Evaluation of the Antibody Response of Two Local Saudi Lines and Commercial Chickens Vaccinated against Newcastle Diseases Virus and Infectious Bursal Disease Virus

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ABSTRACT

Two lines of local Saudi chicken, Hajar-l and Hajar-2, were developed by researchers in King Faisal University. Their physiological and immunological performances are still under investigation. The aim of this study is to evaluate the immunological response of vaccinated and non-vaccinated chicks against Newcastle and infectious bursal diseases in the Saudi lines compared with commercial line. One hundred-eighty one-day-old chicks were divided into three equal groups; Hajarl, Hajar2 and commercial Hisex. Each group was further subdivided into two equal subgroups, vaccinated and non-vaccinated. Blood samples were collected from all chicks at weekly interval starting from the second week of age for four weeks. Indirect enzyme-linked immunosorbent assay (ELISA) technique was used to assess the antibody titer against Newcastle and Infectious bursal disease viruses in addition to measuring weekly body weight.

The results of this study showed that both local Saudi chicken lines had the same body weight gain like the commercial one ($p > 0.05$). Concerning vaccinated groups, Hisex chicken breed showed significant higher antibody titer ($p < 0.05$) against Newcastle and Infectious bursal disease viruses in the second and third weeks samples compared to Hajar-1 and Hajar-2. However, Non-vaccinated local Saudi lines Hajar-1 and Hajar-2 showed better immunity at four weeks old against the two major viral threads compared to the commercial line (Hisex).

It could be concluded that non-vaccinated Hajar-1 and Hajar-2 lines high immunity at older age indicates the potential breeding advantage of these lines as possible source of immunity under non- vaccination condition.

Key Words: Hajar-1; Hajar-2; Hisex; Infectious Bursal Disease Virus; Newcastle Diseases virus.

INTRODUCTION

Two lines of local Saudi chicken, Hajar-l and Hajar-2, were developed, at Animal and Fish Production Department, College of Agriculture and Food Science at King Faisal University (Ahmed and Al-Abbad, 2014). Their physiological and immunological performances are still under investigation because of their distinguished genetic characterization. Local breeds have valuable genetic resources, which need to be maintained and improved. Conservation and characterization should be conducted on these lines (Alexander *et al*., 2004; Luzuriaga -Neira *et al*., 2017). Some arbitrary attempts have been made by researchers in Kingdom of Saudi Arabia to study some of the biological characteristics of these local chicken breeds (Al-Yousef, 2007; Ahmed, 2010, Ahmed and Alamer, 2011; El Sayed *et al*., 2016). However, those researches did not include all immunological aspects and conservation of these breeds. Diseases are controlled by several methods, including biosecurity barriers, biological control protocols (competitive exclusion techniques and vaccination protocols), the use of medicine (antibiotics and anticoccidial) and genetic selection. The application of prophylactic control measures has several limitations especially using medication and antimicrobial agents (World Organization for the Animal Health (OIE), 1998). Economic cost of morbidity and mortality is estimated to be within 10% to 20% of total production costs (Bumstead *et al*., 1991).

Newcastle disease (ND) is a viral disease that affects poultry and other avian species (Kumar and Kumar, 2014). Some velogenic

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strains of NDV are highly contagious, which may spread through inhalation of the virus in contaminated aerosol or ingestion of contaminated food and water. Marked decrease of broiler meat production as a result of NDV outbreaks occurred in Saudi Arabia on 2012 (USDA, 2013). Viral virulence depends on many factors such as the host, age, health status, environmental condition, viral genome sequences, and secondary infection causing high economic loss (Giovambattista *et al*., 2001; Nidzworski *et al*., 2013; Ahmadi *et al*., 2014).

