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With 2 figures in the text

Detection of Lymphoid Leucosis Virus Antigen in Local and Foreign Breeds of Chickens using Enzyme Linked Immuno-sorbent Assay (ELISA)

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ملخص البحث

في هذه الدراسه تم تقصي حقلي لمرض سرطان الخلايا الليمفيه بالدواجن بولاية الخرطوم و فحصت العينات بإختبار المقايسة المناعية المرتبطة بالانزيم غير المباشرة (iELISA) للكشف عن المستضد البروتينى P27و هو بروتين مشترك لجميع تحت مجموعات فيروسي ليكوزيس المجموعات أ ، ب، ج ، د ، هـ وي لحمية الليكوزس . العينات هي كشطات شرجيه وبيض لدجاج بياض بعمر 12 – 36 شهر تم أختيارها من قطعان مشكوك بأصابتها بمرض سرطان الخلايا اليمفيه (الليكوزس). يتكون القطيع من دجاج مستورد و دجاج بلدي. أوضحت النتيجة حمل الدجاج البلدي لحمية الليكوزس بنسبه أكبر من الدجاج المستورد بنسبة 57% (42/24) مقارنة ب 36%(97/35) عند فحص بياض البيض و38.7%(31/12) مقارنة بـ 10.5%(57/6)عند فحص المسحات الشرجيه.

وجد نوع هاي لاين (hyline) 36 الأكثر عرضه للإصابه (37.9%) من بين سلالات الدواجن الأجنبية يليه نوع البوفان (Bovan) بنسبة 32.3% وأخيراً الهايسكس (Hisex) النوع الأبيض بنسبة 16.6% .

Summary

In this study, the occurrence of Lymphoid Leucosis (LL) disease was investigated in Khartoum state. The indirect Enzyme Linked Immunosorbent Assay (iELISA) was used for detection of the protein antigen 27(P27) which is a common protein group for all the lymphoid leucosis virus sub-groups A,B,C,D,E and J.

Samples were selectively taken from flocks suspected of having LL; they included cloacal swabs and eggs. All birds were layers 12-36 month old. Foreign chickens of different breeds and local ones were included. Results revealed that local birds were more positives 57% (24/42) compared to the foreign breeds 36% (35/97) when albumen was tested and 38.7% (12/31) compared to 10.5% (6/57), respectively for cloacal swabs. Amongst the foreign breeds, hyline 36 showed the highest positives (37.9%) followed by Bovans (32.3%) and lastly Hisex white (16.6%).

Introduction

Lymphoid leucosis is a viral disease that may cause tumours of liver, spleen, and other organs. In poultry, the bursa of fabricius is also enlarged. The onset of the disease occurs at 16 weeks of age.

In most cases, mortality is often not a serious problem and, even if birds are not sick enough to die, the disease may affect bird‘s immune

system, productivity, sexual maturity, infertility and hatchability (Gavora *et al*., 1980). The causative agent of LL is the Avian Leucosis (AL) virus. It is an RNA virus of the sub-family oncovirnae of the family retroviridae and are within the genus alpharetrovirus (Fadly and Payne, 2003). AL virus infects the birds vertically through the hatching egg or horizontally from other bird in the same flock. Bird to bird horizontal infection normally induces the production of antibodies. However, this may depend on age of bird or stress factor and hence the bird may become viraemic for the rest of its life.

The use of enzyme linked immunosorbent assay (ELISA) for the detection of group-specific antigens of avian sarcoma and Leucosis viruses have been described by Smith *et al.* (1979) and Clark and Dougherty (1980). Antigen ELISA has been applied by Clark *et al.* (1981) for detection of acute infection and in old laying hens shedding off LL virus

Egg albumen, vaginal and cloacal swabs were more rapid and useful for eradication programmes (Okazaki *et al*., 1975; Crittenden *et al*., 1979 and Spencer *et al*., 1976) and in identifying classes of hens which do not congenitally transmit LL virus (Payne *et al.*, 1979).

In this study, an indirect ELISA was used for detection of the LL virus in congenitally infected birds (shedders) in their eggs and faeces. A comparative study between foreign breeds and local chickens types, as well as between the different foreign breeds of chicken was investigated.

Materials and Methods

Samples:

Fourteen laying poultry farms in Khartoum, Omderman and Khartoum North were visited. Selection of samples was based on clinical signs and gross lesions. Four hundred and thirty-nine cloacal swabs and eighty-two eggs were collected. The cloacal area was carefully swabbed, placed into one ml phosphate buffered saline (PBS) containing antibiotics and kept at -20°C till tested. The affected birds were layers 12-36 month old and belong to the foreign poultry breeds (Hisex white, Hyline 36 and Bovan) and local types. Some birds were kept under close system, whereas others were kept under open system. Flock size ranged from 1000 to 4000 laying birds.

