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Isolation and sero-diagnosis of Newcastle Disease Virus Infection in Human and Chicken Poultry Flocks In Three cities of Middle Euphrates

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Abstract:

This study was planned for detection of Newcastle Disease (ND) infection in human and chicken flocks in Euphrates by the using sero-diagnosis of heamagglutination (HA), heamagglutination inhibition assay (HAI) and competitive ELISA.The NDV was diagnosed in najaf chicken flock by isolation and propagation of virus in chicken egg embryos.

The NDV Ab was detected in serum of local chicken flocks isolated by using competitive ELISA which was indicated the positive high Ab titer with inhibition percent titer more than 40%(PIt>40%) of the total samples.

The fifty eight (58) collected human sera of most poultry associated people in Euphrates in Iraq was determined by NDV competitive ELISA and showed 3 cases from farmers were positive to NDV(PIt>40%).

Key word: NDV, chicken egg embryos, HA, HAI, competitive ELISA**,** PIt**.**

عزل وتشخيص سيرولوجي للاصابة بفايروس النيوكاسل المرضي في الانسان وقطعان الدواجن في ثلاث مدن من منطقة الفرات الاوسط

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الخلاصة:

هذه الدراسة صممت للتحديد الاصابة بالنيوكاسل المرضي في الانسان وقطعان الدواجن في ثلاث مدن من الفرات الاوسط بواسطة استخدام التشخيص المصلي بواسطة الاختبار التلازن الدموي واختبار تثبيت التلازن الدموي والاليزا التنافسي .فايروس النيوكاسل المرضي قد شخص في قطعان الدواجن في النجف بواسطة العزل والتهيئة للفايروس في بيض الدجاج النامي . مستضدات فايروس النيوكاسل المرضي شخصت في مصول قطعان الدواجن المعزول منها العزلة المحلية اصلا بواسطة الاليزا التنافسي الذي اعطى نسبة موجبة عالية للمستضدات بنسبة تثبيط مئوية اعلى من 40% لكل العينات. المصول الثمانية والخمسون للناس الاكثر تعاملا مع الدواجن في ثلاث مدن من الفرات الاوسط في العراق تم تحديدها بواسطة الاليزا التنافسي و اظهر ثلاث حالات موجبة من المزارعين التي كانت موجبة لفايروس النيوكاسل المرضي بنسبة تثبيط مئوية اكثر من 40%.

Introduction:

Newcastle disease (ND) is a highly contagious and fatal disease of chickens. In many developing countries ND is endemic and the disease has the greatest impact on villages where the livelihood of people depends on poultry farming (1). ND can be divided into five pathotypes based on severity of the disease in chickens. These are: Viscerotropic velogenic Newcastle disease (Doyle's form)**,** Neurotropic velogenic Newcastle disease (Beach's form)**,** Mesogenic Newcastle disease (Baudette's form), Lentogenic Newcastle disease (Hitchner's form),and the asymptomatic-enteric form (Ulster type) (2).

The first report in which NDV was described to be a human pathogen was published by Burnet, in 1943. In a review of ND as a zoonosis, (3) recorded 35 published reports of NDV infections of humans between 1948 and 1971**.**

Pedersden et al. (1990) reported significantly higher antibody titres to NDV in people

who had known associations with poultry. Therefore, Newcastle disease is one of a few chicken zoonotic diseases.

Newcastle disease viral replication is the most rapid among the *paramyxoviruses*. (4,5).

Materials and Methods:

The virus was isolated from broiler flock 30-days-old. The clinical signs of NDV were diagnosed by vet. Laboratory and rapid test was given strong NDV with huge mortality.

Collection of pathological specimens from chicken:

A) Collection of tissue samples: After thawing the specimens 100mg of tissue were collected from internal organs like trachea, lung, intestine and brain from several birds then dropped in 1 ml PBS and storage at -43 °C in deep freeze.

B) Collection of blood sample: blood sample 1-2ml was taken from the brachial vein of wing, allowed to clot at room temperature then Centrifuged at 2500 rpm for 15 minutes, and then the sera were collected, labeled and stored at -20 °C for further analysis.

Tissue processing: The tissue was placed in glass beaker and homogenized by sterile automatic homogenizer and adding transport medium thoroughly in concentration of 1:10 w\v with an equal volume of antibiotic 500 IU penicillin and 500 µg streptomycin /ml, then transfer the supe like fluid to a sterile centrifuge tube and Clarified by centrifugation at 1000rpm for 10 minutes at 4°C, the supernatant fluid was stored in a sterile tube in freezer.

Preparation of erythrocytes:

The blood (Chicken RBC) were Collected from the brachial vein (the largest vein under the wing) from healthy chicken in sterile heparinzed tube and then washed three times with PBS after centrifugation at 1000 rpm, 4°C for 10 minutes, the supernatant

fluid was discarded and 0.5 ml of the pelleted RBC was mixed with 49.5 ml PBS to achieve 1% RBC solution.

Materials used in ELISA test:

1. Collection of blood samples

A) Samples from human by cities

Human samples which collected reached to 58 sera samples distributed on cities of middle of Iraq as Adiwaniya (Shamiya) 20 samples, Najaf (Kufa) 22 samples , Asimawa 16 samples which collected from the most people that have close relation with poultry.

Table 2: details of human blood sample.

Cities	**No. of sample**
Adiwaniyah (Shamiyah)	**20**
Najaf (Kufa)	**22**
Asimawa	**16**
Total	**58**

B) Samples from chicken isolate.

Blood samples were collected from five broiler flocks(called hakim flocks) in Najaf city, which the isolate occurred before that . This flocks showed high morbidity and mortality and was tested by NDV rapid test that gave strong positive results in samples which taken to vet. Laboratory. (5-6) blood samples were collected from each flock as (3ml) from each chicken carry on NDV competitive ELISA test.
(NDV competitive ELISA kit Imported from ID.vet France Company).