Another contagious viral disease infection affecting early age chickens is the Infectious bursal disease virus (IBDV) which is immunosuppressive, (Wang *et al*., 2014; Yu *et al*., 2015). The primary target organ of IBDV is the lymphoid tissues especially the bursa of Fabricius (Eterradossi and Saif 2013). IBDV has high mutation rate which may result in the alteration on the viral virulence (Ingrao *et al*., 2013). The incubation period of IBDV is short and ranges from 2-3 days after exposure to the virus followed by appearance the clinical signs of the disease (Van den Berg *et al*., 2000; Eterradossi and Saif, 2013). The morbidity of IBDV is usually high up to 100% while the mortality rate is 30%-40%; however, the mortality is higher in case of the infection with very virulent strains. The economic impact of IBDV could be directly related to mortality from the disease itself or indirectly as a potential interaction between IBDV and other diseases or more importantly due to acquired immunodeficiency (Eterradossi and Saif 2013; OIE, 2014). Maternal antibody of immunized flock by inactivated vaccines will protect the chicks for 1-3 weeks and may extended to 4-5 weeks if breeder were boosted with oil-adjuvant vaccine (Baxendale and Lutticken, 1981). Immunization of young maternally immuned chicks by live attenuated IBDV vaccine still a major problem to determine the proper vaccination time (Block *et al*., 2007).

Thus, the objective of this research is to achieve the first quantitative serological characterization of antibody level against both NDV and IBDV in the newly established Saudi local chicken lines compared with commercial line and also between both local lines. This was achieved by using the ELISA to detect both viruses' antibodies in both local and commercial lines under vaccinated and unvaccinated conditions. It provides the poultry producer and regional research institutes with the information needed to identify and manage health problems effectively. This also allows improving the disease surveillance and vaccination programs and develops the knowledge about regional disease prevention for community service.

MATERIALS AND METHODS

Animals

One hundred and eighty chicks day-old-chicken were obtained from Agriculture Research Station, poultry research unit, King Faisal University. They were classified into three chicken groups; local Saudi chicken lines Hajar-1 (H1), Hajar-2 (H2), as well as commercial Hisex chicken (Hi). Each group comprised both sexes of 60 chicks for each line. The birds of each group were further subdivided into vaccinated and unvaccinated subgroups. At one day old, all chicks were marked by wing tag. Chick line groups were placed in floor pens with wood shaving litter. Birds were fed on commercial starter ration. Both feed and water have been provided with *ad*-libitum consumption. Birds were placed in closed ventilated house. The temperature was adjusted to 33ºC for the first 3 weeks of age, then, it was declined by 2ºC during the fourth week of age. No vaccination program was applied to local parent breeds. However, Hisex parents breed followed the vaccination regimen with oil vaccine against NDV and IBDV before production then monthly against NDV using Lasota vaccine in drinking water. In this study, chicken of vaccinated groups were vaccinated against NDV with eye drop Hitchner B1 at-one-day old then NDV Lasota vaccine in drinking water at one-week old.

They were also vaccinated against IBDV in drinking water at five-days old.
The research on live chicks met the guidelines approved by the institutional animal care and use committee (IACUC), regarding the animal manipulations and blood samples.

Parameters of selection
***Weekly body weight*:**
Starting from day one to the 29th day, all birds were weighted every week using a digital balance. Body weight was measured individually. For confirmation, groups of five chickens were chosen randomly and weighted to calculate mean weight. Body weight was measured to exclude the effect of under- or over- weight performance on immune response.

***Blood samples*:** Whole blood samples were collected at four measuring time intervals for vaccinated and unvaccinated groups every week starting from 7-days-old.

Blood samples were collected from the heart of each bird and kept in a sterile tube for 30 to 60 minutes at room temperature. Then, they were centrifuged at 1000 rpm for 3-5 minutes to obtain serum samples by micropipette to another tube. Serum samples were used for detection of antibody level for NDV and IBDV using indirect ELISA techniques.

Enzyme-Linked immunosorbent Assay (ELISA):
The separated sera were screened with the indirect ELISA for IBD and ND antibodies, according to the manufacturer's instruction. X-OVO FLOCKSCREEN™ Infectious Bursal Disease Antibody ELISA Kit, X-OVO FLOCKSCREEN™ Newcastle Disease Virus – Chicken Antibody ELISA Kit were obtained from x-OvO Limited (Dunfermline, United Kingdom).

