

ASEROLOGIC SURVEY OF BOVINE ROTAVIRUS INFECTION IN EGYPT

(With 2 Tables and 5 Figures)

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مسح سيرولوجى للعدوى بفيرس الروتا فى مصر

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تم تجميع ٢٨٩ عينة سيرم من محافظات مصر المختلفة ممثلة فى الجيزة وبنى سويف والأسكندرية ودمياط والوادي الجديد من العجول القطام والأبقار وذلك لأجراء اختبار التعادل فى السيرم وقد وجد أن نسبة الأجسام المضادة فى أمصال تلك الحيوانات قد وصل معدلها ٨٥.٧% فى العجول ، ٥٠% فى الأبقار فى محافظة الجيزة ، وقد وصل معدلها ٦٦.٦% فى العجول ، ٣٣.٣% فى الأبقار فى محافظة بنى سويف أما محافظة دمياط قد وصلت النسبة ٥٤.٥% فى العجول و٣٦.٤% فى الأبقار وقد وصلت نسبة العدوى فى محافظة الأسكندرية ٣.٨% فى العجول و٣% فى الأبقار ولم تكن هناك أى نسبة للإصابة فى محافظة الوادي الجديد. وقد تم إجراء اختبار الاليزا واختبار الفلورسنت المشع على العينات الايجابية باختبار التعادل فى السيرم وكانت النتائج مطابقة ودلت على وجود العدوى وانتشار مرض الروتا فى مصر حيث أنه لا يوجد تحصين للقاح الروتا فى تلك المزارع من قبل مما يعذى لوجود العدوى ومما يلزم دراسة العترات المحلية وذلك لامكان انتاج لقاح محلى للسيطرة على هذا المرض فى مصر.

SUMMARY

A total of 289 serum samples were collected from calves and adult cows in Giza, Beny Sweif, Alexandria, Demiat and New Valley governorates. A Serum neutralization test (SNT) was conducted by microtitre on MA 104 cells, the results showed that rota virus infection exists in the country, where it reached 85.7% in calves, 50% in adult cows in Beny Sweif governorates, 54.5 in calves and 36.4% in cows in Demiat and reached 3.8% in calves and 3% in cows in Alexandria governorates while new valley governorate was clean. the result obtained by SN was reaffirmed by ELISA and indirect flourecent antibody technique.

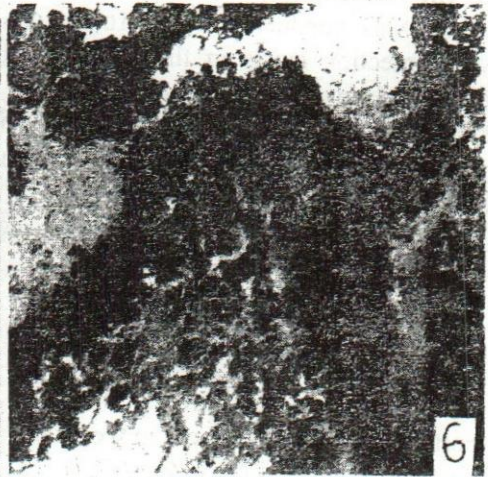
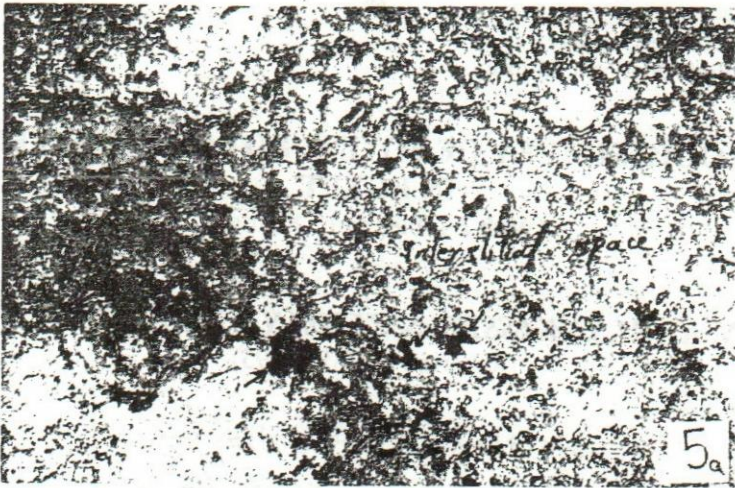
Keywords: Serology-Rotavirus- Bovine

INTRODUCTION

The increasing spread of rota virus infection in neonatal calves appear to be a major cause of diarrhoea, producing high economic loss directly through mortality during the first two weeks of life (CILLI and CASTRUCCI, 1981).

