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DIAGNOSIS OF ROTAVIRUS BY USING CHICKEN EMBRYO AND RT-PCR

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Abstract

Objective—To detection and determine the infectivity of rotavirus in chicken embryo.

Materials and Methods—stool sample from children suspected with ROTA virus infection inoculated in chicken embryonated eggs. In the current study 20 embryonated eggs were used , 16 embryonated eggs inoculated with samples, while 4 eggs were control. The inoculation conducted in into eleven-day-old from incubation. After 72 hours, Eggs were broken and the embryos were inspected. allantoic fluid were inspiration and RT-PCR

Results— out of 16 embryonated eggs 25% (4:16) of embryo were death while 75% (12:16)were dwarfism. According to RT-PCR results Rotavirus genes detected in rate of 75% (12:16) from death and dwarfism embryos.

Conclusion—Chicken embryo model would be useful as an infective model to study the infectivity, pathogenesis and pathogenicity of rotaviruses.

Keywords: chicken embryo; rotavirus ; RT-PCR .

Introduction

Diarrheal disease is one of the most important worldwide causes of morbidity and mortality, accounting for an estimated 1.3 million deaths in children under 5 years of age [1]. Rotavirus is the major cause of acute gastroenteritis and severe dehydrating diarrhea in young children [2]. The majority of morbidity and mortality caused by rotavirus gastroenteritis is experienced by children under 5 years of age in developing countries. Rotavirus causes approximately 453,000 deaths annually in children, most of them occurring in developing countries in Africa and South Asia [3]. Moreover, approximately 40% of

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hospitalizations for diarrheal illness that occur among children under 5 years of age in developing countries are due to rotavirus [4] and [5].

Clinically, rotavirus gastroenteritis is characterized by profuse diarrhea, mild fever and vomiting, leading to mild to severe dehydration. However, the clinical manifestations of rotavirus diarrhea alone are not sufficiently distinctive to permit diagnosis [6] and laboratory testing is the only way to confirm the diagnosis [7].

Diarrheas always treated with antibiotics, irrespective of the causative agent. If infection due to rotavirus can be diagnosed early, the misuse of antibiotics can be avoided [8].

Materials and Methods

Purification of rota virus from Stool sample of children were conducted according to (Imane , et al.,2016) method [9].

Egg Inoculation and Incubation

1. Fertile eggs are obtained, preferably, from specific-pathogen-free (SPF) flocks. Alternatively, fertile eggs may be used that are from healthy flocks free of antibody to the virus of interest .
2. Disinfectant: 70 % ethanol, 3.5 % iodine, 1.5 % sodium iodide.
3. A vibrating engraver (Fisher Scientific) or drill (Dremel) is used to prepare holes in egg shells. Prior to use, disinfect the tip of the engraving tool/drill to prevent contamination of the egg.
4. Plastic cement, glue, tape, or nail varnish are used to seal holes in egg shells after inoculation.
5. Egg flats.
6. Egg candlers are available from a variety of commercial sources.
7. A suitable egg incubator is needed; these are available from a variety of commercial sources. Commercially available egg incubators generally are equipped with heat source, humidifier, and a timer-based mechanical turning system.

Collection of Specimens from Inoculated Eggs

1. Sterile scissors and forceps.
2. Sterile pipettes or 5 ml syringes with 1 in., 18 gauge needles.
3. Sterile plastic tubes, e.g., 12 × 75 mm snap-cap tubes or microcentrifuge tubes.

Allantoic Sac Inoculation

1. Chicken eggs (21 day embryonation period) are generally inoculated at 7-11 days of embryonation.
2. Place eggs in an egg flat with the air-cell up. Candle eggs to ensure viability and mark the edge of the air-cell.
3. Disinfect the area marked on the shell and drill a small hole just above the mark so that the hole penetrates the air-cell, but not the portion of the egg below the air-cell.

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Inderscience Research

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4. A 1-ml syringe with a 25-gauge, 0.5 in. (12 mm) needle is used to inoculate eggs. The needle is inserted to the hub while holding the syringe vertically and 0.1–0.3 ml of inoculum is injected into the allantoic cavity.
5. Seal holes and return eggs to incubator.
6. Incubate eggs for 2-3 days. Evaluate embryos and allantoic fluid for presence of virus as described below.