The following steps were performed:
1. The pre-coated plates were removed from their sealed hags and samples were located on a 12x8 template sheet and run in duplicated manner with the positive and negative controls.
2. 50 µl of the samples were added to the appropriate wells. The plate was covered with an adhesive cover and incubated at +37°C for 30 minutes. Mix on a plate shaker or by gently lapping the side of the plate.
3. The adhesive cover was removed and the plate was washed 4 times with wash buffer (300 µl per well).
4. 50 µl of Enzyme Conjugate Reagent was added to each well and mixed gently.
5. The plate was covered with the adhesive cover and incubated at +37°C for 30 minute.
6. Adhesive cover was removed and the plate was washed 4 times with wash buffer (300 µl per well).
7. 50 µl of the substrate reagent was added to each well of the plate.
8. The plate was covered with the adhesive cover and incubated at +37°C for 15 minutes. Color development was pale pink, which deepened on addition of ELISA Stop Solution.
9. Adhesive cover was removed and 50 µl of Stop Solution was added to each well.
10. The plate was read using ELISA Reader at 550 nm having first blanked on air and the plate was read immediately.

For the test to be valid:
a) Mean Negative control absorbance must be < 0.2.
b) Mean Positive control absorbance must be at least 0.2 OD units greater than the negative control absorbance.

Statistical analysis
Data were analyzed using a one-way analysis of variance (ANOVA), and means were compared using Duncan's Multiple Range Test (Duncan, 1955) by the use of SPSS (2010) program version 19, the level of significance was set at $p < 0.05$.

RESULTS

Antibody response of various chicken lines to Newcastle disease virus (NDV)

The antibody titer against NDV in vaccinated Hajar-1, Hajar-2, and Hisex groups has no significant difference at 8th day. However, it was significantly ($p < 0.05$) higher in Hisex line in comparison with Hajar-1 and Hajar-2 lines at 15th day and ($p < 0.01$) at 22nd day. Insignificant result were found at 29th day old. (Figure 1).

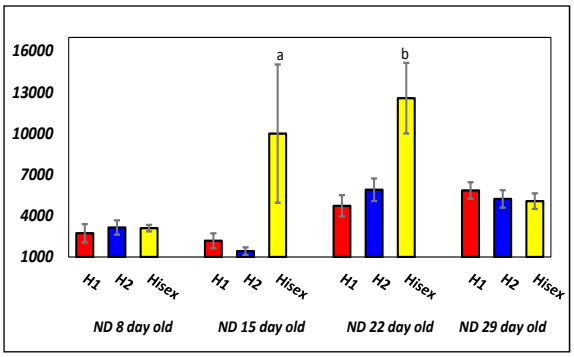

Figure 1: Mean values of Newcastle disease (ND) antibody titer in vaccinated groups (± SE). H1 (Hajar-1), H2 (Hajar -2). 'a' significant at ($P < 0.05$), 'b' significant at ($p < 0.01$) versus H1 and H2 groups.

At 8 day old, there was Significant increase in the antibody titer against NDV in non-vaccinated Hisex group in comparison with Hajar-1 ($p<0.05$), however there was no significant difference between Hajar-2 and both Hajar-1 and Hisex ($p>0.05$). (Figure 2).

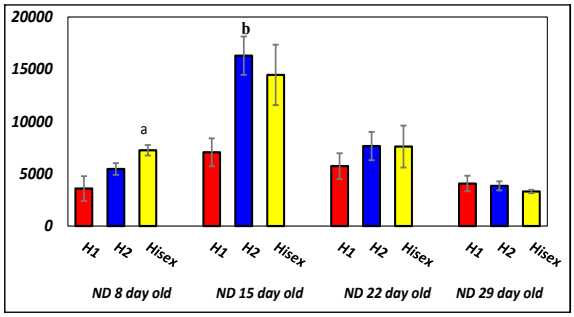

Figure 2: Mean values of Newcastle disease (ND) antibody titer in non-vaccinated groups (± SE). H1 (Hajar-1), H2 (Hajar -2). 'a' significant at ($P < 0.05$), b significant at ($p < 0.01$) versus H1 group.