Isolation of rota virus from feces of newborn diarrhoic calvs in Egypt was reported by SHALABY *et al.* (1981) and WAFAA (1994).

In view of the occurrence of neumours deaths in this winter in newborn calves in many governmental farms in Egypt with symptoms of acute watery mucoid



and bloody diarrhoea and rise of body temperature with high mortality 90% and no vaccination against rota virus, it was suspected that rota virus was the cause in many cases and this infection is widespread throughout different Egyptian farms all over the country.

The aim of this survey is to affirm the presence of bovine rota virus infection in the country, incidence of positive reactors in serum of farm animal in Giza, Beny Sweif, Demiate, Alexandria and New valley governorates and to determine its distribution in the investigated governorates.

So serum samples were collected from different governorates from adult cows and calves.

MATERIALS and METHODS

A. Materials:

Virus strain:-

Bovine rota virus (Nebraska calf diarrhoea virus strain), *NAWAL, et al. (1994)* was used in this work after being propagated in MA 104 cell line using trypsin treatment 5 ug/ml to enhance virus infectivity (*THEIL et al., 1977*). Propagated virus was harvested by subjecting the infected cells three times for freezing and thawing, followed by centrifugation at 3000 rpm/30 minutes at 4°C, original virus titre was calculated to be 10^7 TCID₅₀/ml according to Reed and Muench, (1933), supernatant was concentrated 10 x using polyethylene glycol 6000. This virus concentrate has been used as an antigen for coating ELISA plate at a final dilution 1:50 (*NAWAL et al., 1994*).

Cell culture:-

Cell line fetal Rhesus monkey kidney (MA 104) was grown and maintained as described by *CASTRUCCI et al. (1988)*.

Serums:-

Sera samples were obtained from calves and adult cows from different governorates represent lower Egypt (Dmiate, Alexandria governorates, Upper Egypt (Giza and Beny Sweif governorates) and desert area (New Valley Governorate). The collected sera were kept at -20°C and inactivated at 56°C for 30 minutes.

B. Methods:

1. Serum neutralization tet (SNT) was carried out in 96-well microtitre plate using Nebraska Bovine rota strain as an antigen at its 8th passage level in MA 104 cell culture, each serum sample was diluted in 2 fold steps, to each of the later a 100 TCID₅₀/ml of the antigen in a volume of 0.050 ml/well, following incubation for 60 minutes at 37°C, MAL04 suspended in MEM containing 0.5% volume of fetal calf serum and 5 ug/ml of trypsin were mixed with each serum-virus mixture, Rota virus and positive serum against Rota virus mixture, and a control cells were left as a control, the test was incubated at 37°C and read on the 3rd day (*SATO. et al., 1981*).

2. ELISA Procedure:

Elisa microplates were coated with rota virus antigen with 1:50 dilution in carbonate-bicarbonate Buffer, pH 9.6 followed by incubation of the plates at 4°C for 20 hours, *VALLER, et al., (1976)*, the coated ELISA Microplates

were blocked by using PBS pH 7.4 containing 10% skimmed milk and 0.2% tween 20 and kept for 2 hours at 37°C, the tested bovine sera as well as positive and negative control sera were diluted into 1:10 and dispensed in duplicated raw in the blocked ELISA plates (FOLKEN *et al.*, 1980). The ELISA microplates were incubated at 37°C for 1 hour, the plates then washed 3 times with 5 minute apart with PBS. pH 7.4 containing 0.2% tween 20 (PBS-T). Secondary antiovine peroxidase added to all wells in 1:300 dilution in PBS. pH 7.4 containing 5% skimmed milk and left for 1 hour at 37°C, the plate then washed with PBS-T and then followed by addition of 100 ul of substrate solution (5-amino salicylic acid) and H₂O₂, the developed colour have been read in SLT spectra (USA) Elisa reader at an optical density (FOLKEN *et al.* 1981) and NAWAL *et al.* (1994). The Elisa titre then calculated according to SNYDER *et al.* (1984).

