Antigenic and Pathogenicity Characterization of Rotavirus Commercial Rabbits

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Abstract

The epizootiological investigation of 28 diarrheal outbreaks showed that rotavirus affected about 30% from total diarreal outbreaks with a wide range variation in mortality percentages (2% to 80%). Also the clinical picture vary severity, so rotavirus infection differ in signs and mortality . Local field rotavi detected by monoclonal antigen capture enzyme-linked immunosorbant ass (Mc C-ELISA) which detect rotavirus in 15 fecal samples from total 50 sam in a percent of (30%) using monoclonal antibodies against VP6 which presen the inner shell of rotavirus. Pathogenicity study with local field rotavirus isola indicated that the results began at the 3rd day post inoculation (d.p.i) with 2 de cases then increased in the 4th day post infection (d.p.i)to 3 dead cases a decreased again to just one case in the 5th day. Rabbits after that from 6th to day (d.p.i) became nearly normal with no mortalities but with lower appetite a food conversion rates. Electron microscopy was done to identificate rotavirus the fecal samples of the challenged rabbits and it insured the presence rotavirus that shedded in the feces of infected rabbits. Finally, Histopathologi examination of different parts from small intestine of challenged rabbits ha been done to observe the pathological changes after infection.

Introduction

Rotaviruses are the major etiological agents of acute diarrhea for infants, as v as for young animals of many other mammalian (e.g., monkey, cow, pig, she horse, rabbit, mouse, dog and cat) and avian (e.g., chicken, turkey and pigeo species (Kapikian and Chanock, 1990). Rotaviruses are classified as a ge within the family Reoviridae on a basis of a characteristic morphology. doubled-shelled particles surrounding a genome of 11 segments of dou stranded RNA. Rotaviruses are assigned to seven groups (A to G) on the ba of serology and genome analysis (Pedley et al., 1983 and 1986; McNulty et 1984, and Saif and Theil, 1985). Three types of particles (Double-shell single-shelled and core) are often observed by electron microscope (EM) (Es and Cohen, 1989). The morphologic appearance of rotavirus particles distinctive. Intact virus particles resemble a wheel with short spokes and a w defined rim, when examined by negative-stain EM. The name rotavirus (fr Latin rota, meaning wheel) was suggested on the basis of this characteri feature (Flewet et al., 1974). Rotavirus infection signs differ in severity fr asymptomatic to sever diarrhea and death (Chrystie et al., 1978 and Conne al., 1988). Results of testing several types of rotaviruses showed that the vi like particles (VLPs) are immunogenic when administrated parentrally to rab (Ciarlet et al., 1998).The present study was directed towards epidemiological survey of rabbit rotavirus infection in some governorates Egypt, these included the detection of rotavirus by monoclonal antigen capt

enzyme-linked immunosorbant assay (Mc C-ELISA), pathogenicity study of selected isolates. Identification of rotavirus by electronmicroscopy. and histological examination of small intestine of rabbits infected by selected samples.

Material And Methods

Samples collection for detection of rotavirus:

A total of 50 fecal samples were collected from rabbits suffered from diarrhea , the ages of rabbits ranged between 25-50 days. The samples were collected from Menoufia, Gharbia, Qalyubia, Kafr El-Sheikh and Cairo. The samples were liquid, semi-solid and pasty in consistency and were collected from live and intestinal content of freshly dead rabbits. These samples were stored at -20°C and were used in Monoclonal Capture-ELISA. Also gross lesions in freshly dead rabbits were examined and recorded.

Reference antisera against rotavirus:

a- Polyclonal antibodies to Rotavirus:

Reference polyclonal antibodies against bovine rotavirus (BRV) were kindly supplied by Dr. Hussein Aly Hussein, Department of Virology, Faculty of Veterinary Medicine, Cairo University. The polyclonal antibodies were prepared at Dr. El-Azhary laboratory, Department of Virology, Faculty of Veterinary Medicine, Montreal University, Canada; the lyophilized polyclonal antibodies were resuspended in distilled water and used in a concentration of 1:100 in antigen capture ELISA (AC-ELISA).

b- Monoclonal antibodies to Rotavirus:

VP6 specific Mabs RQ34 (an IgG2a) and RQ64 (an IgG2b) were mixed in a ratio (1:1) and used in a concentration of 1:20. These Mabs were produced and characterized in Dr. El-Azhary laboratory, Department of Virology, Faculty of Veterinary Medicine, Montreal University, Canada. The Mabs were kindly supplied by Dr. Hussein Aly Hussein, Department of Virology, Faculty of Veterinary Medicine, Cairo University.

