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EVALUATION OF THE IMMUNE RESPONSE OF SOME CHICKEN FARMS TO INFECTIOUS BRONCHITIS VIRUS IN AL-AHSA PROVINCE IN SAUDI ARABIA

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ABSTRACT

Infectious bronchitis virus (IBV) still to pose great economic losses among chickens of all ages in many parts in the world. Despite the massive application of IBV vaccines in many countries including Saudi Arabia, several outbreaks are still frequently reported. One of the important challenges that hampered the selection of the right vaccine candidates is the identification of the local circulating strain in a certain area. IBV is recently reported in many chickens flocks in the Eastern region of the Kingdom. The major goals of our study were to evaluate the immune status of some chicken farms in Al-Ahsa to IBV. To achieve this goal, total of (368) serum samples-from chicken farms from Al-Ahsa region were collected. Our results showing high seroprevelance of IBV in the non-IBV vaccinated chicken farms suggesting their exposure to the natural IBV infection. Meanwhile, we were able to detect a high IBV antibody titers in vaccinated chicken farms suggesting the used vaccines induced detectable IBV antibodies. This suggesting the circulation of novel IBV strains in these chicken farms. The overall results confirm the circulating of novel IBV strains in chicken farms in Al-Ahsa region. Further studies are needed to do molecular characterization of these IBV strains

Key words: Infectious bronchitis virus, ELISA, Al-Ahsa, broiler, baladi chickens

INTRODUCTION

Infectious bronchitis virus (IBV) is a highly contagious respiratory viral disease of chickens of all ages. The IBV belongs to the family Coronaviridae (Hackney *et al.*, 2003, Mardani *et al.*, 2006). The IBV infection usually causes high economic losses among poultry industry. It is quite possible to become an endemic in the chicken industry in some regions of the world. The virus has wide tissue tropism including the respiratory, digestive, renal, and reproductive systems of the affected birds. It may affect the oviduct and lead to low production and low quality eggs, or may causes severe renal complications and mortality among the affected birds (Ignjatovic *et al.*, 2006). The IBV infected birds usually shed the virus in their body secretions such as respiratory and the gastrointestinal tract secretions. These birds may remain active shedders of the virus for up to several weeks post infection (Jahantigh *et al.*, 2013). Secondary bacterial infections (Escherichia coli (E. Coli) and Mycoplasma gallisepticum) always exaggerates the viral pathogenesis and auscultates the mortality rates among the infected chicken population (Ji *et al.*, 2011). The IBV infections continues to be a major problem to poultry industry worldwide. In spite of the availability of several IBV vaccines, the virus continues to cause many outbreaks among the chicken farms in both broiler and layer settings (Dhama *et al.*, 2014).

Efforts to control spreading of the IBV infections through vaccination resulted in some variable outcomes. Many IBV strains and serotypes have been emerged since its discovery more than 80 years ago; meanwhile, the misuse of the IBV vaccines complicates the evolution and emergence of new IBV strains (Mase M, 2008). One possible explanation for the emergence of new IBV strains is the poor proof reading capability of the viral RNA polymerase. This resulted in high mutation rates alongside the viral genome. This leads to the emergence of new IBV strains every once in a while (Dolz *et al.*, 2008, Jackwood *et al.*, 2012). Many IBV vaccines are commercially available including inactivated, live attenuated, recombinant etc. The live attenuated vaccines are the most commonly used in all types of poultry; these provide good immune response however, there is a possibility of revert to virulence. Meanwhile, the inactivated vaccines are usually given to layers and breeders before laying as booster vaccines (Jordan, 2017, van Beurden *et al.*, 2018).

Several IBV variants and genotypes are currently circulating in the Middle East, Asia and North Africa such as Iraq, Egypt, Libya, Iran, and Jordan (Zanaty *et al.*, 2016). Furthermore, many outbreaks have been

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reported in Saudi Arabia especially in the central region of the country (Ababneh *et al.*, 2012, Awad *et al.*, 2014, Selim *et al.*, 2013). However, the through molecular characterization of these IBV strains and variants are not well reported yet. The main goal of the current study was to evaluate the immune status of some chicken farms either IBV vaccinated or non-vaccinated using the commercial ELISA kits.