3. Indirect fluorescent antibodies techniques (IFA) IFA was done by using 96 well tissue culture microplates containing MA104 cells infected with 100 TCID₅₀/ml of rota virus, after 24 hours, the infected cells fixed with absolute ethanol for 20 minutes, then Bovine serum samples of 2 fold dilution were added, the plates then kept at 37°C for 45 minutes, then washed with PBS. pH 7.2, a second antiovine conjugated with fluoresceine then added to all wells in dilution of 1:30, the plates then incubated for 1 hour at 37°C then

washed with the PBS then the plates read inverted with fluorescent microscope these method was modified after WELCH and TWIEHOUS, (1973).

RESULTS

The result of serum neutralization test as shown in table 1 and Fig. 1 indicates that rotavirus infection is widespread throughout Egypt. The incidence of positive reactors of serum 1:4 or higher reached 85.7% in calves and 50% in adult cows tested at Giza governorates. In Beny Sweif governorates the Incidence of positive reactors in calves was 54.5% and in adult cow was 36.4%.

In Demiate governorate it reached 6.6 in calves and 33.3 in adult cows.

In Alexandria governorate the incidence of positive reactors reached 38% in calves and 3% in adult cows. The result of SN in new valley governorate showed no positive reactors. This result of SN was emphasized by indirect fluorescent antibodies techniques (IFA) and ELISA.

The incidence of antibodies as measured by ELISA, and the range of titre as shown in Table (2) and Fig. 2,3,4,5. In Giza governorate the incidence of positive reactor reached 62.8% in calves with the 2838 average titre and 29.1% of adult cow with the average titre of 2543, in Beny Sweif it reached 27% with the average titre 4332 in calves

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and 21% in adult cows with the average titre of 7586 in Demiat the incidence of positive reactors was 30% in calves with the average titre of 3004 and it reached 22% in adult cows with average titre of 2292.

In Alexandria the incidence of positive reactors in calves was 3.8% with the average titre of 1416 while it reached 3% in cows with average titre of 1653.

DISCUSSION

Calf diarrhoea has been recognized as a serious disease problem in all areas where calves are reared especially under intensified production method. Serological results reported in this publication clearly indicate that rota virus infection is widespread in Egypt where it poses a serious threat to the calf rearing industry of the country. In this survey, the number of positive reactors of serum dilution 1:4 or higher was higher in all calves than cows which might be related to susceptibility within the species as well as the spread of infection from adult cows to their calves. The higher incidence of infection in Giza governments (EL. Aalamia farm) was due to ineffective sanitary control measure applied in this farm, where as the high incidence of rota infection in Beny Sweif and Demiat governments are due the effect of the season of heavy rainfull and floods that reflect on lowering the immune status of animal and spread of bovine rota infection especially in wet and dirty

farms. The samples obtained from Alexandria that collected from (Dina farm) which applied good sanitary control measures had a lower incidence of Rota virus infection. The new valley governorate reported no Rota virus infection which might be due to the nature of this area of dry air and deserst land.

The result obtained in this study showed that SNT and ELISA one or both can be used for diagnosis of rota virus infection where the ELISA is highly sensitive as it was detected rota virus antibodies in diluted serum up to 1:9682 in serum samples from Demiat governorate.

The previous mentioned interpretation came in agreement with the isolation of Bovine rota virus in Egypt by *SHALABY et al.* (1981) and *WAFAA, (1994)* and *NAWAL et al.* (1994).

The apparent world wide distribution of rota viruses, their enoumerous serotypes and enoumerous host range, *SATO et al.*, (1981) and *CASTUCCI et al.*, (1988), so the prospects of bringing this infection under control do not appear encouraging as active immunization of new born calves is ineffective as a control measure, as a result it is important to vaccinate dam before parturation (*CASTRUCCI et al.*, 1988). So it is important to have a local vaccine of the locally isolated serotypes to be applied on the pregnant dams to protect their neonates.

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Table (1) Distribution of Rotavirus antibodies among adult cows and calves in different governorates

Governorates	No. of serum samples		SNT		IFA	
			% of positive serum $\frac{1}{2}$ or higher		IFA on +ve reactor from SNT	
	Adult cow	Calves	Adult	Calves	Adult	Calves
Giza	24	35	50	85.7	+	+
Beny Sweif	33	33	36.4	54.5	+	+
Demiate	27	27	33.3	66.6	+	+
Alexandria	32	26	3.1	3.8	+	+
New Valley	N.D.	52	N.D.	0	N.D.	-

SNT = Serum neutralization test.