Rabbits used in Pathogenicity studies:

A total number of 21 rabbits of mixed sex and types; White Newzealand, Giant, German and Flemish. Their body weights were ranged from 700 to 800 gram. The rabbits were caged reared under natural day light in 2 isolated experimental rooms which previously cleaned and disinfected. Infected group were 18 in number reared in 4 cages; each cage measured 50×60 cm to contain 4-5 rabbits. Control group which were 3 in number reared in one cage in another room. Water was provided all time by nibbles (2 nibbles in each cage) and they feed twice per day by a fattening ration (as pellets) in the feeders. The rabbits were used for the following purposes

1- Pathogenicity studies of Rotavirus of selected positive samples to Mc C-ELISA.

2- Follow up the clinical signs and the mortality rate as a result of rotavirus infection.

3- Virus identification in experimental infected rabbits using electron microscope (EM) examination.

4- Histopathological studies of small intestine of infected and control rabbits.

Rotaviruses:

Two selected samples which were highly positive for rotavirus by AC-EL Ten folds dilution in a total of 10 ml (1 gm feces in 9 ml PBS) of feces were c inoculated for experimental infection as 1ml for each rabbit by sterile syringe

Phosphate buffered saline (PBS) pH 7.2-7.4:

Sodium Chloride [Sigma]	7.2 gram
Potassium Chloride [Sigma]	0.2 gram
Potassium Dihydrogen Phosphate [Sigma]	0.36 gram
Disodium Hydrogen Orthophosphate [Sigma]	3.83 gram
Distilled water up to	800 ml

- Adjust the pH to 7.2-7.4 using 1N HCL and complete the volume to 1 sterilized by autoclaving.

Antigen Capture ELISA:

VP6 specific Mabs RQ34 (IgG2a) and RQ64 (IgG2b) were mixed at a (1:1) and concentration of (1:20). The Mabs were the base of detecting gro rotavirus in the fecal samples. The indirect Monoclonal antibodies (Mabs) b antigen capture ELISA were done according to Hussein et al., 1995.

Electron Microscopy Examination of collected fecal samples experimentally infected rabbits:

The fecal samples collected from the 3rd and the 4th days post inoculati experimentally infected rabbits were examined in the EM unit in Vacse order to prove the virus shedding.

Material used in histopathological examination:

The duodenum, jejunum and ileum of the experimentally infected, and cc non infected rabbits were fixed in 10% neutral buffered formalin solution. samples were prepared for histopathological examination.

Experimental Design of Pathogenicity of selected rotavirus-positive samples designated: rabbit rotavirus 49/Menoufia/2008 and rabbit rota 50/Menoufia/2008.

Group No.	Number of Rabbits	Inoculum	Criteria adapted for evaluation of pathogenacity stu		
			Observation for 7 days from 3rd to 9th d.p.i.	Electron Microscopy	Histopathologi Examination
1	18	1 ml/rabbit orally From mixed fecal samples No. (49) &(50)	1-Clinical signs. 2-Mortality percentage. 3-Gross lesions.	*Rotavirus Identification in fecal samples by E.M	**For pathological lesions in sm intestine (duodenum, jejunum a ileum) of infec group.
2	3	Control Non Challenged			

d.p.i.= days post inoculation.

Inoculation at the age of 28 days (4 weeks).

* One mixed fecal sample was taken from the samples of the 3rd and 4th d.p.i.

** 8 fecal samples; one was taken as a control before rotavirus inoculation and 7 samples fror 9th d.p.i.

Result

Table (1) History and locality of examined farms:

Code No.	Date of collection	Location	Age	Code No.	Date of collection	Location	Age
1	May,2007	Menoufia	40d	26	Nov,2007	Menoufia	25d
2	Jun,2007	Menoufia	38d	27	Nov,2007	Menoufia	32d
3	Jun,2007	Menoufia	45d	28	Nov,2007	Menoufia	40d
4	Jun,2007	Menoufia	29d	29	Nov,2007	Cairo	29d
5	June,2007	Gharbia	40d	30	Nov,2007	Cairo	35d
6	June,2007	Gharbia	45d	31	Dec,2007	Menoufia	35d
7	June,2007	Menoufia	40d	32	Dec,2007	Menoufia	30d
8	Jun,2007	Menoufia	30d	33	Dec,2007	K. El Sheikh	35d
9	Aug,2007	Gharbia	40d	34	Dec,2007	K. Sheikh	45d
10	Aug,2007	Gharbia	30d	35	Dec,2007	Menoufia	35d
11	Aug,2007	Menoufia	32d	36	Jan,2008	Menoufia	40d
12	Aug,2007	Menoufia	29d	37	Jan,2008	Menoufia	30d
13	Aug,2007	Menoufia	35d	38	Jan,2008	Qalyubia	25d
14	Aug,2007	K. Sheikh	35d	39	Jan,2008	Qalyubia	35d
15	Aug,2007	K. El Sheikh	35d	40	Jan,2008	Qalyubia	50d
16	Aug,2007	K. Sheikh	45d	41	Feb,2008	Menoufia	40d
17	Aug,2007	K. Sheikh	35d	42	Feb,2008	Gharbia	30d
18	Aug,2007	K. Sheikh	40d	43	Feb,2008	Gharbia	45d
19	Aug,2007	K. Sheikh	40d	44	Feb,2008	Gharbia	50d
20	Aug,2007	K. Sheikh	35d	45	Mar,2008	Menoufia	28d

