is the most abundant protein and an integral part of the virion capsid while VP2 (24 kDa) is a non-structural protein having phosphatase activity and also helps in virion assembly (Peters *et al*., 2002). VP3 (13.6 kDa) is the smallest protein of the virion and capable of inducing apoptosis in transformed cells (Noteborn *et al*., 1991).

CAV infection in chicken causes aplastic anemia leading to the deficiency of all major blood cell types. Due to lymphoid atrophy and immunosuppression, the occurrence of secondary bacterial, viral and parasitic infections are often high. During severe condition, the mortality and morbidity rates increase up to 55% and 80%, respectively (Schat, 2009). Clinical symptoms comprise of pale comb and wattle, loss of appetite, lethargy followed by dullness and depression. The virus spreads mainly by vertical transmission but horizontal transmission also contributes to its epidemiology. The infection occurs in varied age groups but clinical signs are predominantly found in young chickens due to vertical transmission. Vertical transmission of maternal antibodies from hens to chicks against certain pathogens provides a crucial mean of protection up to a certain age (Ali, 2018; Gharaibeh *et al.*, 2008). Also, the level of these inherited antibodies is of major importance when serology for disease diagnosis is considered (Sharma, 2003). Sero-negative hens get infected during egg production. Newborn chicks positive for CAV specific maternal antibodies show reactivity against the virus (Hoop, 1992). Older chicks (>14 days) get infected horizontally by feco-oral route. The subclinical form of the disease is found in birds of two week or more age due to horizontal transmission (Sommer and Cardona, 2003).

The first isolate of CAV was reported in Japan in the year 1979 (Yuasa *et al*., 1979). Subsequently, the virus was reported from various regions worldwide (AboElkhair *et al*., 2014; Bhatt *et al*., 2011; De Herdt *et al*., 2001; Ledesma *et al*., 2001; Olszewska-Tomczyk *et al*., 2015; Zhou *et al*., 1996). In developing regions including Southeast Asia (SEA), traditional, small-scale, extensive backyard poultry is in practice. Due to unconfined poultry rearing, disease control is very difficult and thus the virus is thought to be omnipresent in SEA. In a study in Cambodia, out of 33 spleen samples collected from dead village chickens from the backyard farms, three CAV were classified as genotype II (Han *et al.,* 2018). In South Korea, 32 sequences of CAV from various flocks of the breeder and commercial chickens were genetically characterized and segregated into groups II, IIIa, and IIIb. Besides these, seven CAV genomes that were similar to vaccine strains (26P4 strain) have also been reported (Kim *et al.*, 2010). Some of the recent CAV isolates were reported from China, India and Iran (Ganar *et al*., 2017; Kaffashi *et al*., 2017; Li *et al*., 2017). In Bangladesh, the first CAV isolate was reported in the year 2002 (Islam *et al*., 2002). The mortality and morbidity caused by CAV infection lead to huge economic losses to the broiler industry (Sommer. and Cardona, 2003). Specific pathogen-free eggs used to study poultry viruses are affected due to vertical transmission of the virus (Miller *et al*., 2003). Infected backyard chickens have a direct impact on the economy of the farmer. Developing countries are at the major risk as poultry farming is the primary occupation in the rural areas.

The low titer in cell culture and embryonated egg makes it difficult to develop a vaccine against CAV. Recombinant viruses, subunit vaccines and DNA vaccines act as a potential preventive measure against CAV infections (Moeini *et al*., 2011; Sawant *et al*., 2015; Shen *et al*., 2015). Failure in appropriate storage condition and mishandling during transport also decrease the efficiency of veterinary vaccines (Kumar, 2015; Kumar and Koul, 2016). The persistent occurrence of CAV outbreaks is mainly due to vaccine failure. Lack of knowledge on the epidemiology of CAV from developing countries

misleads the vaccine implementing strategy. Initially only one serotype was proposed based on cross neutralization test (Yuasa and Imai, 1986). However, a possible second serotype has been suggested, alarming the urgency for rapid vaccine development (Spackman *et al.*, 2002).