Preparation of albumen:

The air sac of the blunt end of the egg was marked and disinfected. Using sterile scissors, the shell over the air sac was removed, the albumen was expunged using sterile disposable syringes and about five ml of light albumen was collected from each egg in sterile tubes. It was frozen and thawed to help reducing its viscosity.

ELISA Kit:

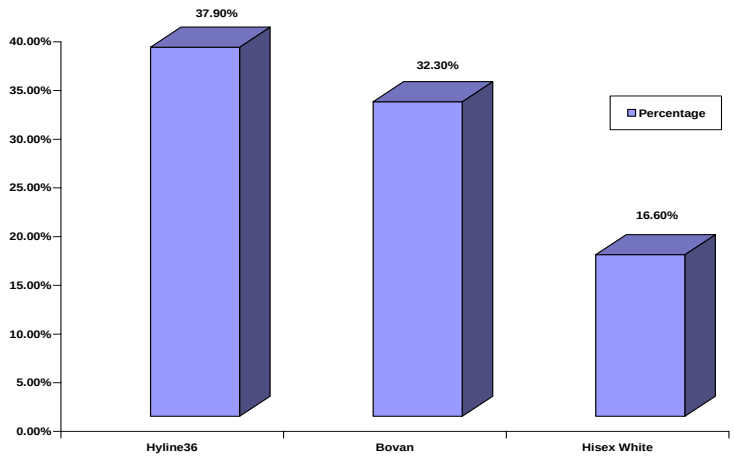
Ag indirect ELISA kit for the detection of the avian lymphoid virus antigen p27 (IDEXX- USA) was used.

The assay procedure:

The test was carried out according to the manufacturer´s instructions. It is designed to detect AL virus protein 27, an antigen common to all sub-groups of AL virus including endogenous viruses. Anti (P27) antibodies were coated into 96-well plates. The recommended samples were light albumen and cloacal swab (100 µl). Cloacal swabs and albumen suspected to contain the virus were added each to a coated microplate which were then incubated for one hour at room temperature. The plates were washed three times with PBS and Tween 20 (T_{20}) to remove the unbound material. Thereafter, 100 µl of anti P27 Horseradish Peroxidase (HRPO) conjugate was added to each well and incubated for one hour at room temperature. The plate was then washed and 100 µl of enzyme substrate OPD was added to each well. Reaction was stopped using 100 µl H_2SO_4 and the plates were read using ELISA reader multiscan machine at 650 nm wavelength. The presence or absence of P27 antigen is determined by relating the A 650 value of the unknown to the mean positive control. The positive control has been standardized and represents significant antigen levels (approximately 10 ng / ml). The relative level of antigen in the unknown was determined by calculation the ratio of the sample value to positive control value (S/P). If the ratio is less than or equal to 0.2, the sample should be considered negative and when S/P ratio is greater than 0.2% it indicates the presence of P27 antigen.

Results

Fig. 1 shows the number and percentage of positives for AL virus antigen. The local type birds had the highest percentage of positives 57% (24/42) compared to 36% (35/97) for foreign breeds when albumen was tested, and 38.7% (12/31) compared to 10.5% (6/57) when cloacal swabs were examined. Fig. 2 shows the results of comparative study on some foreign breeds; hyline 36 breed showed the highest positivity (37.9%) followed by Bovans (32.3%) and lowest
in Hisex white (16.6%).



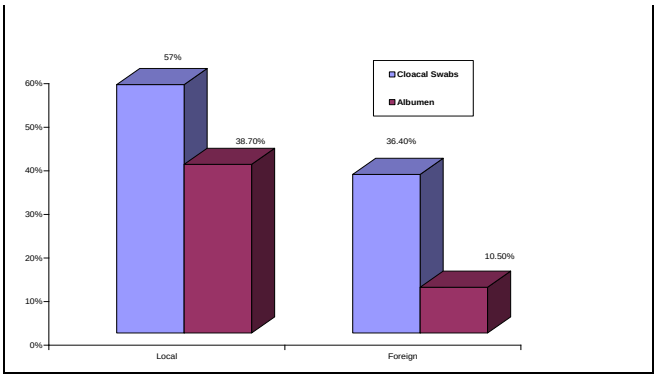
breed

Fig. 1: the percentage of AL virus (P27) antigen positive cases among foreign breeds of chickens using ELISA test.

