

A SEROLOGICAL SURVEY ON THE PRESENCE OF NEWCASTLE DISEASE ANTIBODIES IN THE HUMAN IN AL-KUT COUNTRYSIDE ⁺

التقصي السيرولوجي عن وجود الأضداد المثبطة لتلازن خلايا الدم الحمر لمرض نيوكاسل المتواجدة في الإنسان في أرياف مدينة الكوت

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Abstract:

The present article was designed to estimate the epidemiological distribution of Newcastle Disease (ND) as a Zoonotic disease at Al-Kut countryside. For this purpose, one hundred and sixty blood samples were collected from rural poultry with no history of vaccination against ND. Sixty avian blood sample (10 chickens, 10 turkeys, 10 geese, and 10 ducks) from the four cardinal points around the city, another sixty blood samples obtained from the owners (male and female) of these domesticated birds, fifteen blood sample from each site. Sera were separated from these blood samples and submitted to Haemagglutination Inhibition (HI) test to detect the presence, and quantity of HI antibodies.

The results of the serological test showed that there were positive result in most samples of man (especially in females) and turkeys, this elevation was statistically significant at the level $p < 0.05$. This positive result was less obvious in samples of geese and ducks; whereas, almost all samples of chicken were negative, so, the fact is that, this disease is an endemic disease at the site of study, and the virus epidemiological cycle may involves latency period and shading of this virus from man to rural and backyard poultry and vice versa.

المستخلص:

أجريت هذه الدراسة لمعرفة خارطة التوزيع الوبائي لمرض نيوكاسل كمرض مشترك بين الإنسان والطيور في ضواحي مدينة الكوت عند الجهات الأربع. إذ تم جمع ١٦٠ عينة دم من الطيور الداجنة غير الملقحة سابقاً ضد مرض نيوكاسل و المربية في الأرياف حول المدينة ، بواقع ٦٠ عينة دم لكل موقع (١٠ عينات من الدجاج و ١٠ عينات من الديك الرومي و ١٠ عينات من الإوز و ١٠ عينات من البط)، و ٦٠ عينة دم أخرى (١٥ من كل موقع) أخذت من الأشخاص المربين لتلك الطيور (نساء و رجال). فصلت مصول الدم عن العينات لغرض إجراء اختبار اثباط تلازن خلايا الدم الحمر لغرض تحديد وجود وكمية الأضداد المثبطة لتلازن خلايا الدم الحمر.

بينت نتائج اختبار اثباط تلازن خلايا الدم الحمر وجود أجسام مناعية مثبطة لتلازن خلايا الدم الحمر في معظم العينات المأخوذة من الإنسان أكثر وضوحاً بصورة معنوية في النساء عنه في الرجال كذلك في الديك الرومي عند

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التحليل الإحصائي لها مع تفوق معنوي عند مستوى $p < 0.05$ عن باقي العينات؛ بينما كانت العينات المأخوذة من البط والإوز ذات نتائج موجبة مع أقل مقارنة بباقي النتائج؛ في حين أن في الدجاج كانت جميع العينات المأخوذة منه تقريباً خالية من الأضداد مثبطة لتلازن خلايا الدم الحمر في أمصالها ولمعظم العينات، استخلصت الدراسة أن مرض نيوكاسل هو مرض مستوطن في المنطقة مكان البحث، وأن الدورة الوبائية للفايروس قد تتضمن طرحه من الإنسان إلى طيور المزرعة، والطيور المنزلية والعكس بالعكس مما يخدم وبائية المرض.

Introduction:

Newcastle disease (ND) is a viral disease of many species of wild and captive birds [1]. This disease is caused by Newcastle disease virus (NDV) which is synonymous with avian paramyxovirus serotype 1 (APMV-1), other eight serotypes of the virus are recorded to infect birds other than chickens named (APMV 2-9). This virus is a nonsegmented, single-stranded, negative-sense RNA virus, showing helical capsid symmetry. The virus is a member of the genus *Rubulavirus*; subfamily *Paramyxovirinae*; in the family *Paramyxoviridae*; order *Mononegavirales* [2]. Newcastle disease is enzootic in some areas of the world and a constant threat to most birds reared domestically [3]. Kaleta and Baldauf [4] concluded that in addition to the domestic avian species, natural and experimental infection with NDV has been demonstrated in at least 241 species from 27 of the 50 orders of birds, so, many species of birds showed sufficient antigen divergence that it could be compartmentalised, more or less as a different virus [5]. The great impact of ND may well be on village or backyard chicken production in developing countries, since, village poultry is an extremely important assist representing a significant source of protein in the form of eggs and meat [6]. However, ND is frequently responsible for devastating losses in village chickens due to high mortality rate and endemicity [3].