Materials and methods

The present study has been carried out to understand the existing scenario of CAV and its seroprevalence in the broilers in Bangladesh. A total of seven broiler parent stock farms were selected based on availability of broiler parent stock farm from seven districts (Panchagar, Rangpur, Bogura, Joypurhat, Gazipur, Mymensingh, and Chattogram) stretching from north to south-east part of Bangladesh with approximately 4,000 birds each (Figure 1). The broiler parent stocks were vaccinated single time with live Nobilis® CAV P4 (MSD Animal Health) by intramuscular route at 80 days of age and sample collection was done at 0, 6th weeks and after that 8 weeks interval for next 4 times (at 14, 22, 30 and 38 weeks). An average of 15 samples was collected per farm at each time point. In the second phase, broiler-chicks hatched (unvaccinated) from eggs of 49th weeks old corresponding broiler breeders were selected for sample collection three times at 15 days interval (at 0, 15 and 30 days), with an average seven samples collected each time from each broiler-chicks farm. The antibody titer was assessed using a commercially available indirect ELISA test (BioChek, Netherlands).

All the birds were serologically analyzed for CAV antibodies on day zero (11 weeks post hatching) both for experimental and control birds. The significant CAV antibody titer on day zero post-vaccination was observed in all the birds including the control birds (Figure 2).

Results and Discussion

The birds in farm 3 showed the highest titer of >2500 on week six followed by birds in farm 5 and 4. The birds in farm 1 and 7 showed the similar CAV antibody titer on week 6 post-vaccination. Lowest CAV antibody titer was observed in farm 2 at 6 weeks post-vaccination. At 14 weeks post vaccination, the birds in farm 3, 4 and 5 have the CAV antibody titer higher (>1000) while those in farm 1, 2, 6 and 7 have lower titer (<1000). In all the farms, CAV antibody titer has increased from 22 weeks onwards.

With regards to the maternal CAV antibodies, at zero day, all the farms exhibit CAV antibody titer above 500, in fact, three farms have CAV antibody titer higher than 1500 (Figure 3). The birds in farm 5 showed the highest CAV antibody titer followed by farms 3 and 2. At 15 day, the antibody titer though decreased about half the initial value but still higher than 500. All farms at 30 days, showed a decline in the CAV antibody repertoire. The control farm did not show any indication of maternal CAV antibodies. No adverse reaction was observed in any of the birds during the course of the study. A substantial maternal antibody titer has been observed in all groups of chicken hatched out from the earlier vaccinated birds.

Bangladesh with a total population of around 163.05 million (1115 people per km^2) has the highest population density in the world. Poultry fulfill half of the total meat demand of the country which requires an estimated total of 338 million poultry population. About 1.4 million people, who are substantially involved in poultry production abode in the rural area. The CAV has emerged as a threat to the poultry population in Bangladesh. The study related to the CAV in the broiler industries has not been done. The present work reports the first comprehensive CAV vaccine related study from seven different locations in Bangladesh. Regular CAV outbreaks have been reported from different parts of Bangladesh (our unpublished data). The serum of all the parent birds showed antibodies against CAV even on day zero, which could be due to natural environmental exposure of these birds to the virus (Biđin *et al.*, 2010). Our results showed the high titer of antibody in the parent stock from 22 weeks post vaccination could be because of concurrent CAV infection. It has been shown that the CAV could infect the flock when the antibody concentration is going below a threshold level or in case of a novel variant against which the antibody is not responsive (De Herdt *et al.*, 2001).