21	Sept,2007	Menoufia	40d	46	Mar,2008	Menoufia	35d
22	Sept,2007	Gharbia	45d	47	Apr,2008	Menoufia	35d
23	Sept,2007	Gharbia	40d	48	Apr,2008	Menoufia	50d
24	Nov,2007	Menoufia	32d	49	May,2008	Menoufia	30d
25	Nov,2007	Menoufia	35d	50	May,2008	Menoufia	40d

Table (2) Mortality rates in examined farms:

Code No.	Location	Age	Mort %	Code No.	Location	Age	Mort. %
1	Menoufia	40d	80%	26	Menoufia	25d	70%
2	Menoufia	38d	50%	27	Menoufia	32d	40%
3	Menoufia	45d	2%	28	Menoufia	40d	2%
4	Menoufia	29d	90%	29	Cairo	29d	20%
5	Gharbia	40d	50%	30	Cairo	35d	30%
6	Gharbia	45d	3%	31	Menoufia	35d	90%
7	Menoufia	40d	30%	32	Menoufia	30d	10%
8	Menoufia	30d	90%	33	K. Sheikh	35d	3%
9	Gharbia	40d	15%	34	K. Sheikh	45d	5%
10	Gharbia	30d	15%	35	Menoufia	35d	70%
11	Menoufia	32d	90%	36	Menoufia	40d	10%
12	Menoufia	29d	90%	37	Menoufia	30d	15%
13	Menoufia	35d	2%	38	Qalyubia	25d	50%
14	K. Sheikh	35d	30%	39	Qalyubia	35d	6%
15	K. Sheikh	35d	10%	40	Qalyubia	50d	4%
16	K. Sheikh	45d	10%	41	Menoufia	40d	40%
17	K. Sheikh	35d	30%	42	Gharbia	30d	70%

18	K. Sheikh	40d	30%	43	Gharbia	45d	4%
19	K. Sheikh	40d	60%	44	Gharbia	50d	70%
20	K. Sheikh	35d	80%	45	Menoufia	28d	80%
21	Menoufia	40d	5%	46	Menoufia	35d	70%
22	Gharbia	45d	20%	47	Menoufia	35d	60%
23	Gharbia	40d	2%	48	Menoufia	50d	8%
24	Menoufia	32d	50%	49	Menoufia	30d	80%
25	Menoufia	35d	30%	50	Menoufia	40d	70%

Table (3) Clinical symptoms in examined farms:

Code No.	Clinical symptoms	Code No.	Clinical symptoms
1	-Brown diarrhea, stained abdomen, lameness, bloat.	26	-Sever diarrhea, emaciation, lameness, off food.
2	-Diarrhea, emaciation, bloat.	27	-Nervous signs, diarrhea, bloat.
3	-Normal signs.	28	-Normal signs.
4	-Sever diarrhea, nervous signs, emaciation, stained abdomen.	29	-Diarrhea with bloat, less food consumption.
5	-Brownish diarrhea with offensive odor, emaciation.	30	-Mucoid diarrhea, general depression.
6	-Some cases with soft feces.	31	-sever diarrhea, stained abdomen, off food.
7	-Mucoid diarrhea, depression, bloat.	32	-stick feces into anus, soft feces.
8	-Sever diarrhea with offensive odor, bloat and emaciation.	33	-Normal signs.
9	-Diarrhea, bloat.	34	-Normal signs.
10	-Diarrhea, bloat.	35	-Sever lameness, Mucoid diarrhea, emaciation.
11	-Mucoid diarrhea, emaciation, stained abdomen, off food.	36	-Cases of diarrhea with bloat.
12	-Bloody diarrhea, emaciation, bloat, lameness.	37	-Diarrhea with bloat.

13	-Normal signs.	38	-Brownish diarrhea, offensive odor, stained abdomen.
14	-Diarrhea in some cases, bloat.	39	-some cases with soft feces.
15	-Soft feces, low food consumption.	40	-Slight soft feces with normal signs.
16	-Sudden deaths, soft feces.	41	-Mucoid diarrhea, bloat, some with nervous signs.
17	-Diarrhea with offensive odor, bloat.	42	-Sever diarrhea, emaciation, bloat, stained abdomen.
18	-Diarrhea , bloat, stained abdomen.	43	-Normal signs.
19	-Mucoid diarrhea, off food, emaciation.	44	-Diarrhea, bloat, emaciation, lameness.
20	-Sever diarrhea, emaciation, lameness, nervous signs, off food.	45	-Sever diarrhea, bloat, lameness, stained abdomen.
21	-Normal signs.	46	-Diarrhea with bloat.
22	-sudden deaths, slight diarrhea.	47	-Mucoid diarrhea, emaciation, bloat, stained abdomen.
23	Normal signs.	48	-Some diarrhea, bloat.
24	-Mucoid, bloody diarrhea with bloat.	49	-Sever diarrhea, bloat, emaciation.
25	-Diarrhea with offensive odor, bloat.	50	-Sever diarrhea, bloat, emaciation.