At 15 day old there was Significant increase in the antibody titer against NDV in non-vaccinated Hajar-2 in comparison with Hajar-1 ($p<0.01$), however there was no significant difference between Hajar-2 and Hisex ($p>0.05$).

There was no significant difference between the three genetic lines at 22 and 29 day old ($P > 0.05$).

Antibody response of various chicken lines to Infectious bursal disease virus (IBDV)

The antibodies titer in vaccinated breeds of IBDV has no significant difference at 8th day but significant differences between the three tested breeds at 15, 22, and 29 day old were observed.

Hisex and Hajar-1showed heighted antibodies titer in comparison with Hajar-2 at 15 day old. On the other hand, hisex breed showed higher antibodies level at 22nd day old in comparison with Hajar-1and Hajar-2. At the last week of the experiment Hajar-2 breed displayed the heighted antibody titer followed by Hajar-1in comparison with hisex breed (Figure 3).

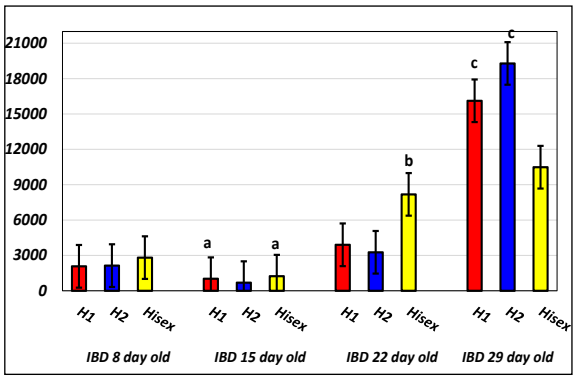

Figure 3: Mean values of Infectious Bursa Disease (IBD) antibody titer in vaccinated groups (± SE). H1 (Hajar-1), H2 (Hajar -2).
'a' significant at ($P < 0.05$) versus H2 group.
'b' significant at ($p < 0.01$) versus H1 and H2 groups.
'c' significant at ($P < 0.001$) versus Hisex group.

There was no significant impact of breeds in case of the IBDV antibodies titer in non-vaccinated chickens during 8, 22, and 29 day old ($p > 0.05$). However, there was significant increase in IBD antibodies in hisex breed in comparison with Hajar-1and Hajar-2 at 15 day old ($p < 0.001$) (Figure 4).

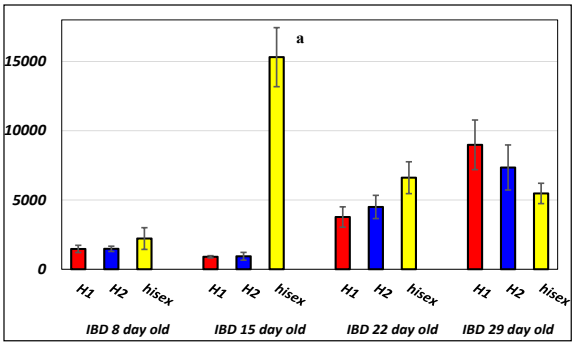

Figure 4: Mean values of Infectious Bursa Disease (IBD) antibody titer in non-vaccinated groups (± SE). H1 (Hajar-1), H2 (Hajar -2). 'a' significant at (P <0.001) versus H1 and H2 groups.

Effect of different chicken line on daily weight gain

The daily weight gain did not show significant difference among the three chicken genetic lines (Hajar-1, Hajar-2 and Hisex) in vaccinated groups (P > 0.05) (Figure 5).