MATERIALS AND METHODS

Processing of serum samples

A total of 368 sera from broilers (184) and layers (184) were collected from seven chicken farms (Table 1). Blood samples were collected randomly prior to each exposure. Specimens were placed for 12 hours at room temperature and centrifuged at 5000 RPM for 10 min. The serum was aspirated by micropipettes. Heat inactivation of sera at 56C for 30 min was done in a water bath to inactivate the non-specific inhibitors. The separated sera were and stored at -80°C for further testing (Praveen & Narasimha, 2016, Hemida *et al.*, 2017).

Enzyme-linked immunosorbent assay (ELISA)

Evaluation of the IBV antibody titters from birds was done by using commercial available total antibody ELISA (IDVet Screen Infectious Bronchitis Virus Competition, insert 0913, lot 555). ELISA procedures were done as per the kits instructions with minor modifications. Simply, each sample was tested in triplicate. Specimen-to-positive ratios (S/P ratios) was calculated as the percent inhibition as per the following formula.

$$(PI\%) = \frac{OD(NC) - OD\ (sample)}{OD\ (NC)} \times 100$$ (NC: Negative Control, OD: optical density).

The optical density of samples presenting a PI% greater than 40% were considered positive while the OD of samples between 30-40% were considered doubtful, However, the OD of samples less than 30% were considered negative. Individual serum titres were calculated from these S/P ratios, evaluated as positive or negative, and expressed as OD 450 nm values according to the manufacturer's instructions. Allow all reagents to come to room temperature before use.

Table 1: Summary of the collected specimens and their geographical distribution 2014-2016.

No.	Type	Collection age / Date	Clinical signs	Type of vaccine	No. of serum	Organs	Farm No.
1	B	35 days / 11/2014	APN	-IBV (H120) & NDV, at 0 days -NDV at 10 days -IBV at 16 days	92	NA	1
2	B	38 days / 11/2014	APN	-IBV (H120) &ND at 0 days -NDV at 10 days -IBV at 16 days	92	NA	2
3	L	150 days / 11/2014	APN	-IBV (H120) and ND at 7 days -IBDV at 15 days -NDV (LaSota strain) & IBDV at 19 days -NDV (LaSota strain) at 30 days -AI and NDV at 38 days -IBV (H120) at 42 days -Fowlpox at 52 days - AI & NDV at70 days -IBV (H120) and NDV at 100 days -NDV at 120 days	92	NA	6
4	L	180-210 days / 1/2015	RS	NV	13	Trachea, lung and kidney	7
5	L	210-240 days / 1/2015	RS	NV	41	Trachea, lung and kidney	5
6	L	180-210 days / 1/2015	RS	NV	24	Trachea, lung and kidney	3
7	L	270-300 days / 2/2015	APN	NV	14	Trachea, lung and kidney	6
		Total			**368**	**276**	**6**

B= broilers, L= layers, APN= apparently normal, RS= respiratory signs, IBV=infectious bronchitis virus, AI=avian influenza, IBDV= infectious bursal diseases virus, NDV= newcastle disease virus, VTC= veterinary training centre, NV= non vaccinated (did not receive any vaccine for viral diseases).

RESULTS

Seroprevalence of IBV in some chicken farms in Al-Hasa

A total of 368 chicken serum samples were tested for the presence of IBV antibodies. Specimens were collected from chicken farms on six regions in Al-Hasa. Our results are showing that 23/92 (25%) non-vaccinated chicken sera and 257/276 (93.12%) (Figure. 5), the vaccinated sera were positive for IBV antibodies (Figure. 6). However, seven samples out of 13 tested from non-vaccinated chicken in Farm No.7 were positive (53, 85%).Our results are showing that 8/41 (19.51%) in farm No.5 and test 6/24 (25%) in Farm No.3 sera were positive. However, two samples from chickens in Veterinary Training Centre was IBV antibodies positive out of 14 tested samples (14.29%) non-vaccinated. In contrast, the antibody IBV ELISA reported much higher 90/92 (93,83%) in farm No.1 and same in farm No.2 result was 86/92 (93.48%) chicken vaccinated. We test 81/92 (88.04%) chicken vaccinated were positive from the Veterinary Training Centre (Table 2).