Table (4) The results of the positive rotavirus and negative samples b AC-ELISA:

Code No.	Age	Location	ELISA test results	Code No.	Age	Location	ELISA test results
1	40d	Menoufia	-ve	26	25d	Menoufia	-ve
2	38d		-ve	27	32d		+ve
3	45d		-ve	28	40d		+ve
4	29d		-ve	29	29d	Cairo	+ve
5	40d	Gharbia	-ve	30	35d		+ve
6	45d		-ve	31	35d	Menoufia	-ve
7	40d	Menoufia	-ve	32	30d		-ve
8	30d		-ve	33	35d	Kafr El-Sheikh	-ve
9	40d	Gharbia	-ve	34	45d		-ve
10	30d		-ve	35	35d	Menoufia	-ve
11	32d	Menoufia	-ve	36	40d		-ve
12	29d		-ve	37	25d		+ve
13	35d		-ve	38	25d	Qalyubia	+ve
14	35d	Kafr	-ve	39	35d		-ve

15	35d	El-Sheikh	-ve	40	50d		-ve
16	45d		-ve	41	40d	Menoufia	-ve
17	35d		-ve	42	30d	Gharbia	+ve
18	40d		-ve	43	45d		+ve
19	40d		-ve	44	50d		+ve
20	35d		-ve	45	28d	Menoufia	+ve
21	40d	Menoufia	-ve	46	35d		+ve
22	45d	Gharbia	-ve	47	35d		+ve
23	40d		-ve	48	50d		+ve
24	32d	Menoufia	-ve	49	30d		+ve
25	35d		-ve	50	40d		+ve

Table (5): The mortality percentage in the ELISA positive samples for rotavirus:

Code No.	Age	mortality	Location
27	32d	40%	Menoufia
28	40d	2%	
29	29d	20%	Cairo
30	35d	30%	
37	30d	15%	
38	25d	50%	Qalyubia
42	30d	70%	Gharbia
43	45d	4%	
44	50d	70%	
45	28d	80%	Menoufia
46	35d	70%	
47	35d	60%	
48	50d	8%	
49	30d	80%	
50	40d	70%	

Table (6) Pathogenicity studies of weaned rabbits challenged orally with 2 selected rotavirus positive samples designated as (rabbit rotavirus 49/Menoufia/2008 and rabbit rotavirus 50/Menoufia/2008):

Group No.	Treatment	Number	Inoculum	Mortality observation at day post inoculation										
													Mortality	
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	Total	Percentage
I	Infected	18	1 ml/rabbit orally From mixed fecal samples No. (49) &(50)											
II	Control	3		0	0	3	2	1	0	0	0	0	6	33.3%

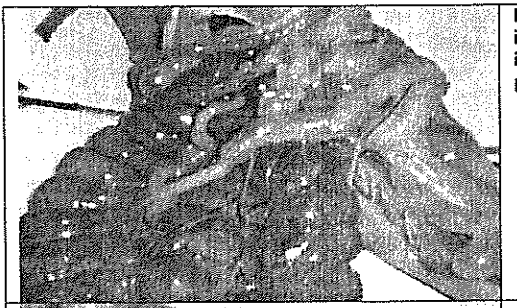


Fig.(1): Congested intestine, the small intestine is filled with gases 5 days post inoculation.

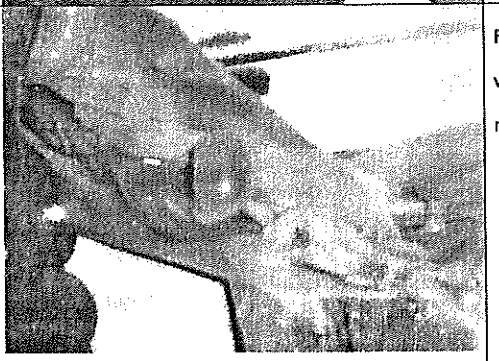
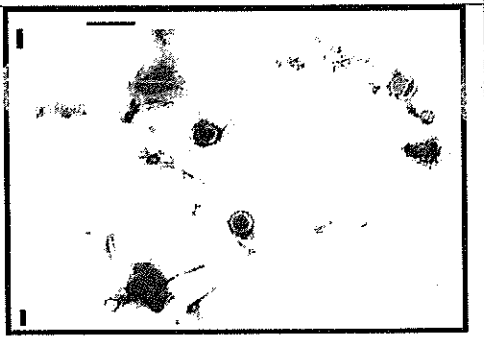


Fig.(2): The small intestine has thin wall andfilled with gases in P.M .of experimentally infected rabbits with rotavirus 4 days post inoculation.