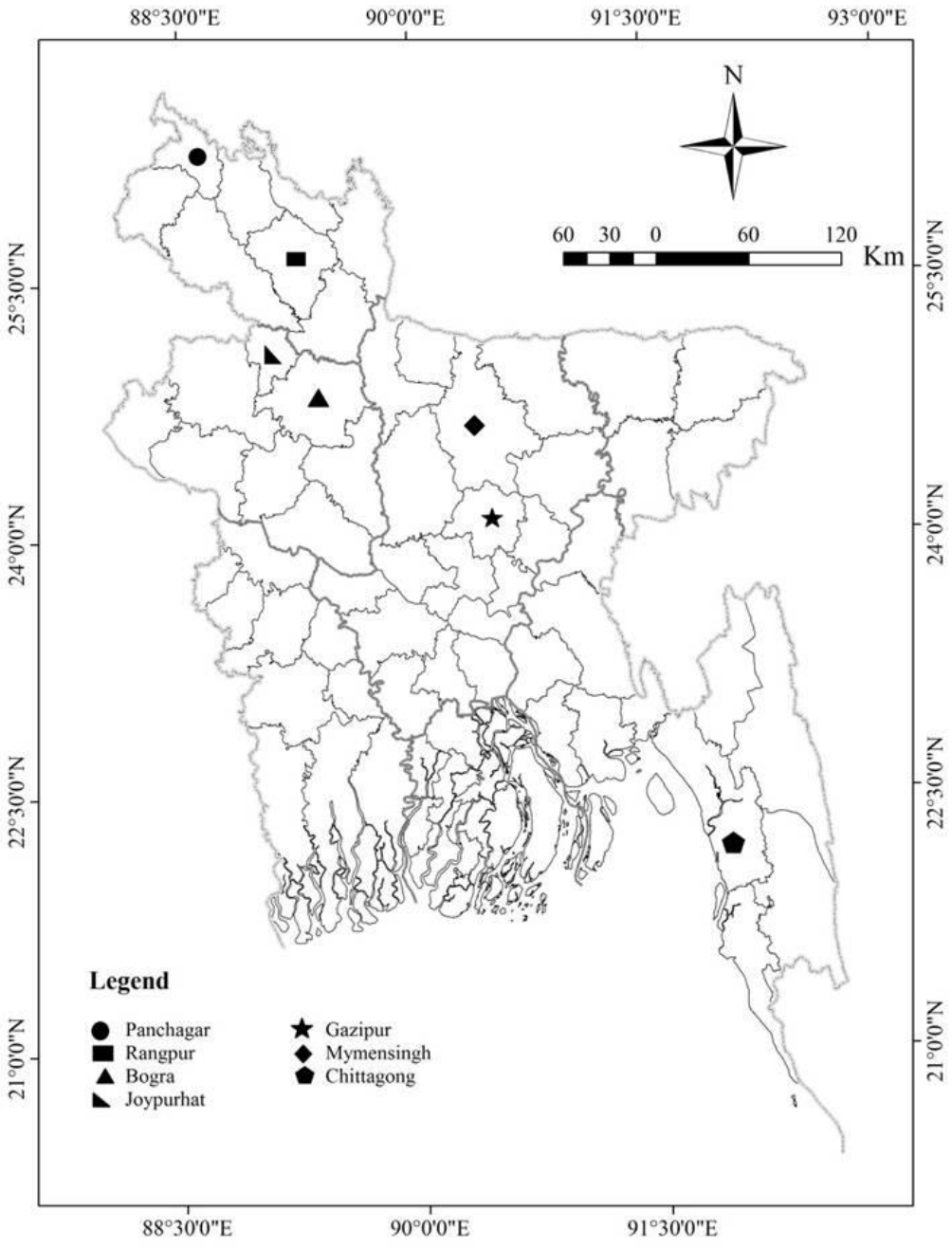

Figure 1. Different regions in Bangladesh used in the study

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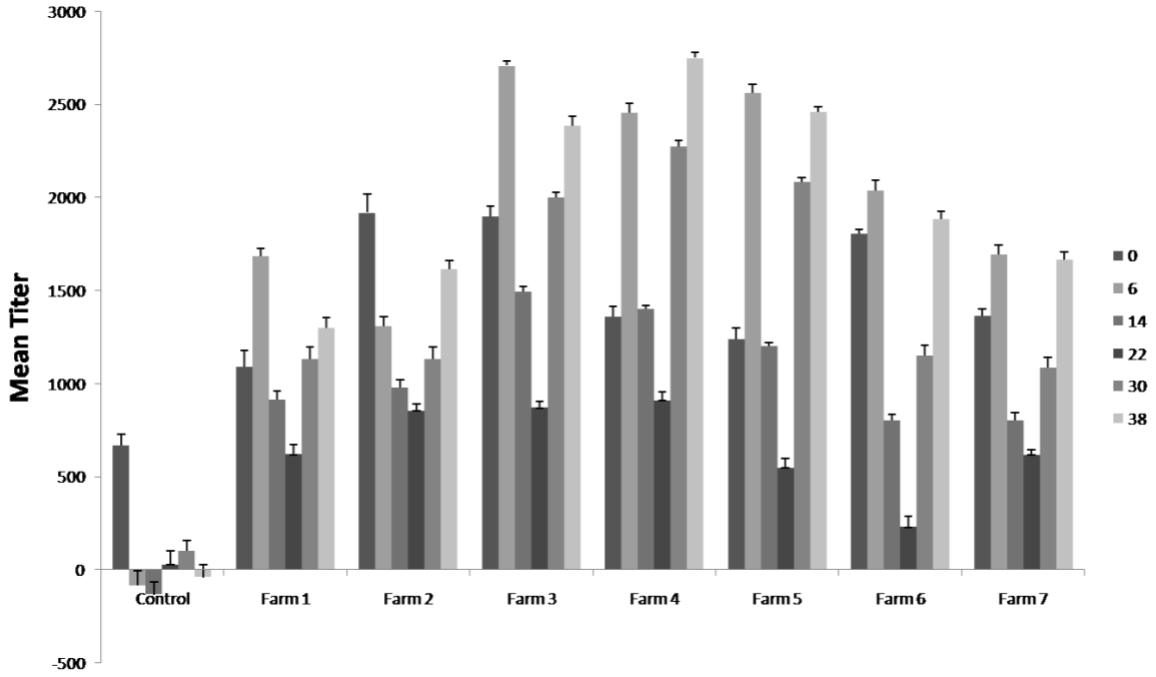

Figure 2. Mean antibody titer of the serum samples collected from the parent stock at different time interval post vaccination.

