Efficacy of Latex Agglutination Test for Detection of Avian Rotavirus in Broiler Chickens

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Abstract

The presence of rotavirus infection in broiler chickens (1-4 weeks old) was investigated using latex agglutination test. A total of 200 faecal specimens were collected from broiler chicks and examined by latex agglutination test for the presence of rotavirus. This test is comparable with dot-ELISA for the same purpose. One week old broiler chicks were subjected to infection orally with supernatant of fecal suspension of one of rotavirus positive sample. Possibility of contact exposure was also investigated. Virus shedding and seroconversion was screened using latex agglutination test and haemagglutination test and haemagglutination inhibition test respectively. Virus shedding and seroconversion were confirmed both in orally infected birds and contact exposed chicks. The obtained results revealed that the latex agglutination test is clearly a reliable, rapid and easy performed for detection of samples of broiler chicks. The HI test is a good tool for detection of rotavirus affection in broiler flocks using rotavirus group A antigen.

Introduction

Rotaviruses infect many species of domesticated birds may be associated with enteritis, dehydration, anorexia, low weight gain, and increased mortality however, subclinical infections are also common (١٦ **and 27**). The economic significance of rotaviral enteritis to the poultry industry has not yet been defined, but by analogy with the situation in mammals, it is likely to be significant. Some mammalian rotaviruses have limited ability to infect other mammalian species, and rotaviruses from turkeys and pheasants can infect chickens (**29**).

As a member of the *Reoviridae* family, the rotavirus particle is icosahedral and naked and comprises two concentric capsids of characteristic morphology surrounding a core shell with its 11 double-stranded RNA genomic segments. The rotaviruses are antigenically complex and are ubiquitous among mammals and birds. According to the antigenic specificity of the VP6 protein, which constitutes the internal capsid, the rotavirus strains are classified into seven groups (A to G) and at least four subgroups. The outer capsid proteins VP7 and VP4 determine the viral G and P serotypes, respectively (**6**). Group A is the conventional or typical rotaviruses termed that includes mammalian and avian rotaviruses, while atypical rotaviruses, include nongroup A rotaviruses B, C, D, E, F, and G (**3, 11, 21 and 25**). Group A rotaviruses have been isolated from mammals and birds, but so far groups B, C, and E have been found only in mammals, and groups D, F, and G have been detected only in birds (**16**). Some avian rotaviruses show an antigenic relationship with mammalian group A rotaviruses (**17, 19, 27 and 28**). Those avian rotaviruses antigenically related to mammalian group A rotaviruses are referred to as avian group A rotaviruses. This relationship originally was assumed to occur through simple sharing of the mammalian rotavirus group A antigen, it appears that epitopes on VP6 of group A avian and mammalian rotaviruses exist and responsible for that cross reactivity (**16**).

Avian rotavirus has been isolated from a wide variety of avian species, including turkeys, chickens, and pheasants (**7; 8; 16; 22; 23; and 27**). In field conditions, rotavirus infections in poultry may induce subclinical manifestations, or they may be associated with enteritis, dehydration, anorexia, low weight gain, and increased mortality (**16 and 26**). The economic significance of rotaviral enteritis to the poultry industry has not yet been defined, but by analogy with the situation in mammals it is likely to be significant.

Several tests are used routinely in diagnostic laboratories for the detection of rotavirus in fecal samples. These include enzyme-linked

immunosorbent assay (ELISA) (**1; 4 and 14**), electron microscopy, virus isolation (VI), passive hemagglutination, immunoelectrophoresis, and latex agglutination assays (**5; 9; 12; 13, 21** and **2**).

This study was undertaken to investigate the efficacy of Latex agglutination test as a rapid and simple test for detection of the rotavirus among broiler chicks.

Materials and Methods

Samples: A total of two hundreds diarrheal specimens and cloacal swabs have been collected from broiler chicks (1-4 weeks of age) at two Governorates (Kafr El-Sheikh and Gharbia, Egypt). The collected samples were prepared as 20% suspension in phosphate buffer saline (PBS 0.01M pH 7.2) and centrifuged at 1000g for 10 minutes.