IFA = Indirect fluorescent antibody techniques

+ = Positive

- = -ve

% = Percent

N.D. = Not done.

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Table (2): Level and distribution of antibodies to Bovine rotavirus as measured by indirect ELISA in different Governorates.

	Adult cows						Calves					
	No.	Titre range	No. of +ve	Average	% of +ve	No.	Titre range	No. of +ve	Average	Titre	% of +ve	
Giza	24	2000-3000	7	2543	29.1	35	2000-3000	22	2838	62.8		
		3000-4000	3	3553	12.5		4000-5000	5	4323	14.2		
		6000-8000	2	7742	8.3		7000-8000	3	7123	8.6		
Beny Sweif	33	3000-4000	7	3163	9	33	4000-5000	9	4333	27		
		7000-8000	2	7586	21		7000-8000	4	7332	12		
		8000-9000	5	8690	6		9000-10000	5	9240	15		
Demiate	27	2000-3000	6	2292	22	27	2000-4000	8	3004	30		
		3000-6000	3	6513	11		4000-5000	4	4562	15		
							5000-6000	2	5322	7		
							6000-7000	1	6856	4		
							7000-8000	1	7943	4		
							9000-10000	2	9682	7		
Alexnabria	32	1000-2000	9	1653		3	1000-2000	10	1416	3.8		

Titre expressed as the reciprocal of log serum dilution
+ ve positive reactor.