Table 2: Summary of the seroprevalence of IBV in some chicken farms in Al-Hasa.

No.	Type of chicken	Collection of age	Type of vaccine	No. of serum	No. of		No. of		Farm No.
					+	%	-	%	
1	B	35 days	-IBV (H120) & NDV, at 0 days -NDV at 10 days -IBV at 16 days	92	90	97.83 %	2	2.17 %	1
2	B	38 days	-IBV (H120) &ND at 0 days -NDV at 10 days -IBV at 16 days	92	86	93.48 %	6	6.52 %	2
3	L	150 days	-IBV (H120) and ND at 7 days -IBDV at 15 days -NDV (LaSota strain) & IBDV at 19 days -NDV (LaSota strain) at 30 days -AI and NDV at 38 days -IBV (H120) at 42 days -Fowlpox at 52 days - AI & NDV at70 days -IBV (H120) and NDV at 100 days -NDV at 120 days	92	81	88.04 %	11	11.96 %	6
4	L	180-210 days	NV	13	7	53.85 %	6	46.15 %	7
5	L	210-240 days	NV	41	8	19.51 %	33	80.49 %	5
6	L	180-210 days	NV	24	6	25 %	18	75 %	3
7	L	270-300 days	NV	14	2	14.29 %	12	85.71 %	6
			Total	368	280	76.09 %	88	23.91 %	6

B= broilers, L= layers, APN= apparently normal, RS= respiratory signs, IBV=infectious bronchitis virus, AI=avian influenza, IBDV= infectious bursal diseases virus, NDV= Newcastle disease virus, VTC= veterinary training centre, NV= non vaccinated (did not receive any vaccine for viral diseases).

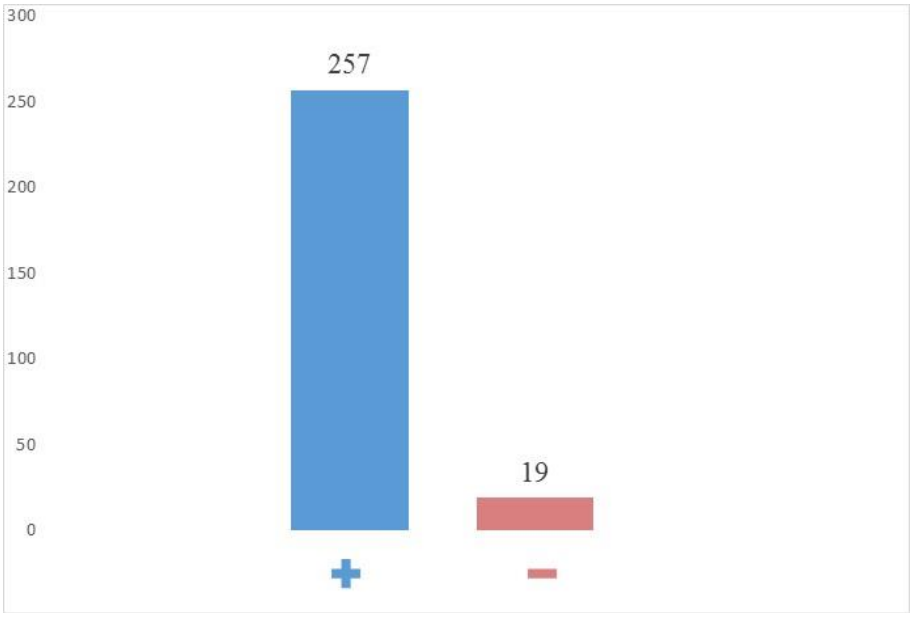

Figure 1: ELISA results of some vaccinated chicken farms in Al-Hasa.