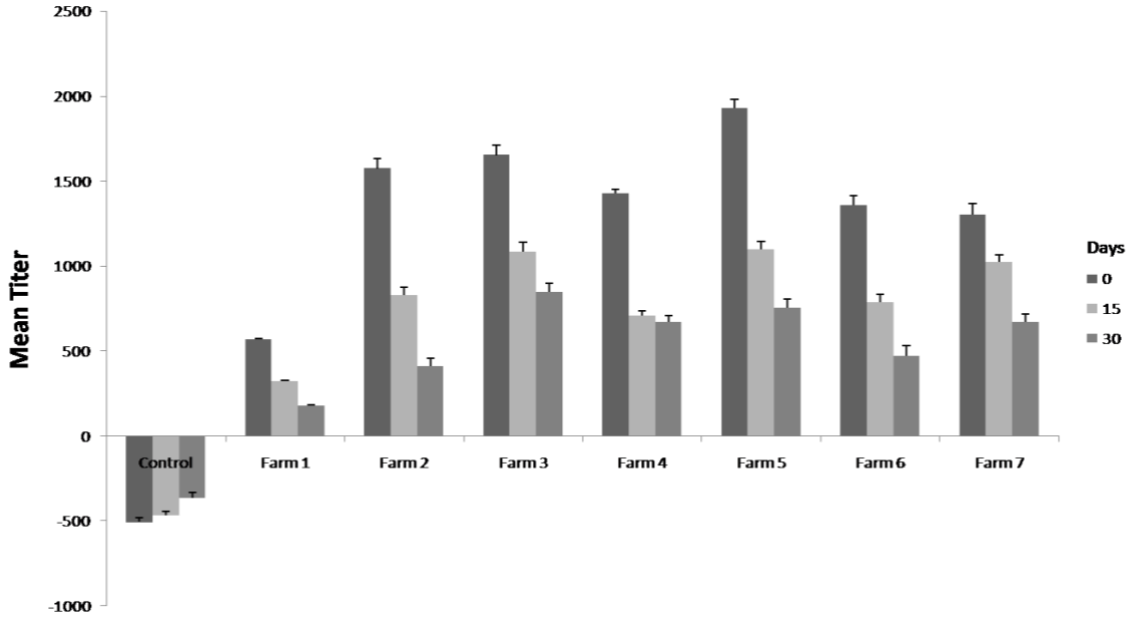

Figure 3. Mean antibody titer of the serum samples collected from the hatched stock at different time interval post vaccination.

Maternal antibodies to CAV usually confer complete protection against the disease and the industry practice to ensure the protection of chicks from CAV infection is usually done through vaccinating or exposing the breeders before lay (Ali and Hasan, 2018; Gharaibeh *et al.*, 2008). Chicks vaccinated while having high levels of maternal antibodies resulted in vaccine failure, due to neutralization of the live vaccine and level of maternal antibodies plays a role in determining the level of response in chicks to early vaccination (Ali *et al.*, 2019; Al-Natour *et al.*, 2004; Mondal and Naqi, 2001). Our results of maternally transferred CAV antibody titer in hatched chicks suggests that this could be sufficient to protect the birds to initial 30 days of their life. Although, the titer was visible in the assay, its protective efficacy requires a challenge experiment. Moreover, the kinetics of antibody titer suggests that the antibody titer would go down after 10 weeks post infection. Perhaps, the titer of the antibody should require validation in terms of CAV neutralization ability.

Conclusions

The study reports the efficacy of CAV vaccination in Bangladesh and its possible implications in further optimizing the strategy for its vaccination. Furthermore, study also insights about the probable role of serological titers when comes to diagnosis of CAV in field condition. It is concluded that the kinetics of antibody titer become declined after 10 weeks of post vaccination to broiler parent stock against CAV. In case of maternally derived antibody, the chicks can protect with significant antibody titer until 30 days of age. The study will pave a way to understand the less explored poultry epidemiology in Bangladesh.

Conflict of interest

The authors declare no conflict of interest.

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