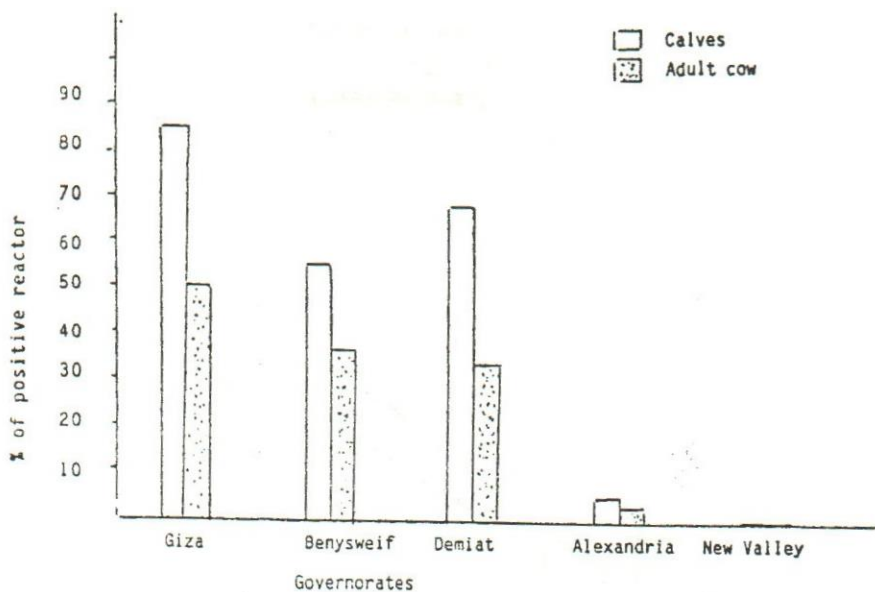
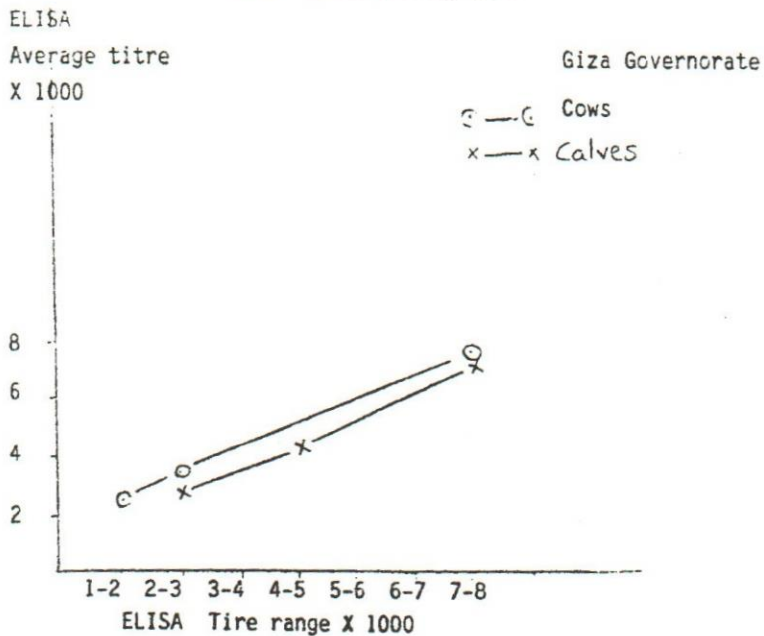
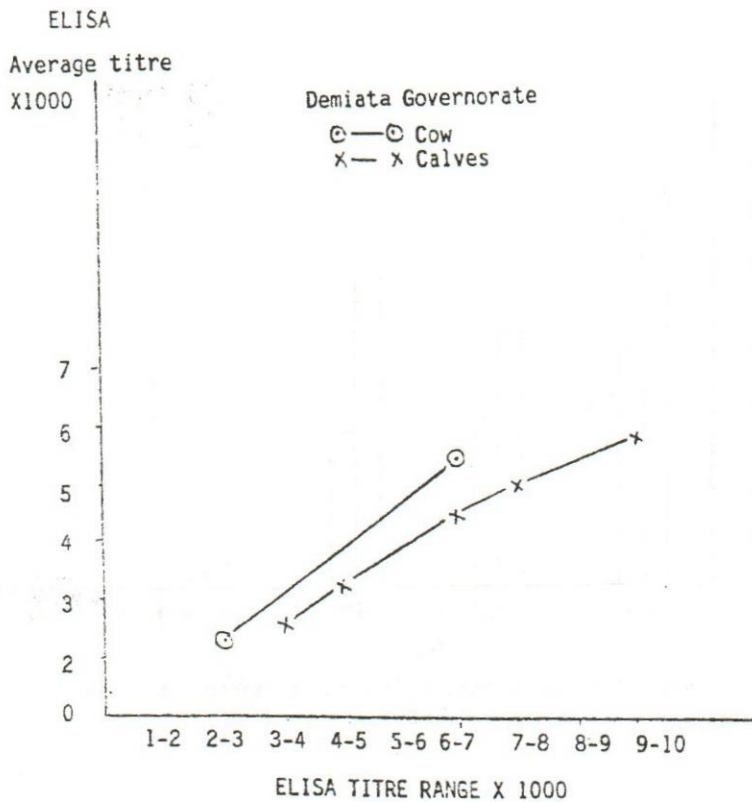
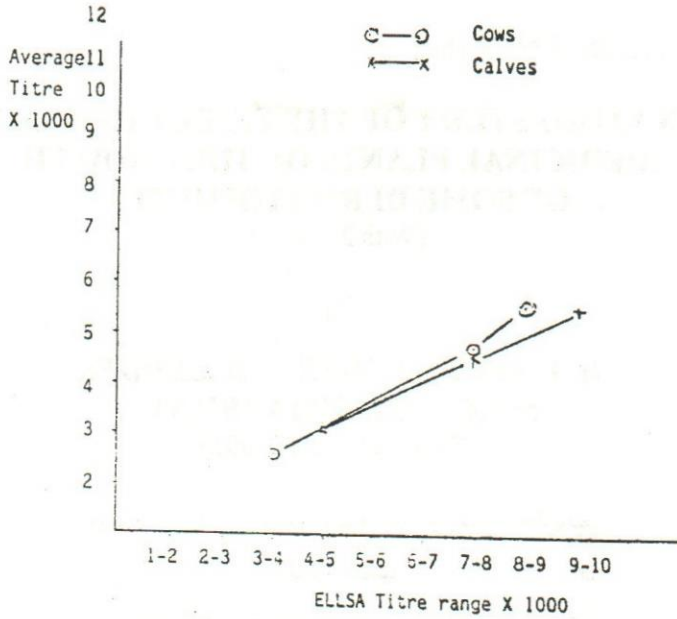


Fig. (1): Distribution of positive reactors to rotavirus infection using SNT.

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benisweir Governorate



Alexandria governorate