Latex agglutination slide test: The LAT for rotavirus detection in chicken fecal samples was performed with the VIROTECT® (OMEGA DIAGNOSTICS LTD. England) following the manufacturer's instructions for human fecal samples. This is a rapid slide test in which latex particles are coated with rabbit antibodies specific for group A rotavirus antigens present in a fecal supernatant. This test is read with the naked eye in 5 min. Briefly, specimen was centrifuged at approx 1000g for 10 minutes. 50µl of supernatant was transferred onto each of two wells on a test slide. One drop of suspension was added to the first circle (Test Circle). One drop of the control latex reagent was added to the second circle (Control Circle). The contents of each circle were mixed using a separate disposable stirrer ensuring coverage of the test circle with the mixture for 2 minutes. The slide was examined for agglutination.

Haemagglutination and haemagglutination-inhibition tests: HA and HI tests were performed in polystyrene 'U' well microtitre plates by the method of (**15**). For HA tests with erythrocytes from chicken erythrocytes to 0.5 %. Four HA units of antigen in 25µl were used in the HI test. For all HI testing, phosphate buffered saline (PBS; 0.01 M-Na2 HPO4/NaH 2 PO4, 0-15 M-

NaCI pH 7-2) was the diluent for serum, antigen, and erythrocytes. Tests were incubated at room temp.

Dot ELISA test: It was performed in according to **(10)**.

Experimental infection: One hundred and twenty 1-4 weeks old broiler chicks were obtained from commercial hatchery and used for experimental infection with rotavirus. The chickens were randomly assorted into three trial groups (40 chicks per each) namely G1 (orally infected with supernatants of fecal suspensions diagnosed positive for rotavirus), G2 (contact infected group) and G3 (control non-infected group). Fecal samples and cloacal swabs have been collected from the experimental chickens periodically at 1, 3, 5,7,9,11,13 and 15 days post-infection and assayed for the presence of rotavirus using LAT as previously mentioned. The prevalence of haemagglutination-inhibition antibody titers against roravirus in the sera of the experimentally infected chickens was assessed using HI test as described by **(15)**.

Results and Discussion

A rapid, simple, sensitive, and specific diagnostic technique for the detection of viral agents causing gastroenteritis is needed to facilitate rapid diagnosis. Transmission electron microscopy has been used in many diagnostic laboratories as the gold standard for virus detection. The electron microscope (EM) detects virus only if large numbers of particles are present **(21)**. It is expensive and requires special equipment thus; it is not suited for routine examination of specimens.

Commercial ELISAs and LAT are available for the routine diagnostic detection of rotavirus from several mammalian species, many of which possess the group A antigen. Most of the kits available on the market are designed primarily for humans and are not approved for veterinary diagnostic applications. **(2)** Evaluated LAT used for human rotavirus detection for detection of bovine rotavirus antigen and compared it with enzyme-linked immunosorbent assay (ELISA), and virus isolation (VI) and showed high correlation with high specificity and sensitivity. ELISA is also good for laboratories handling large numbers of specimens on a daily basis; however, it is less sensitive than latex agglutination. Because low numbers

of viruses can be amplified with repeated passages, VI is more sensitive than latex agglutination and ELISA; however, this is time-consuming, as it takes from 3 to 8 days for a cytopathic effect to develop for each passage. In our study, we use the VIROTECT for the detection of avian RV in clinical fecal samples.

A total of two hundreds faecal specimens were collected from two governorates in Egypt (Kafr El-Sheikh and Gharbia) from infected chicks (1-4 weeks old). They were examined by latex agglutination test for the presence of rotavirus. Data in table (1) revealed that 67 (67%) out of 100 tested faecal samples were positive in Kafr El-Sheikh Governorate, while percentage was higher in Gharbia Governorate (87%). The absence of the rotavirus antigen in negative samples may be due to the low titres of the virus particles in those samples. Table (2) shows the intensity of the reaction by latex agglutination test among the positive samples.

On using the Dot-ELISA for the same purpose, the obtained results among samples of Kafr El-Sheikh Governorate vary for some extent from those obtained by latex agglutination test (Table 3). The results revealed that 28 (80%) out of 40 tested samples were positive. On the other hand the percentage of positive was nearly the same between the used tests among sample of Gharbia Governorate (88% by dot-ELISA).