Electron microscopy picture of Rotavirus detected in fecal samples using negative stain technique:

Fig.(3): Characteristic features of rotavirus particles with EM 4 days post inoculation (rounded particle with a thin, smooth, well-defined outer capsid surrounding the spoke-like projections of the inner capsid).

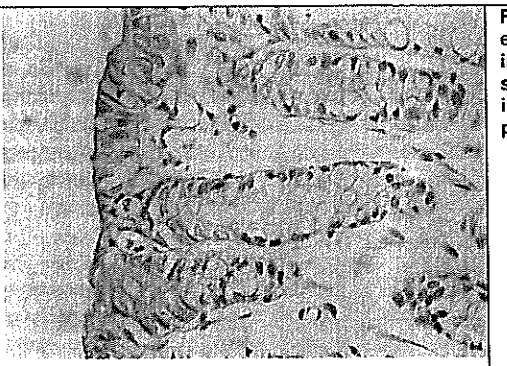


Fig.(4): Vacules in the epithelial cells of the villi and in the duodenal glands in the small intestine with inflammatory oedema 3 days post inoculation (H&E X40).

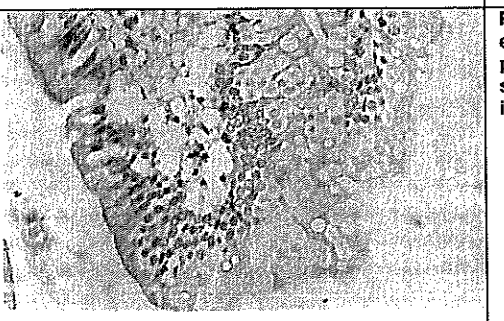


Fig.(5): Hyperplasia of the epithelial cells with the presence of vacules in the small intestine 3 days post inoculation.

Discussion

Rotaviruses are the major etiological agents of acute diarrhea for infa
well as for young animals of many other mammalian species including
and avian species (Kapikian and Chanock, 1990). Rotaviruses are mem
the family Reoviridae and represent the most important etiologic agents
gastroenteritis in the young of many animal species, including humans (K
et al., 2001). To investigate the nature of rotavirus outbreaks in rabbi
and to detect rotavirus presence in Egypt, 22 diarrheal outbreaks ir
different types of rabbits at the age ranged from(25 to 50 days)
governorates over the period 2007 and 2008 were studied as showed in
& 2). Affected rabbits showed several forms of the clinical signs, some
mild signs which were defined as slightly soft, wet fecal stained anus.
showed moderate signs which were defined as soft to semi-liquid feces v
fecal stained abdomens and legs and sever signs in some cases as ob
liquid feces with fecal stained perineum with anorexia were found as sh
table (3).

This variation in signs severity was due to both viral and host fac
reported by (Conner and Raming, 1997). Among the viral factors are: (1
VP4 alleles may be associated with asymptomatic disease (Flores et al.,
(2) Virus strains can be attenuated. Attenuation generally results in a re

ability to replicate and cause disease in the host (Hall et al., 1993). (3) Virus strains seem to be adapted for growth in particular host species (Host range) (Broome et al., 1993).

Several host factors also can affect the severity of rotavirus disease including: (1) Malnutrition can increase the severity of rotavirus diarrhea (Steel and Torres-Medina, 1984), that it delays the small intestinal recovery (Zhang et al., 2000) and intestinal inflammatory response (Zijlstra et al., 1999). (2) Rotavirus symptoms are generally age restricted (Conner and Raming, 1997). Age restriction may be related to immunity, as neutralizing antibodies increase with age and virus exposure (Morris et al., 1999). (3) Rotavirus disease may be related to age-dependent protease expression, as viral infectivity requires protease cleavage of VP4 and new-borns have low levels of protease in the gut (Graham et al., 1984). The expression of intestinal mucins and the rate of epithelial cell replacement and fluid absorption are both age dependent and have been shown to affect rotavirus infection and disease in the host (Moon, 1994 and Yolken et al., 1992). The investigations showed that 30% of these outbreaks were naturally infected with rotavirus as showed in table (4). The infected rabbits showed several signs; some were asymptomatic similar to described by (Chrystie et al., 1978), others showed mild, moderate and sever signs like findings that were reported by (Conner et al., 1988). Gross lesions ranged from normal to typical gross lesions as described by (Wary et al., 1981, b).

The dead rabbits showed congested intestine with presence of fluid and gas in the small intestine. The most susceptible age was around weaning period with different morbidity and mortality rates as reported by (Peeters et al., 1982; DiGiacomo and Thouless, 1984 and Schoeb et al., 1986), that there were a wide range of mortality rates in infected flocks with rotavirus (from 2% to 80%) as shown in table (5).The biosecurity, management and hygiene were suboptimal in many investigated farms. Some were land rearing with bad cleaning and disinfection so the disease easily transmitted among rabbits. Other farms suffer from bad ventilation which affects the viability and immunity of the rabbit. Rotavirus was detected by Mc C-ELISA, rotavirus antigen was detected in 3o% (15/50) of total collected fecal samples as showed in Table (4).