Results and Discussion:

Isolation of the virus in embryonated chicken eggs:

The results of tissue suspension that were inoculated in to the allantoic fluid of 8 to 10 days embryonated chicken eggs was showed that isolated virus kill embryos in different times like(24 hrs , 48 hrs , 60hrs , 72 hrs)with marked sever hemorrhage in infected embryos in contrast to control uninfected embryos that inoculated with PBS remained living for more than 96 hrs post inoculation . (Table . 1)

This virus was passaged five times and show same result in infected embryos and they were congestion and sever hemorrhage, while control had normal embryos. Singh *et al*.(2005)(6) considered that Embryonic death within 24 h of inoculation was considered non-specific, and such eggs were discarded. Eggs showing embryonic death after 24 h and up to day 4 were chilled, this was described by several researcher (7 - 6).

In this study at first passage the mean death time (MDT) specified to velogenic strains (kill embryos in less than 60 h).

The mean death time (MDT) test is based on the experience that virulent viruses kill embryos quicker than those with lower virulence. Velogenic strains kill embryos in less than 60 h, mesogenic strains in 60–90 h and lentogenic strains in > 90 h (8).

Table (1): percentage of embryos death no./hrs in five passages.

Passages	No. of inoculated eggs	Time of embryo dead				Total no. of embryo dead	%
		24hrs	48hrs	60hrs	72hrs		
P1	15	1	4	3	5	13	86.6
P2	16	2	5	2	2	11	68.7
P3	16	0	6	4	0	10	62.5
P4	24	0	2	5	4	11	45.8
P5	23	0	0	8	2	10	43.4

Hemagglutination test :

The isolated virus (allantoic fluid from dead embryos) was positive for agglutination activity of chicken RBCs (0.5%) and the titration for five passages was given different results. Table (2).

Table (2): Hemagglutination test in 60 hrs.

Diagnostic tests	Titer of isolated virus				
HA	**P1**	**P2**	**P3**	**P4**	**P5**
	256	**512**	**256**	**128**	**1024**

Hemagglutination Inhibition test:

Isolated NDV tested by Hemagglutination Inhibition on chicken RBCs using Reference Monoclonal Antibodies showed positive results. Table (3).

Table (3): Hemagglutination Inhibition test in 60 hrs.

Diagnostic tests	Titer of isolated virus				
HI	P1	P2	P3	P4	P5
	64	64	32	32	128

In this study hemagglutination activity test was measured in five passages individually to detect the HA activity of ND virus that showed the high activity of this isolate which reach to 2^{10} which demonstrate the high activity of the virus with the aim of haemagglutination inhibition (HI) test with Reference NDV standard monoclonal antibody and the purpose of identification this isolate with the intention of indicate HI titer 128<256 in highest value in passage five that indicate the virulence of the isolate as referred to that Gough *et al*.(1974) and Rezaeianzadeh *et al*.(2011)and Jahangir *et al.* (2009)(9-10-11).

NDV detection by using competitive ELISA test in infected isolate flock:

The results showed high titer for NDV in all these flock (herds) that mean value of the positive control has PIt (percent inhibition titer) greater than 40% . The percent inhibition (PI) value was used for understanding of results. PIt >40% were considered positive, PIt = 30-40% considered doubtful and PIt <30% considered negative. This results showed in table (4).

This agreed with Aziz and Ahmed, 2010 (12)that conduct serological survey of Newcastle disease virus by using competitive ELISA that demonstrated the high percent inhibition (PIt) in the infected chickens survey, and Bronzoni *et al*.(2001)(13) that was recommended to using an antigen-competitive ELISA for detection of avian disease in experimentally infected chickens because the ability and the potential of this technique of this type of ELISA to detect avian disease, highlights to possibility of using this method for detection of avian viruses like NDV

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Table (4). Results of NDV competitive ELISA test in isolates flock.

Test	Antibody titer of NDV%				
	herd (1)	herd (2)	herd (3)	herd (4)	herd (5)
ELISA	53.2	55.96	59.3	60.6	51.9

Note: This study was planned on same flock (herds) that the new NDV strain was isolated.
Note: **PIt**pc**>40%**

Detection of anti- NDV antibodies in human samples by competitive ELISA assay:

In this study blood samples were collected from 58 person in three cities in middle Euphrates of Iraq to detect the possibility of NDV infection in four groups of most exposed people like Veterinarian, Poultry workers, Farmers, Poultry salesman's who may infected previously . The results showed presence of positive samples from number of farmers who exposed to NDV previously. The details of positive samples and cities showed in table (5).

This agreed with Allawi (2004)(14) that was isolated NDV from infected conjunctiva of one flock worker that actually encourage the possibility of zoonotivity of NDV that agreed with Pedersden *et al*.(1990)(15) that make

human survey by ELISA test to detection of antibody of avian viruses (IBV, NDV , IBDV) in two groups: people associated with poultry, and people having limited association and the result show differences between the two study groups were evident: people having a known association with poultry showed significantly higher levels of antibodies to Newcastle disease virus and the antibodies detected may be due to virus exposure rather than zoonoses, but chang .,(1981)(3) considered NDV as zoonotic disease that cause conjunctivitis and respiratory disorder.

Table (5). The details of NDV competitive ELISA test in human.

Cities	No. of sample	No. of positive sample	Percentage %
Al-diwaniya(shamiya)	**20**	**2**	**10%**
Najaf(kufa)	**22**	**0**	**0%**
Al-simawa	**16**	**1**	**6.25%**
Total	**58**	**3**	**5.17%**

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