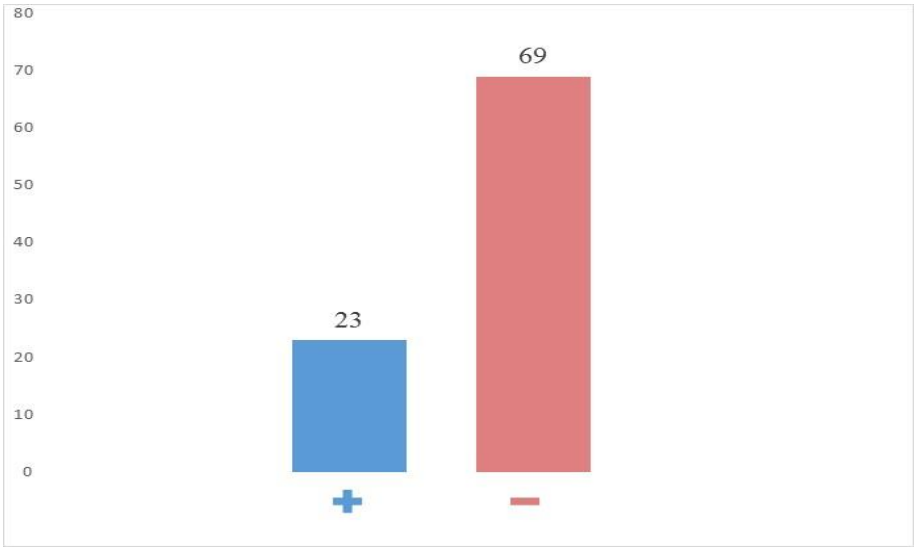

Figure 2: ELISA results of some non-vaccinated chicken farms in Al-Hasa.

DISCUSSION

Infectious bronchitis virus (IBV) is one of the major viral threats of poultry industry worldwide. There are large number of IBV genotypes identified in many parts around the globe (Khataby *et al.*, 2016, Fellahi *et al.*, 2016). IBV is one of the family *Coronaviridae.* The IBV viral genome is believed to mutate at a high frequency due to the poor proof reading capabilities of its viral RNA polymerases. Both mutations and recombinations provides ample genetic diversity among the IBV population throughout the world (Jackwood *et al.*, 1992, Najafi *et al.*, 2015, Xu *et al.*, 2016a). Although IBV vaccination is a common practice throughout most poultry farms across the globe, many IBV outbreaks still reported in commercial poultry farms. This resulted in high economic losses to poultry raisers and industry (Mohajer Shojai *et al.*, 2016, Sun & Liu, 2016). Isolation of many IBV strains and antigenic variants from vaccinated commercial chickens was reported in many cases frequently (Xu *et al.*, 2016b). Recombination occurs among different IBV genes and was reported so frequently especially within the S gene (Promkuntod *et al.*, 2015, Makhija & Kumar, 2015). The N protein plays important roles in the pathogenesis and replication cycle of most coronaviruses. The N protein has many conserved motifs showing similarity of more than 94% among different coronaviruses (Sapats *et al.*, 1996). The N protein is an important diagnostic marker which have been used for diagnostic purposes of many coronaviruses including IBV for long time by the RT-PCR technique. Meanwhile, it is most frequently used