The results from this study show that the LAT is a valuable tool in the diagnosis of ARV infection The assay has a number of advantages, including its simple format, rapidity, and low cost, and it can be performed without the need for trained personnel or expensive equipment (**9**). In addition, the LAT has the advantage that it can be read with the naked eye, making it easy to perform in every laboratory. Management of diarrheal diseases demands rapid, accurate diagnosis; therefore, the use of the LAT to detect viral antigen from diluted fecal samples is a good alternative to both VI and ELISA.

Detection rate of avian rotavirus is high in clinical samples, lower rate of rotavirus infection have been documented by (**18**) where 40% of chicken farms and 59% of bird on these farms in USA were rotavirus seropositive. (**25**) reported that rotavirus detection rate 50% of farms sampled and 58% of the flocks positive on these farms. (**17**) further reported that 70% of serum

samples from broiler breeder from 14 farms in Ireland were seropositive for rotavirus-like virus.

One week old broiler chicks were subjected to infection orally with supernatant of fecal suspension of one of rotavirus positive sample. Possibility of contact exposure was also investigated. Virus shedding and seroconversion was screened using latex agglutination test and haemagglutination inhibition test respectively. Virus shedding and seroconversion were confirmed both in orally infected birds and contact exposed chicks (Tables: 5, 6 and 7). On comparing between results of the latex agglutination test and haemagglutination test for detection of rotavirus it samples of experimentally infected birds as showed in table 5 and 6, it is clear that the latex agglutination test is more sensitive than haemaggltination test.

In conclusion, our study showed that latex agglutination is clearly a reliable and rapid method for the detection of ARV. From the obtained results, we can recommend the use of latex agglutination test as a repid field diagnostic test because it is rapid, sensitive, specific and accurate and easy to perform. Further studies should be performed to develop an even more sensitive and specific latex agglutination assay for the diagnosis of ARV infection.

Table (1): Results of LAT on the collected faecal specimen and cloacal swabs:

Locality	Numbers of samples	Positive	Negative	Percentages
Kafer El-Sheikh	100	67	33	67%
Gharbia	100	87	13	87%
Total	200	154	46	-

Table (2): Intensity of the positive reaction by latex agglutination test among the positive samples:

Locality	Numbers of positive samples	3+	2+	1+
Kafer El-Sheikh	67	19	27	21
Gharbia	87	23	38	26

3+ Large clumping with clear background
2+ Moderate clumping with fluid slightly opaque in background
1+ Small clumping opaque fluid in background

Table (3): Results of Dot-ELISA on the collected faecal specimen and cloacal swabs:

Locality	Numbers of samples	Positive	Negative	Percentages
Kafer El-Sheikh	40	28	12	80
Gharbia	40	36	4	88
Total	80	64	16	-

Table (4): Intensity of the positive reaction among the positive samples:

Locality	Numbers of positive samples	3+	2+	1+
Kafer El-Sheikh	28	6	10	12
Gharbia	36	8	17	11

3+ Large clumping with clear background
2+ Moderate clumping with fluid slightly opaque in background
1+ Small clumping opaque fluid in background

Fig (1): Results of Dot-ELISA and LAT on faecal specimen of young chicks.

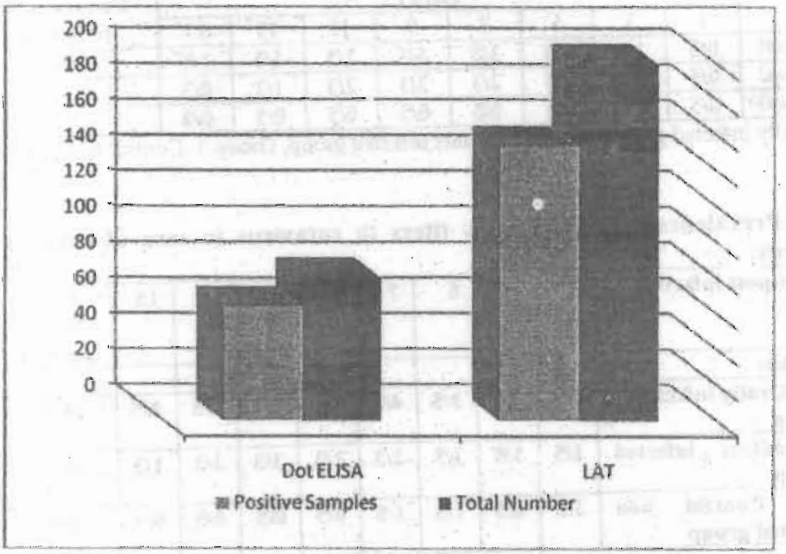


Table (5): Results of LAT on the faecal suspension and cloacal swabs of experimentally infected birds:

	Days PI								Cumulative positive
	1	3	5	7	9	11	13	15	
Group 1	4/5	5/5	4/5	4/5	3/5	2/5	2/5	1/5	25/40
Group 2	0/5	1/5	3/5	2/3	2/3	2/3	1/3	1/3	12/30
Group 3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/40

Group 1: Orally infected group, Group 2: contact infected group, Group 3: Control non infected group

Table (6): Results of Haemagglutination test on the faecal suspension and cloacal swabs of experimentally infected birds:

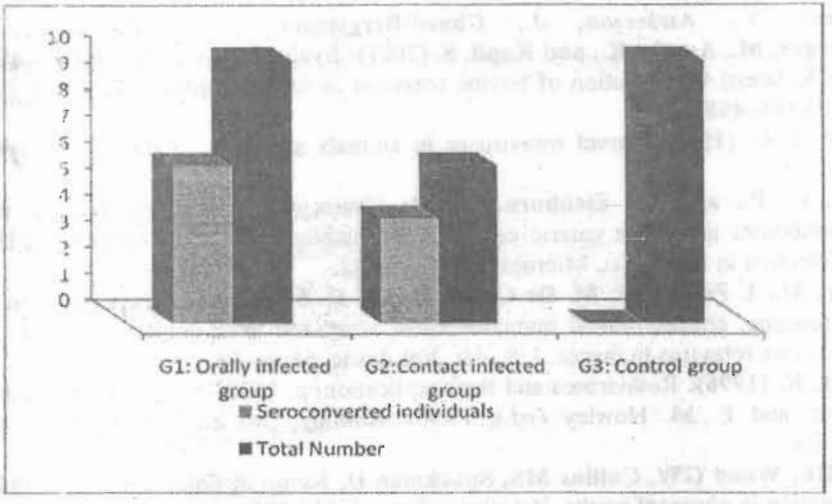
	Days PI								Cumulative positive
	1	3	5	7	9	11	13	15	
Group1	0/5	1/5	3/5	3/5	5/5	2/5	1/5	1/5	18/40
Group2	0/5	0/5	0/5	2/3	2/3	2/3	1/3	0/3	7/30
Group3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/40

Group 1: Orally infected group, Group 2: contact infected group, Group 3: Control non infected group

Table (7): Prevalence of HI antibody titers to rotavirus in sera of experimentally infected birds:

Days-post infection	1	3	5	7	9	11	13	15	Total No. of positive
Groups									
G1: Orally infected group	2/5	3/5	3/5	4/5	5/5	5/5	4/5	2/5	28/40
G2:contact infected group	1/5	1/5	3/5	2/3	2/3	3/3	3/3	1/3	16/30
G3: Control non infected group	3/5	2/5	1/5	1/5	0/5	0/5	0/5	0/5	7/40

Fig (2): Prevalence of HI antibody titers to rotavirus in sera of experimentally infected birds.



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فاعلية اختبار التلزن في تحديد وجود فيروس الروتا في بدارى التسمين

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الملخص العربي

تم تجميع عينات براز ومسحات لفتحة المجمع من كتاكيت تسمين في محافظتين مختلفتين (كفر الشيخ والغربية) وذلك لاستخدامها في تحديد وجود فيروس الروتا في العينات المجمعة من المزارع في تلك المحافظتين باستخدام اختبار التلزن (اللاتيكس) ، وقد أظهرت النتائج ايجابية بعض العينات بنسب مختلفة . بعد ذلك تم احداث عدوى تجريبية في عدد ١٤٠ كتكوت تم تقسيمهم الى ثلاث مجموعات بمعدل ٤٠ كتكوت لكل مجموعة وتم استخدام اختبار التلزن للكشف عن وجود الفيروس في براز تلك الكتاكيت المعدية وأيضاً تم الكشف عن وجود الأجسام المضادة لفيروس الروتا في أمصالها باستخدام اختبار مثبط التلزن الدموي. وقد خلصت النتائج الى امكانية استخدام اختبار التلزن للكشف عن هذا الفيروس في الطيور المصابة كاختبار بسيط وسريع في الأداء وأقل في التكلفة.