Collection of Allantoic Fluid from Eggs Inoculated by Allantoic Route

1. Candle eggs once daily after inoculation. Discard all eggs with embryos that die within the first 24 h after inoculation.
2. Collect allantoic fluid from all eggs with embryos that die >24 h after inoculation and from eggs with embryos that survive through the specified incubation period. Eggs with live embryos following the specified incubation period are refrigerated at 4 °C for at least 4 h, or overnight, prior to collection of allantoic fluid.
3. Place eggs in an egg flat with the air-cell up. Disinfect the portion of the egg shell that covers the air cell, and use sterile forceps to crack and remove egg shell over air cell.
4. Use forceps to gently dissect through the shell membrane and CAM to expose the allantoic fluid. Use forceps to depress membranes within the allantoic cavity so that allantoic fluid pools around the tip of the forceps. Use a pipette or syringe with needle to aspirate fluid. Place fluid in sterile, 12 × 75 mm snap-cap tubes, or other vials. Store at −70 °C .
5. Examine allantoic fluid for presence of rotavirus using RT-PCR .

Results

In the current study 20 embryonated eggs were used , 16 embryonated eggs inoculated with samples, while 4 eggs were control. showed that out of 16 embryonated eggs 25% of embryo were death while 75% were Dwarfism**.**

Table (1) Results of embryonated eggs inoculation

	No. of chicken embryo	**Death**	**Dwarfism**
Inoculation	**16**	**4(25 %)**	**12(75%)**
Control	**4**	**0**	**0**
Total	**20**		

Any case of death or dwarfism of embryo considered positive case.

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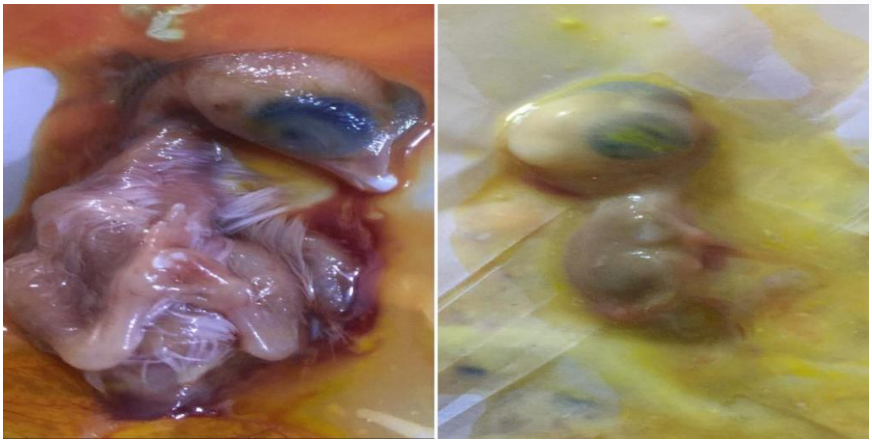

Figure (1). Showed sings of dwarfism .

Table (2) relationship between embryonated eggs inoculation and PCR test.

Results of RCR test	**Inculcated embryo with sample**	
	Death	**Dwarfism**
+ve	**4(100%)**	**8 (66.6%)**
-ve	**0 (0%)**	**4 (33.3%)**
Total	**4**	**12**

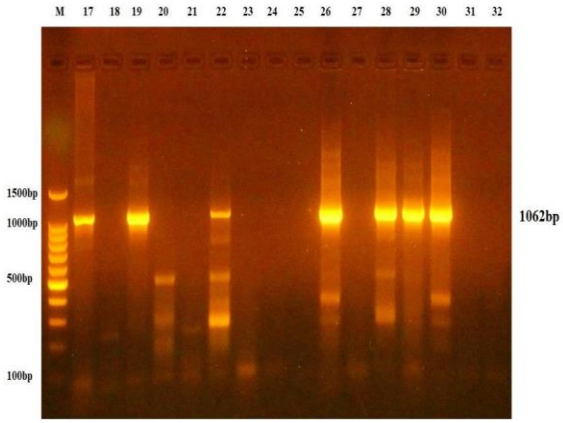

Figure (2) showed the result of PCR to conformation the results of chicken embryo inoculation.

Discussion

Our results firmly established clinical and virological parameters of group A rotavirus infection in embryonated eggs by showing that (1) viral replication and disease of rotavirus occurred in embryonated eggs as measured by virus: (2) disease was age dependent: (3) histopathological changes found in the intestine of chicken embryo: (4) rotavirus infection of eggs resulted in reduced growth of embryo. Analysis of allantoic fluid demonstrated that a complete virus replication occurred. The eggs model is being used to define parameters of infection, pathology and disease, According to the results

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of virus-inoculated seven, nine and eleven-days-old. In conclusion, chick embryo model that we described here will be very useful as an infective model to the study of infectivity test of avian rotavirus and mechanism of rotavirus gastrointestinal pathogenesis.

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