Discussion

Avian leucosis, Marek's disease and reticuloendotheliosis viruses are among the oncoretroviruses which are tumours-causing viruses (Bagust, 1993; Witter, 1999). They have a worldwide distribution. In the Sudan, LL and both classical and visceral forms of Marek's disease were reported (Anon, 1956; Khogali 1969; Kheir *et al.* 1992; Salim *et al.*, 2001). They were also observed during the routine work at the Avian Pathology and Diagnosis Department (APDD), Central Veterinary Research Laboratories (CVRL). In the Sudan, at the mid-nineties, seven outbreaks of visceral tumour that caused high losses to the poultry industry were reported (Salim *et al*., 2001). According to the APDD at the CVRL, the Marek's disease mild vaccine HVT was incriminated for heavy losses in the country during the early nineties.

The HVT vaccine was thereafter replaced by a bivalent vaccine (SB1+HVT) and since then the prevalence of the disease decreased. However, sporadic cases in layers (12-34 months) associated with loss of weight, abnormal gait and decreased egg production together with various visceral tumours continued to be recorded at the APDD. Recently, Maaz and Abd Elmalik (2005) has confirmed the presence of the AL virus sub-groups (A-D and J) using the polymerase chain reaction (PCR) technique. ELISA test for the detection of the virus protein was used by many wokers (Clark and Dougherty, 1980; Payne *et al*., 1982; Smith *et al*., 1990). It is useful for the detection of the P27 antigen which is located in the gag gene region.



Breeds

Fig. 2: The comparative results between different foreign breeds of chickens to AL virus antigen (P27) using Ag ELISA test.

It could also help in the detection of shedder birds (Clark *et al*., 1981) and useful in the eradication programmes. As long as the objective of this study was to determine the occurrence of LL in Khartoum State. AL virus Ag ELISA was found to be useful test and was arbitrary chosen to determine the presence or absence of AL virus in a particular sample. PCR can be used to determine the exact subgroup. Based on study made by Spencer *et al.* (1976), it was found that the ELISA absorbance values (O.D) were usually 0.5 or greater when egg albumen or cloacal swabs were used for the detection of exogenous AL virus. Vagino-cloacal swab and albumen were the suitable samples for the detection of the virus. It is believed that AL virus can be of high concentration in the magnum of the oviduct and in Lieberkuhn´s glands in the intestine in the host from which the virus can pass to the albumen and to the intestinal contents (Bagust *et al*., 2004).

It is true that exogenous subgroups A-D and J are congenitally transmitted to the egg albumen. Most of these chickens will become immunologically tolerant, viraemic (V+ve) without antibody (Ab-ve) and shedders, the virus, therefore, can be detected in cloacal, vaginal swabs and gg albumen using AgELISA test. Those chickens are considered to be a serious danger to the uninfected hatch mates.

Horizontal infection can occur and it depends on the bird age at the time of infection. The bird could be either vireamic positive and hence antibodies negative and positive shedder or antibody positive. Stress factors and concurrent infections are known to enhance tumor formation (Zavala, 2001). Numerous studies have been directed to find the optimum method for the detection of hens and embryos infected with LL. Examination of vagino-

cloacal swabs has been found by several groups to be useful for detection of hens shedding LL virus and/or egg and embryos (Spencer *et al*., 1976; Okazaki *et al.*, 1975; Payne *et al*., 1979; De Boer *et al*., 1980; Fadly *et al*., 1981). In this study, Hyline 36 gave the highest percentage of positives followed by Bovans and then the Hisex white. It worthmentioning that both Bovans and Hisex white are amongst the first imported poultry breeds in the Sudan, while Hyline36 has been introduced recently. This high percentage might be due to a congenital infection introduced with the Hyline 36 breed. As for the Bovans and Hisex white, infection might be due to a horizontal rather than a congenital infection. Local chickens in the Sudan are usually owned by families, they are usually left to live free in the village or sometimes within the premises of the house, whereas foreign breeds are kept in close intensive or semi-intensive system. In this study, high percentage of positives was detected in local chickens. Similarly wild birds, such as *Passer domesticus*, are sometimes kept free in the environment especially in poultry farms, where feed and water are available. Varejka and Tomsik (1974) identified Rous Sarcoma virus neutralizes activity in one blood serum from three sparrows captured in a poultry farm. This type of free range living for both local and wild birds may play a role in the transmission of LL virus. In this study, however, wild birds were not considered but their role in transmission of LL virus disease should not be underestimated. In the future, wild birds in poultry farms should be examined for the presence of LL virus. Furthermore, the role of local chickens that do not show clinical signs and may transmit infection to foreign chickens should be investigated.

In conclusion, this work confirms the presence of LL in the Sudan. It supports the results of Anon (1956), Khogali (1969), Salim *et al*., (2001) and Maaz and Khalafalla (2005). Further investigation on local and wild birds could have an important role in the disease transmission to foreign birds.

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