There were two ELISAs described for rotavirus antigen in feces, which were designed to be as sensitive and specific as possible and easy to use anywhere. Both are indirect methods, using the antibody capture method. In our study, we used the assay which utilizes rotavirus group-specific monoclonal "detecting" antibody instead of the hyperimmune polyvalent guinea pig antisera used in the other assay, this like what reported by (Beards et al., 1984) In this study, Monoclonal ELISA was used. This assay primarily detect epitopes specific-antibodies directed against the highly conserved inner capcid protein VP6 as reporter by (Macartney and Offit, 2000).

Group A rotavirus which infect rabbits are routinely detected by ELISA, which identifies the group-specific antigen (James et al., 1998). Utelizing ELISA based on the monoclonal antibodies have proved its efficacy and sensitivity in

detecting rabbit rotavirus directly in fecal samples. The success in ELISA u current study was previously reported in similar study on BRV in Qu Canada by (Hussein et al., 1995).The assay was based on the combinat two VP6 specific monoclonal antibodies. Several factors contributed in ELISA have been modified including Mabs concentration, the dilute conjugate and the substrate. The VP6-ELISA used in this study has prove presence of rabbit rotavirus in young rabbits with a percentage of 30% (1 Yet, ELISA is more efficient and sensitive than other techniques (Yolken 1978). The pathogenicity study by selected fecal samples was done. 4- old rabbits were challenged by selected detected rotavirus of samples No and (50) by ELISA from collected field samples, using the highly p samples to make an inoculum. The single dose for one rabbit was 1 ml diluted selected samples (1 gm/ 9 ml PBS) orally by a blunt syringe, control group in a separate room in order to record the effects of the ro infection. The mortality and the clinical signs observation was recorded fro 3rd d.p.i. there were 2 dead cases. In the 4th d.p.i. there were 3 dead case deaths decreased in the 5th d.p.i. to just 1 dead case. From 6th to 9th d.p.i were not any dead cases as showed in table (6). Infected rabbits recover with less appetite and food conversion rates. Diarrhea was the main clinic observed, it varies from slightly soft to soft semi-liquid to liquid fec described by (Conner et al., 1988).

Gross lesions on post-mortem examination were mainly in the intestine, congested as in figure (1& 2) with the presence of gases and fluid a reported by (Wary et al., 1981, b and Thouless et al., 1988). The el microscopy was used to detect and identificate rotavirus in experim infected rabbits. Initially direct visualization of stool material by e microscopy was employed for rotavirus detection (Flewett et al., 197 Brandt et al., 1981). Electron microscopy is important in the detec rotaviruses which have a distinctive morphologic appearance, also EM most rapid diagnostic method on fecal samples by staining phosphotungestic acid and examined directly within few minutes of coll EM examination permits detection of rotavirus in 80% to 90% of virus p fecal samples (Brandt et al., 1981 and Nakata et al., 1987). Fecal samples 3rd and 4th days post inoculation were mixed and ultracentrifugated to r.p.m. to concentrate the virus then examined directly by EM which ga characteristic morphological appearance of double shelled layers of rotav figures (3) That confirmed the rotaviral shedding in the feces experimentally infected rabbits.Histological examination of the experim infected rabbits was done to record the histological effects of rotavirus in on young rabbits.

Different parts from the small intestine were taken from the 3rd to the 9 and examined under light microscope. In the 3rd d.p.i , there was a hyperp the epithelial cells with vaculation as in figures (4&5). In the 4th d.p.i., the more sever hyperplasia with vaculation and inflammatory oedema. In d.p.i., mild hyperplasia with lymphocytes infiltration in the lamina propri observed. Lesions in the 6th d.p.i became milder and to the 9th d.p.i. ther mild lymphocytes infiltration with mild vaculation. Differentiated epithelial c

villi in the small intestine (enterocytes) are the main target of rotavirus infection (Osborne et al., 1988 and Lundgren et al., 2000). Rotaviruses replicate in the non dividing mature enterocytes near the tips of the villi, and the pathological changes are almost exclusively limited to the small intestine. There is no absolute correlation between histological lesions and disease symptoms (Conner and Raming, 1997). Finally, the present study reports the existence of the rotavirus infection in rabbits in Egypt and in some flocks with very high rates up to 80% causes sever economic losses with no critical treatment, so it needs to give more attention to make a special vaccination program for rotavirus to control the rotavirus infection in young rabbits in Egypt.

References

Beards, G.; A. Campbell; N. Cottrell; J. Peiris; R. Sanders; J. Shirley; H. Woode and T. Flewett (1984): ELISAs based on polyclonal and monoclonal antibodies for rotavirus detection. J. Clin. Microbiol. 19(2):248-254.