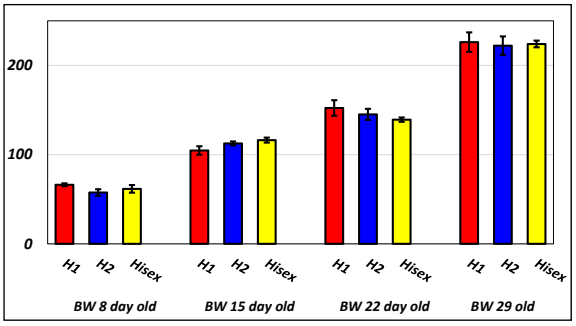

Figure 5: Mean values of body weight (BW) ± SE in vaccinated H1 (Hajar-1), H2 (Hajar -2) and Hisex groups. No significant difference among groups

Also, the daily weight gain in non-vaccinated Hajar-1, Hajar-2 and Hisex groups has no significant difference between the three genetic lines at different timings (P < 0.05) (Figure 6).

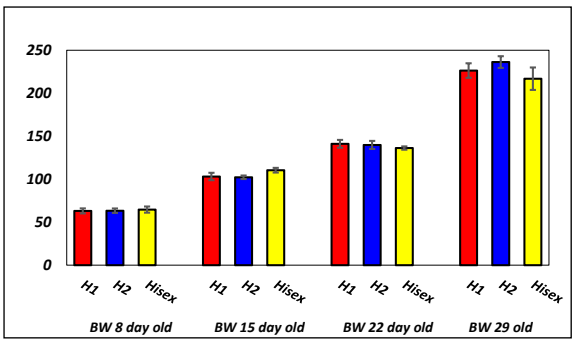

Figure 6: Mean values of Body weight (BW) ± SE in non-vaccinated H1 (Hajar-1), H2 (Hajar -2) and Hisex groups. No significant difference among groups.

DISCUSSION

This study was planned to measure the antibody titer against Newcastle disease NDV and Infectious bursal disease viruses IBDV in some local Saudi breeds (Hajar-1 and Hajar-2) in comparison with commercial chick breed (Hisex) using ELISA technique. The non-vaccinated Hajar-2 line had significant higher titer against NDV in comparison with Hajar-1 at 15th day old. Local vaccinated Saudi breeds showed significant higher antibody titer in comparison with commercial Hisex chick breed at 29th day old for IBD. Meanwhile, no significant difference was detected at the first week of age in the NDV and IBDV antibody titer for all vaccinated breeds. The body weight gain showed no significant difference among the three chick lines among the first four weeks of life. The body weight was measured to exclude the effect of under- or over- weight performance on immune response.

Ganapathy *et al*. (2014) had similar results when studying the immune response of chickens in tropical countries. They reported that the effectiveness of either live or inactivated vaccine against NDV depends on the virulence of the field strain, immunological state of the birds and the methods of vaccine application. Eterradossi and Saif (2013), administered different types of vaccines and reported that Lasota vaccines against NDV did not interfere with protection gained by other live vaccine. They also mentioned that the immune response of chicken to IBDV depends on the age, breed sensitivity, strain virulence and the degree of passive immunity.

There is a general concept about rural chick as it can resist infection more than commercial chicks since it grows in an open environment, and exposed to pathogens without veterinary control. This was supported by Fathi *et al*. (2017), who concluded that native chicken breeds have several genes adjusted to hard environmental conditions that exhibit solid natural immunity for common infections.

Natural selection of local breed takes place in the rural environments where the chickens with better immunity can survive (Giovambattista *et al*., 2001).

The chicken immune system in not fully developed in the first few week of life and the birds depend on the maternal immunity until the complete development of the adaptive immune system. (Hamal *et al*., 2006; Davison *et al*., 2011). Regarding to the maternal immunity of non-vaccinated chickens, antibodies detected in the early life of chicken in our study passes from hen to egg as confirmed by Al-Natour *et al*. (2004) and Hamal *et al*. (2006).

Concerning the two local Saudi chickens (Hajar-1 and Hajar-2), it was reported that the transfer of the maternal antibodies against NDV from hens to chickens quantitatively depends on hens' maternal antibody level. Thus, the genetic background can affect maternal antibody transfer against several antigens among various breeds at different age (Ahmed, 2011). The physiological and genetic dissimilarities between Hajar-1 and Hajar-2 Saudi lines have been emphasized earlier (Ahmed and Al-Abbad, 2014).