Newcastle disease virus is a recognized human pathogen in its own right [7]. Reports of disease have often been anecdotal but the best substantiated clinical signs in human infections have been eye infections, including conjunctivitis with eyelids edema, but without corneal effects [8]. More recent reports postulating more obvious systemic signs; include chills, headache, and fever [6]. This infection is usually due to direct contact with infected birds or carcasses [9], now it's clearly noticed that all workers in commercial poultry industry have had sufficient antibody level to NDV in their own sera, this positive result was due to subjection to virulent (of chicken) NDV that causes infection, or avirulent ones that came from vaccination isolates [10]. In the other hand, there is no scientific survey dealing with the presence of the ND antibodies in poultry owners sera in Al-Kut countryside at present.

Material and methods:

Blood samples were taken from rural un-vaccinated poultry against ND as well as their owners in countryside. The procedure includes collection of samples at the four cardinal points around the city of al-Kut (Al-Battar village at the north, As-Suada village at the east, Al-Mazag village at the west, and As-Sudan village at the south). For this purpose sixty random human blood samples from males and females were collected from the brachial vein; fifteen blood samples from each site, at the same time.

Other 160 blood samples were collected from rural poultry, thus, sixty blood samples from domesticated birds 10 chickens, 10 turkeys, 10 geeses, and 10 ducks from wing vein at the same sites. All blood samples humans' and avians' samples were submitted to centrifugation at 3000 rpm for 5 min. [11], to collect their sera, then the sera were examined

for the presence and quantity of Haemagglutination Inhibition (HI) antibodies by applying HI test.

- **Samples of the trail:**

- A. Human blood samples:**

Total of sixty random blood samples (male and female) were collected, fifteen samples from each of the four cardinal points at countryside of the city of al-Kut. The collection procedure was applied on brachial vein using sterile and disposable syringes and needles to obtain 5ml of venous blood, the blood samples then put in sterile plain test tube in slant position till be clotted, then the samples transported to the lab by cool box.

- B. Poultry blood samples:**

Total of 160 avian blood samples were collected, sixty blood samples (10 chickens, 10 turkeys, 10 geese, and 10 ducks) were collected from each area of collection, the procedure was applied by using sterile and disposable syringes and needles, to obtain a quantity of about 2.5ml of venous blood, then the blood samples put in sterile plain test tubes in slant position till be collected, and then transported to the lab by cool box as same time as human blood samples, and with the same conditions (*see above*).

- **Haemagglutination test (HA):**

This test was carried out by using the ND vaccination isolate clone 30 as an antigen (manufactured by Intervet Co., Holland). The viral isolate was dispensed in 5ml of sterile normal saline; then diluted by twofold dilution with sterile normal saline, in U-bottom microtiter plate (Persix plate Co., UK) with automated equipment (Volac autotiter II, Liverpool, UK). The dilution started at 1:2 using 50 µl of the virus antigen with a same volume of saline, after awhile, 50 µl of a 2% suspension of three-times-washed chicken erythrocytes was added to each well, and the plates were incubated for 15 minutes at room temperature. The wells of agglutinated virus with RBCs suspension were counted, then the original viral antigen was diluted till each 50 µl of suspension was contained 8HA [12].

- **Haemagglutination Inhibition test (HI):**

This tests were done by the beta-procedure in U-bottom microtiter plate (Persix plate Co., UK) with automated equipment (Volac autotiter II, Liverpool, UK) using 8 HA units of NDV antigen. Duplicate twofold serial dilution of serum in saline starting with 1:2 were prepared using 50 µl per well. Fifty µl of 8 hemagglutinating units of antigen were added to each well, and the mixture was incubated for 30 min. at 37 C. Fifty µl of a 2% suspension of three-times-washed chicken erythrocytes was added to each well, and the plates were incubated for 15 min. at room temperature. The geometric mean of each group was then determined [12].

- **Statistical analysis:**

All data had been submitted to analysis with F-test for comparison among differences of quantitative values, and *Chi* square-test for qualitative values, this procedure was followed by application of space of confidences for all data at 95% to detect the lesser differences among groups at ($p > 0.05$) [13].

Results:

a. Haemagglutination Inhibition test of human blood samples:

There were significant differences at $p>0.05$ in HI antibody titers between human females and males blood samples (Table 1), these differences were noticed as a peak elevation in HI antibody titers of the females blood samples and less obvious in males blood samples (Figure 2).