Brandt, C. D.; H. W. Kim and W. J. Rodriguez (1981): Comparison of direct electron microscopy, Immune electron microscopy and rotavirus enzyme-linked immunosorbant assay for detection of gastroenteritis viruses. J. Clin. Microbiol. 13:976-981.

Broome, R. L.; P. T. Vo; R. L. Ward; H. F. Clark and H. B. Greenberg (1993): Murine rotavirus genes encoding outer capsid proteins VP4 and VP7 are not major determinants of host range restriction and virulence. J. Virol. 67:2448-2455.

Chrystie, I. L.; B. M. Totterdell and J. E. Banatvala (1978): Asymptomatic endemic rotavirus infections of the newborn. Lancet i: 1176-1178.

Ciarlet M.; Sue E. Crowford; C. Barone; A. B. Ciarlet; R. F. Raming; M. K. Estes and M. E. Conner (1998): Subunit rotavirus vaccine administered parenterally to rabbits inducesactive protective immunity. American Society for Microbiology. Journal of Virology, November 1998, p. 9233-9246, Vol. 72.

Conner M. E.; M. K. Estes and D. Y. Graham (1988): Rabbit model of rotavirus infection. American Society for Microbiology. Journal of Virology, May 1988, p. 1625-1633.

Conner, M. E. and R. F. Ramig (1997): Viral enteric diseases, p. 713-743. In N. Nathanson (ed.), Viral pathogenesis. Lippincott-Raven Publishers, Philadelphia, Pa.

DiGiacomo, R. F. and M. E. Thouless (1984): Age-related antibodies to rotavirus in New Zealand rabbits. J. Clin. Microbiol. 19:710-711.

Estes M. K.. and J. Cohen (1989): Rotavirus Gene structure and function. American Society for microbiology. Microbiological Reviews, Vol. 53, P. 410-449.

Flewett, T. H.; A. S. Bryden and H. Davies (1974): Diagnostic electron microscopy of feces. J. Clin. Pathol. 27:603-614.

Flewett, T. H.; A. S. Bryden and H. Davies (1972): Virus particles in gastroenteritis (letter) Lancet 2:1497.

Flores, J.; K. Midthun; Y. Hoshino; K. Green; M. Gorziglia; A. Z. Kapikian and R. M. Chanock (1986): Conservation of the fourth gene among rotaviruses recovered from asymptomatic newborn infants and its possible role in attenuation. J. Virol. 60:972-979.

Graham, D. Y.; J. W. Sackman and M. K. Estes (1984): Pathogenesis of rotavirus-induced diarrhea: Preliminary studies in miniature swine piglet. Dig. Dis. Sci. 29:1028-1035.

Hall, G. A.; J. C. Bridger; K. R. Parsons and R. Cook (1993): Variation in rotaviruses virulence: A comparison of pathogenesis in calves between two rotaviruses in different virulence. Vet. Pathol. 30:223-233.

Hussein, H. A.; E. Cornaglia; M. S. Saber and Y. El-Azhary (1995, a): Prevalence of serotypes G6 and G10 group A rotaviruses in dairy calves in Quebec. Canadian. J. Vet. Res. 59:235-237.

James, V. L. A.; P. R. Lambden; E. O. Xaul and I. N. Clarke (1998): Enzyme-linked immunosorbant assay based on recombinant human group C rotavirus inner capsid protein VP6 to detect human group C rotaviruses in fecal samples. J. Clin. Microbiol. 36(11):3178-3181.

Kapikian, A. Z., and R. M. Chanock (1990): Rotaviruses, p. 1353- 1404. In B. N. Fields, D. M. Knipe, R. M. Chanock, M. S.Hirsch, J. L. Melnick, T.P. Monath and B. Roizman (ed.), Virology, 2nd ed. Raven Press, New York.

Kapikian, A. Z.; Y. Hoshino and R. M. Chanock (2004): Rotaviruses, p. 1787-1834. In D. M. Kr M. Howley; D. E. Griffin; R. A. Lamb; M. A. Martin; B. Roizman and S. E. Straus (ed.), virology, 4th ed. Lippincott/The Williams & Wilkins Co., Philadelphia, Pa.

Lundgren, O.; A. T. Peregrin; K. Persson; S. Kordasti; I. Uhnoo and L. Svensson (2000): Role enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. S 287:491-495.

Macartney, K. K. and P. A. Offit (2000): Immunologic correlates of protection. In Rotav Methods and Protocol, J. Gray and U. Desselberger (ed.), Human press, Totow Jersey, p.121.

McNulty, M. S.; D. Todd; G. M. Allaqn; J. B. McFerran and J. A. Greene (1984): Epidemic rotavirus infection in broiler chickens: recognition of four serogroups. Arch. Virol. 81:11

Moon, H. W. (1994): Pathophysiology of viral diarrhea, p. 27-52. In A. Z. Kapikian (ed. infections of the gastrointestinal tract. Marcel Dekker, Inc. New York.