Previous study was performed to compare between Hajar-1 and Hajar-2 and its crossbreeds of Hisex with either of them from 4th to 16th weeks of age with four weeks intervals only against NDV. It was found that Hajar-2 and its crossbreed gave higher titer than Hajar-1 and its crossbreed (El Sayed *et al*., 2016). Their study differs from the current research as it studied the chickens' age starting from one day up to four weeks old with one week intervals against NDV and IBDV.

CONCLUSION

The current study concluded that the non-vaccinated local Saudi breeds (Hajar-1 & Hajar-2 lines) had better immunity than the commercial line (Hisex) at older age of life (at four weeks). However, their performance in vaccinated groups is almost similar to the commercial line. The Hajar-1 and Hajar-2 maintained the same body weight gain as Hisex in all samples.

The present study is one of the primary investigations about the antibody response profile for such birds with unique genetic pool. Based on the current study, further studies considering different aspects of the immune response for those local lines are planned.

Conflict of interest: The authors declare that they have no conflict of interest

ACKNOWLEDGMENT

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تقييم مستويات الأجسام المضادة للنيوكاسيل والجمبورو في خطوط الدجاج المحلي السعودي والدجاج التجاري

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استلام 8 ابريل 2018م - قبول 13 أكتوبر 2018م

الملخص

تم تصنيف خطين من الدجاج السعودي المحلي من قبل باحثين في جامعة الملك فيصل: هجر-1 وهجر-2، ولا يزال أداؤهم الفسيولوجي والمناعي قيد البحث، وقد هدفت هذه الدراسة إلى تقييم الاستجابة المناعية ضد مرضيّ النيوكاسل والجمبورو في الخطوط السعودية مقارنة بالخط التجاري للكتاكيت المحصنة وغير المحصنة، وتم تقسيم 180 كتكوتًا من عُمْر يوم إلى ثلاث مجموعات متساوية كالتالي: هجر-1 وهجر-2 والتجاري (الهايسكس).

تم تقسيم كتاكيت كل سلالة إلى مجموعتين فرعيتين متساويتين؛ محصنة وغير محصنة، وتم جمع عينات الدم من جميع الكتاكيت، واستخدمت تقنية ELISA لتقييم عيارية الأجسام المناعية ضد كل من فيروسي النيوكاسل والجمبورو بالإضافة إلى قياس وزن الجسم الأسبوعي للطيور أسبوعيًّا أربع مرات بدءًا من الأسبوع الثاني من العمر.

أظهرت نتائج الدراسة أن سلالتي الدجاج المحلي السعودي حققتا زيادة وزن الجسم نفسها مثل السلالة التجارية؛ حيث كانت بمستوى معنوي ($p> 0.05$)، كما وُجد ارتفاع كبير معنوي للأجسام المناعية لكتاكيت الهايسكس المحصنة ضد كل من فيروسي النيوكاسل والجمبورو في الأسبوعين الثاني والثالث من العمر بالمقارنة مع الكتاكيت المحصنة من السلالات السعودية المحلية هجر-1 وهجر-2 ($P <0.05$). من جهة أخرى كان المستوى المناعي ضد كل من فيروسي النيوكاسل والجمبورو للسلالات السعودية المحلية غير المحصنة هجر-1 وهجر-2 أفضل بالمقارنة مع الخط التجاري الهايسكس في عمر أربعة أسابيع.

ويمكن استنتاج أن ارتفاع مستوى المناعة لسلالتي هجر-1 وهجر-2 في العمر المتقدم تحت ظروف عدم التحصين يظهر فرصة استخدامهما كمصدر محتمل لتوريث المناعة تحت ظروف عدم التحصين.

الكلمات المفتاحية: هجر-1 ، هجر-2، هايسكس، فيروس الجمبورو، فيروس النيوكاسيل.

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