to study the evolution of IBV strains and genotypes (Chang *et al.*, 2016). The live IBV attenuated vaccines are effective tools for controlling IBV when used properly. Improper IBV vaccine distribution within chicken flocks and insufficient vaccine dosage favors the reversion of IBV vaccines to virulence. In some cases using the IBV live vaccine in chickens may increase the susceptibility of birds to be infected with the virus rather than protected from its field infection as planned. (Lim *et al.*, 2015, Reddy *et al.*, 2016, Seger *et al.*, 2016). A serosurveillance study was conducted among some IBV vaccinated and non-vaccinated chickens in some chicken farms across Al-Ahsa region. A total of 368 including 276 IBV vaccinated and 92 non-IBV vaccinated chicken sera were collected. Sera were tested for the presence of IBV antibodies by the commercial available ELISA kits. Our date is showing that 257/276 (93.12%) (Figure.1) vaccinated chicken were positive while 23/92 (25%) non-vaccinated sera were positive for IBV (Figure.2). Presence of IBV antibodies in vaccinated chicken sera indicating the IBV vaccines were able to induce antibodies against the IBV antigen. In contrast to the detection of IBV antibodies in non-vaccinated chicken sera indicating that, those birds were exposed to a recent IBV natural infection.

CONCLUSIONS

Our results confirmed the detection of specific antibodies against IBV in non-vaccinated chickens in Al-Ahsa province. This suggesting those chickens were exposed to an IBV natural infection.

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COMPETING OF INTEREST

The author declare there is no competing of interest exist

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تقييم الاستجابة المناعية لفيروس التهاب الشعب الهوائية في بعض قطعان الدواجن بمحافظة الاحساء بالمملكة العربية السعودية

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يصنف فيروس التهاب الشعب الهوائية من أهم مسببات الأمراض المعدية والذي يصيب الجهاز التنفسي في مختلف الاعمار في الدواجن. كما يسبب هذا الفيروس إصابات شديدة للجهاز التناسلي للدجاج البياض مما يؤدى الى انخفاض شديد في معدلات إنتاج البيض بين القطعان المصابة. بالإضافة الى أن بعض العترات من الفيروس تسبب إصابات بالغة للكليتين في الدواجن المصابة مما قد يؤدي الى نفوق العديد من الطيور المصابة بهذه العترات. ينتمي فيروس التهاب الشعب الهوائية الى عائلة الفيروسات التاجية (كورونا) وقد أثبتت أكثر من دراسة سابقة وجود إصابات بفيروس التهاب الشعب الهوائية في قطعان الدواجن بالمملكة العربية السعودية. على الرغم من وجود العديد من اللقاحات ضد هذا الفيروس الا أنه مازال متفشي في العديد من مزارع الدواجن في مناطق مختلفة من المملكة وخاصة في الأحساء. تم تصميم هذه الدراسة لتقييم الاستجابة المناعية لبعض قطعان الدواجن بالأحساء لهذا الفيروس ولتحقيق هذه الاهداف، تم اختبار عدد ٤٦٨ عينة من بعض مزارع الدواجن بالأحساء من نوفمبر ٢٠١٤ إلى أبريل ٢٠١٦. وقد شملت هذه العينات عدد ٣٦٨ سيرمات اختبر منها عدد ٢٧٦ عينة من السيرم لبعض الدواجن المحصنة ضد فيروس التهاب الشعب الهوائية وكذلك عدد ٩٢ عينة لدواجن غير محصنة ضد الفيروس والتي تم جمعها من ستة مزارع للكشف عن الأجسام المضادة للفيروس باستخدام اختبار الاليزا. وقد كانت النتيجة على النحو التالي لعينات السيرم وجدت اجسام مضادة للفيروس في ٢٧٦/٢٥٧ (٩٣,١٢٪) في الدواجن المحصنة بينما كانت النتيجة للدواجن الغير محصنة ٩٢/٢٣ (٢٥٪). توصى الدراسة الحالية بضرورة إجراء المزيد من الدراسات المستقبلية لعمل المزيد من التوصيف البيولوجي الجزيئي الكامل لعترات هذا الفيروس مع استمرار إجراء المزيد من المسوحات التي تعتمد على البيولوجيا الجزيئية للفيروس. كما توصي بالاعتماد على التقنيات البيولوجية والسيرولوجية الحديثة في إجراء التحصينات الدورية لمزارع الدواجن ضد فيروس التهاب الشعب الهوائي.