Morris, A. P.; J. K. Scott; J. M. Ball; C. Q. Zeng; W. K. O'Neal and M. K. Estes (1999): NSP4 age-dependent diarrhea and Ca^{+2} mediated I⁻ influx into intestinal crypts of CF mice. Physiol. 277:G431-G444.

Nakata, S.; B. L. Petrie; E. P. Calomeni and M. K. Estes (1987): Electron microscopy pro influences detection of rotaviruses. J. Clin. Microbiol. 25(10):1902-1906.

Osborne, M. P.; S. J. Haddon; A. J. Spencer; J. Collins; W. G. Starkey; T. S. Wallis; G. M. C J. Worton; D. C. Candy and J. Stephen (1988): An electron microscopic investigation related changes in the intestine of neonatal mice infected with murine rotavirus. J. Gastroenterol. Nutr. 7:236-284.

Pedley, S.; F. Hundley; I. Chrystie; M. A. McCrae and U. Desselberger (1983): The genc rotaviruses isolated from chronically infected immunodeficient children. J. Gen 65:1141-1150.

Pedley, S.; J. C. Bridger; D. Chasey and M. A. McCrae (1986):Definition of two groups of rotavirus. J. Gen. Virol. 67:131-137.

Peeters, J. E.; G. Charlier and E. Opdenbosch (1982): Rotavirus in commercial suckling some preliminary observations. Vet. Bull. 52:724.

Saif, L. J. and K. W. Theil (1985): Antigenically distinct rotaviruses of human and animal o 208-214. In S. Tsipori (ed), Infectious diarrhea in the young. Elsevier Science Publish New York.

Schoeb, T. R.; D. B. Casebolt; V. E. Walker; L. N. D. Potgieter; M. E. Thouless and R. F. Di (1986): Rotavirus-associated diarrhea in a commercial rabbitry. Lab. Anim. Sci. 36:14

Steel, R. B. and A. Torres-Medina (1984): Effects of environmental and dietary factors on rotavirus infection in gnotobiotic piglets. Infect. Immun. 43:906-911.

Wary, C.; M. Dawson; A. Afshar and W. Sojka (1981, b): Experimental diarrhea in lambs. Z Med. B, 31:381-390.

Yolken, R. H.; B. Babbour; R. G. Wyatt; A. R. Kalica; A. Z. Kapikian and R. M. Chanock Enzyme linked immunosorbant assay for identification of rotaviruses from differen species. Science, 201:259-262.

Yolken, R. H.; L. A. Peterson; S. L. Vondrfecht; E. T. Fouts; K. Midthun and D. S. Newburg Human milk mucin inhibits rotavirus replication and prevents experimental gastroent Clin. Investig. 90:1984-1991.

Zhang, M. C. Q-Y. Zeng; A. P. Morris and M. K. Estes (2000): A functional NSP4 enterotoxin secreted from rotavirus-infected cells. J. Virol. 74:11663-11670.

Zijlstra, R. T.; B. A. McCracken; J. Odle; S. M. Donovan; H. B. Gelberg; B. W. Petscho Zuckermann and H. R. Gaskins (1999): Malnutrition modified pig small i inflammatory responses to rotavirus. J. Nutr. 129:838-843.

الملخص العربى

التوصيف الجينى والمرضى لفيروس الروتا فى الارانب التجارية

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أجريت دراسات وبائية على حالات الإسهال فى مزارع الأرانب والتى حدثت خلال الفترة من ٢٠٠٧ الى ٢٠٠٨ .

وقد اشتملت الدراسات على عزل فيروس الروتا من العينات الحقلية باستخدام اختبار الأجسام المضادة الوحيدة النسلية وكذلك تم تأكيد وجود الاصابة بفيروس الروتا فى ١٥ عينة من عدد كلى يبلغ ٥٠ عينة.وكانت نسب النفوق تختلف فى معدلاتها بين ٢% الى ٨٠% فى مختلف الأنواع من الأرانب.

أجريت العدوى الاصطناعية بالمعزولات الحقلية رقم ٤٩ و ٥٠ والتى اكدت أعلى ايجابية لوجود الفيروس لأرانب عمر ٤ أسابيع وقد أسفرت نتائج العدوى التجريبية عن حدوث وفيات بدأ من اليوم الثالث بعد العدوى يتمثل فى حالتين ويتزايد العدد فى اليوم الرابع ليصبح ثلاث ثم يعود للانخفاض مرة أخرى فى اليوم الخامس الى حالة واحدة فقط.

ولقد تم التأكد من نزول فيروس الروتا فى منزلات الارانب المختبرة بالكشف بواسطة الميكروسكوب الالكترونى وتمييزه بشكله الحلقى.

تم اجراء فحص للأمعاء الرفيعة للأرانب المختبرة والمعدية اصطناعيا ولقد وجدت تغيرات على مستوى الخلايا ووجود فجوات وسوائل بين الخلايا بالميكروسكوب الضوئى.