Table 1: HI antibody titers in human sera:

The four cardinal points	Male (mean $\pm$ SE)	Female (mean $\pm$ SE)
North (Al-Battar village)	1.7 $\pm$ 0.65 A b*	6.5 $\pm$ 2.2 A a
South (As-Sudan village)	0.433 $\pm$ 0.073 C b	4.9 $\pm$ 1.414 C a
East (As-Suada village)	0.0 $\pm$ 0.0 E b	5.5 $\pm$ 1.89 B a
West (Al-Mazag village)	0.11 $\pm$ 0.033 D b	5.33 $\pm$ 1.56 B a
Average	0.603 $\pm$ 0.189 B b	5.57 $\pm$ 1.766 B a

(*): Figures with different superscripts in the vertical and horizontal columns were significantly different at $p>0.05$.

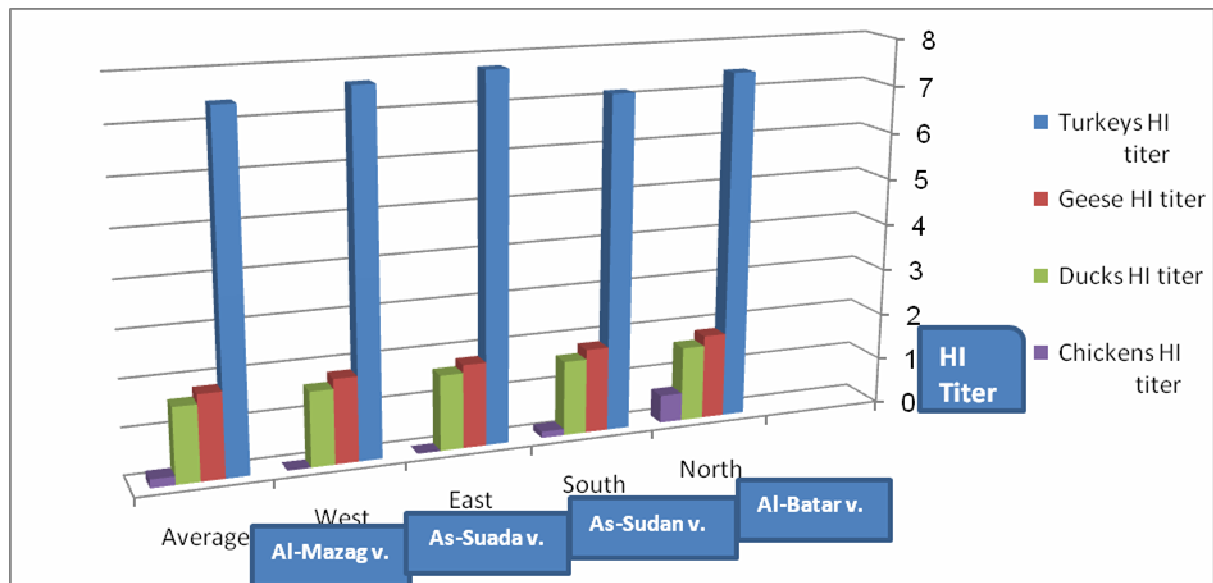
b. Haemagglutination Inhibition test of avian blood samples:

There were significant differences in HI antibody titers in the samples of serum among groups of birds, these differences emerged in turkeys serum samples in all sites at the level $p>0.05$, then be noticed in geese sera; then after, ducks were with less remarkable HI antibody titer in their sera, samples obtained from chickens almost did not show a detectable HI antibody titer in all (Table 2).

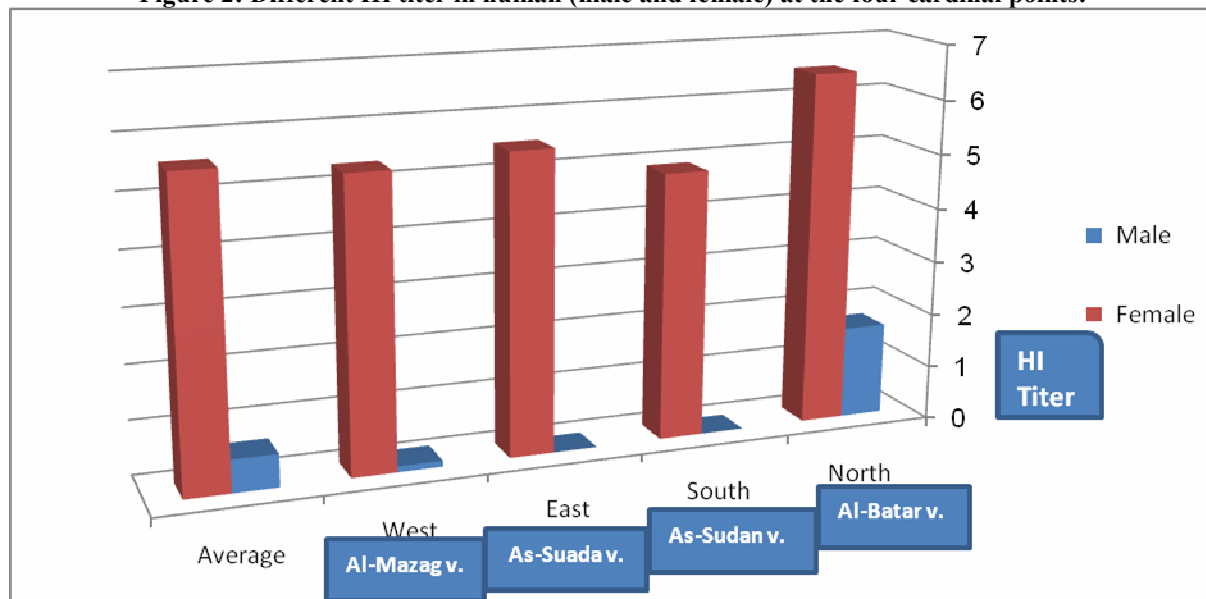
Table 2: HI antibody titers in rural poultry sera at the four sides around the city:

The four cardinal points	Turkeys HI titer (mean $\pm$ SE)	Geese HI titer (mean $\pm$ SE)	Ducks HI titer (mean $\pm$ SE)	Chickens HI titer (mean $\pm$ SE)
North (Al-Battar village)	7.41 $\pm$ 1.6 B a*	1.816 $\pm$ 0.5778 Ab	1.602 $\pm$ 0.4989 Ac	0.6 $\pm$ 0.042 Ad
South (As-Sudan village)	7.133 $\pm$ 1.876 Ca	1.808 $\pm$ 0.6055 Bb	1.5996 $\pm$ 0.5111B Cc	0.134 $\pm$ 0.022 Cd
East (As-Suada village)	7.766 $\pm$ 1.733 Aa	1.7812 $\pm$ 0.5473 Cb	1.61 $\pm$ 0.4954 ABb	0.0 $\pm$ 0.0 Dc
West (Al-Mazag village)	7.616 $\pm$ 1.5666 Aa	1.798 $\pm$ 0.4903 Cb	1.5891 $\pm$ 0.4766 Cc	0.0 $\pm$ 0.0 Dd
Average	7.4 $\pm$ 1.6879 Ba	1.8 $\pm$ 0.5538 Bb	1.6 $\pm$ 0.4989 Ab	0.183 $\pm$ 0.28 Bc

(*): Figures with different superscripts in the vertical and horizontal columns were significantly different at $p<0.05$.

Figure 1: Haemagglutination inhibition titer in poultry at the four cardinal points:

*X-axis: The four cardinal points around the city of Al-Kut.
Y-axis: Antibody titer.*

Figure 2: Different HI titer in human (male and female) at the four cardinal points:

*X-axis: The four cardinal points around the city of Al-Kut.
Y-axis: Antibody titer.*

Discussion:

Newcastle disease is a zoonotic disease; infects most avian species and human as well [14]. The recurrent infection had been reported in man [7 and 15], nevertheless, no transmission of the infection from man to man was recorded yet [7].

As showed in table 2, women were shown much higher HI antibodies level in their sera (Figure 2), this elevation was significantly deferent at $p > 0.05$ if compared with that of men (Table 1), which may attribute to the result that management and care of rural birds is undertaken by countrywomen themselves, which lead to direct contact with infected birds, or carcasses of dead birds [3, 14, and 15]. On the other hand, turkeys exhibited the highest HI

antibodies level in their sera if compared with other poultry (Table 2 and Figure 1) [16]. This may be explained by the relative resistance to NDV infection by the local breed of turkeys [17], that's because turkeys usually infect with APMV-3, in much higher prevalence than with APMV-1 which is the initial pathogen of ND in poultry [7 and 17], the low level of HI antibodies in the sera of aquatic birds may be referred to that these birds are not readily infected by APMV-1 [8]. Also the results showed that there were no detectable HI antibodies in the sera of majority of tested chickens sera, this may attribute to that the local viral serotype is a velogenic, highly virulent virus; lead to high mortality rate to infected birds may be reach up to 100 % in chickens (Table 2)[18]. So, morbidity and mortality are nearly the same when ND hits chickens; and there are no survival birds after infection, coincidentally the rural chickens rarely vaccinate with NDV to survive the disease, this agreed with the fact that serology may often be a poor indicator of the presence of highly pathogenic strains of ND in unvaccinated chicken populations [19].

Unlike commercial flocks, backyard or rural flocks are with multi-aged individuals; this system provides enough susceptible birds within one area to sustain an explosive spread of virus with numerous deaths [20].

The conclusion is that the NDV can be serologically detected in human serum with HI test; the turkeys are refractory to infection, and the rural chickens exhibit no HI titer in their sera, either due to lack of ND vaccination programs or being submitted to depopulation by ND once or twice a year.

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