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A Comparative Study on Three Methods for Detection of Rotavirus from Patients with Diarrhea and Farm Animals

A Thesis

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1. Introduction

Introduction:

Today, Rotaviruses are recognized as the single most significant cause of severe gastroenteritis, malnutrition and diarrhea in young children in both developed and developing countries worldwide (Bishop, 2009). Although RV can infect older children and adults, diarrheal disease is primarily observed in children under 2 years of age (Bernstein, 2009). It accounts for 5% of all deaths in children younger than 5 years in developing countries. Mortality rates in developed countries on the other hand are very low and illness is usually self limiting (Black *et al*., 2010; Frieden *et al*., 2011).

The RV belongs to the *Reoviridae* family, exhibits icosahedral symmetry, it is not enveloped and was first identified by electron microscopy by Bishop *et al.*(1973). The viral particle consists of three layers of protein and the viral genome consists of 11 segments of double-stranded RNA (dsRNA), which encode six structural proteins, VP1-4, VP6 and VP7 and six non-structural proteins, NSP1-6 (Pesavento *et al*., 2006). The VP6 protein, located in the inner capsid of the virus contains the antigenic determinants, which allow their classification into seven serogroups of A to G, with group A being the most common agent of childhood diarrhea (Parashar *et al*., 2006). At present, 5 rotavirus serotypes (G1, G2, G3, G4, G9) are the predominant circulating strains, accounting for approximately 95% of strains worldwide, although there is considerable geographic variability (Santos and Hoshino, 2005; O'Ryan, 2009).

RVs are transmitted by fecal-oral spread, and possibly also by the respiratory route (Kapikian *et al*., 2001). It has an incubation period of 18-36 hours. Following ingestion, the virus attaches to and then replicates in the differentiated villous columnar epithelial cells (enterocytes) lining the upper small intestine. Death and desquamation of infected enterocytes impairs the normal digestive and absorptive processes, outcomes a watery diarrhea of 2-7 days duration, resulting in loss of fluids and electrolytes, which may lead to

severe or fatal dehydration. Rotavirus induced diarrhea is thought to be caused by a combination of factors (Lorrot and Vasseur, 2007), which include a reduction in epithelial surface area, replacement of mature enterocytes by immature cells, an osmotic effect resulting from incomplete absorption of carbohydrates from the intestinal lumen with bacterial fermentation of these non-absorbed compounds, secretion of intestinal fluid and electrolytes through activation of the enteric nervous system (Lundgren and Svensson, 2001; Ruiz *et al*., 2009), and the effect of the RV non-structural protein 4 (NSP4), which is thought to act as a viral enterotoxin (Borghan *et al*., 2007).

Rotaviruses are generally species-specific, but cross-species transmission is possible, causing economically significant malady in neonates of many domestic animals (Cook *et al*., 2004; Dhama *et al*., 2009). There is increasing evidence that animal rotaviruses can infect humans, either by direct transmission of the virus or by contributing one or several genes to reassortmants with essentially a human strain genetic background, as mixed infections are prerequisite for reassortment events (Gentsch *et al*., 2005; Muller and Johne, 2007). Therefore, animal rotaviruses are regarded as a potential reservoir for genetic exchange with human rotaviruses (Martella *et al*., 2010).

Initially, diagnosis of rotavirus infection was performed using electron microscopy, by visualization and observation of the rotavirus wheel-like appearance (Arcangeletti *et al*., 2005). Nowadays, the laboratory diagnosis of rotavirus infection is usually performed by antigen detection, using latex agglutination techniques, which is affirmed to be a good tool for the simple and rapid detection of RV in stool specimens (Kohno *et al*., 2000; Pirkooh and Shahrabadi, 2007). Enzyme-linked immunosorbant assays which are the most preferably used due to their high sensitivity and specificity for detection of RV in different pathological specimens (Ghazi *et al*., 2005;Ferreira *et al*., 2006; Vainio *et al*., 2009). More sensitive and newer methods are molecular techniques such as polyacrylamide gel electrophoresis (PAGE) and reverse

transcription-polymerase chain reaction (RT-PCR), which are used to determine the RV-RNA migration patterns and virus genotyping, respectively (Buesa *et al*., 1996).

RV-induced diarrhea now is considered to be a disease that can be prevented through vaccination (Vesikari, 2009). Two live attenuated oral rotavirus vaccines were licensed in 2006: Rotarix (GlaxoSmithKline), a human rotavirus vaccine with G1P[8] serotype characteristics, and RotaTeq (Merck), a bovine-human reassortant vaccine expressing human G1-4 and P[8] antigens. Rotarix is an oral live-attenuated human RV vaccine containing a single G1P[8] strain. It afforded sustained high protection against severe RV gastroenteritis during the first 2 years of life in a region with a changing pattern of wild-type RV circulation (O'Ryan and Linhares, 2009). RotaTeq is a three-dose, orally administered, live, pentavalent human-bovine reassortant RV vaccine used for the active immunization of infants for prevention of RV gastroenteritis (Plosker, 2010). With the introduction of these two vaccines in many countries, it appears that the total number of hospitalizations due to RV infections is being reduced, at least in developed countries that implemented a universal immunization program (Greenberg and Estes, 2009).

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Aims of the study

The present study was conducted to achieve the following aims.

1. The diagnostic capability of different laboratory techniques namely; rapid latex agglutination test, enzyme-linked immunosorbant assay, and polymerase chain reaction for the detection of RV in stool specimens of human and certain domestic animal species.

2. The effects of certain demographic factors on the detection rate by these laboratory techniques.

الخلاصة

أجريت هذه الدراسة للمدة من 2010/8/1 ولغاية 2011/8/30 في مدينة بعقوبة. هدفت الدراسة الى التحري عن القدرات التشخيصية للتقنيات المختبرية المختلفة و هي :تقنية التلازن المباشر، تقنية الاليزا و تقنية تفاعل البلمرة المتسلسل على الكشف عن الفيروس العجلي في نماذج البراز المأخوذة من الإنسان و بعض انواع الحيوانات الداجنة، و كذلك التحري عن تأثير بعض العوامل الديموغرافية على نسب الكشف بواسطة هذه التقنيات المختبرية.

شملت هذه الدراسة 120 مريضا ممن يعانون من الإسهال الحاد 70 من الذكور و 50 من الإناث تتراوح أعمارهم بين شهرين إلى خمس سنوات. جمعت معلومات ديموغرافية عن المرضى من قبل ذويهم فيما يتعلق بـ العمر، الجنس، محل السكن، نوع التغذية و مصدر مياه الشرب. فضلا عن ذلك شملت الدراسة 60 من الحيوانات الداجنة السليمة ظاهريا.

جمعت عينات البراز من كل المرضى و الحيوانات الداجنة. اجري الكشف عن الفيروس العجلي في نماذج البراز بواسطة اختبار التلازن المباشر، اختبار الاليزا و اختبار تفاعل البلمرة المتسلسل. اختبار الاليزا اجري في وحدة الفيروسات / مختبر الصحة المركزي في بعقوبة، بينما اختبار تفاعل البلمرة المتسلسل اجري في مختبر الفيروسات / كلية الطب البيطري / جامعة ديالى.

أظهرت نتائج الدراسة الحالية ارتفاعا في نسب الإصابة الكلية بالفيروس العجلي بين المرضى المصابين بالإسهال الحاد و باستخدام اختبار التلازن المباشر، اختبار الاليزا و اختبار تفاعل البلمرة المتسلسل حيث كانت 70%، 93.33% و 93.88% على التوالي. سجلت نسب إصابة عالية بين أولئك المرضى ممن تتراوح أعمارهم 15≤-10<اشهر وبوساطة الاختبارات