Epidemiological Studies On Rift Valley Fever

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ABSTRACT

An epidemiological investigation was carried out to study seroservalence of Rift valley fever virus (RVFv) antibodies among cattle, buffalo, sheep and goat herds by Serum Neutralization Test (SNT) and by Capture Enzyme linked immunosorbant assay IgG (IgG ELISA).Blood samples were collected from animals vaccinated with inactivated RVF vaccine at Dakhlia and Sharkia governorates. The serum samples were prepared from collected blood samples during a period from 17/2/2006 to 03/10/2006. The percent of positivity at Dakhlia governorate was 77.9%by SNT and 79.7 %by ELISA. The percent of positivity at Sharkia governorate was 62.8%by SNT and 64 %by ELISA. The total protection percent was good in Dakhlia governorate that indicates the big effort done by General Organization of veterinary services. This may be due to the fact that many farmers or animals owners at Sharkia governorate are not responding to vaccination campaigns, so Levels of the protective antibody titer of "immunized animals" was not good, thus making them highly vulnerable to RVF and resulting in the possibility of rapid dissemination of infection, particularly in densely populated areas which may begin in it epidemic wave. In Egypt vaccine is obligatory, in which control of RVF relies predominantly on vaccination and disinfection. So Virucidal efficacy was evaluated for available disinfectants on RVF Virus *in vivo*. VirkonS (1% potassium peroxymonosulfate), and Sodium hypochlorite (3%) and 4-5% acetic acid and Phenol 0.5% .These disinfectants can aid in controlling transmission of viruses.

INTRODUCTION

Rift valley fever (RVF) regarded as communicable disease by the World Organization for Animal Health through the Office International des Epizootics (OIE). The disease is serious and rapidly spread irrespective of national borders; moreover it is of a major importance in the international trade of animals and animal products. . RVFV is a negative-strand RNA virus which belongs to the family Bunyaviridae, genus Phlebovirus. The virus is native and occurs in climatic conditions favoring the breeding of mosquito vectors. It is most severe in sheep, goats and cattle, in which it produces abortions in pregnant animals and a high mortality rate in the newborn. Older nonpregnant animals, although susceptible to infection, are more resistant to clinical disease *(1)*. Studying the pattern of RVF occurrence revealed that it consists of epizootic and interepizootic cycles *(2)*. Epizootic cycle occurred often when unusually heavy rainfall was observed. However, the disease does not have a regular yearly or seasonal distribution *(3)*.

In Egypt the major epidemics occurred in 1977, 1993, 1998, and more recently in 2003 *(4)*. These epidemics were usually accompanied with great animal losses and human infection. Therefore, prevention and control of RVF by a sustained program of animal vaccination was practiced through live attenuated and killed vaccines *(5)*.

The indirect ELISA test is a reliable and sensitive test and can provide results within hours and is a test for both IgM and IgG antibodies. An index case in an outbreak situation, the low-level serological cross-reactions with other members of the *Phlebovirus* genus may cause problems. Doubtful results should therefore be interpreted with caution and may need to be confirmed by serum neutralization (SNT) test at a reference laboratory *(6)*. The IgG sandwich ELISA was more sensitive in detection of the earliest immunological responses to infection or

vaccination with Rift Valley fever virus. Its sensitivity and specificity derived from field data sets ranged in different ruminant species from 99.05 to 100% and from 99.1 to 99.9%, respectively *(7)*.

Disinfection plays a major role in the control of animal diseases by decontamination of farm environment *(8)*. Furthermore, for medical use the disinfectants should have high virucidal activity, short exposure times, and nontoxic by-products and should expose to further investigation *(9)*.

Hypochlorite has been used as a disinfectant for more than 100 years *(10)*. It fulfills many requirements as an ideal disinfectants and has an excellent cleaning action, but their effectiveness for disinfection depends on the concentration of available chlorine and the pH of the solution *(11)*.

Virkon S is abrand name of a powerful multi-purose disinfectant consists mainly from potassium peroxy monosulphate. It is a strong oxidizing disinfectant, has high concentration coefficient and a wide range of action against viruses and is essentially non irritant and is easily biodegradable *(12)*. The solution of virkon S is widely used as a virucidal spray in many fields, including hospitals, laboratories, nursing homes, funeral homes, medical, dental and veterinary facilities *(13)*, on the other hand organic matter even in small amount reduced the activity of disinfectant *(14)*. Virkon®S in particular represents a rapid and safe way to decontaminate *(15)*.

The aim of this work was evaluation herd immunity against RVF at Dakahlia and Sharkia Governirates and to evaluate virucidal efficacy of available disinfectants on RVF Virus *in vivo*.

MATERIAL AND METHODS

Experiment (1): Evaluation herd immunity against RVF using Serum Neutralization Test (SNT)

1.Blood samples

A total of 2822 blood samples were collected from different species of apparently healthy farm animals (895 cattle, 673 buffaloes, 689 sheep and 565 goats). The studied animals were varied in age and sex. The animals belonged to known farms distributed allover Dakahlia and Sharkia provinces. The blood sample was collected from the period of 17/2/2006 to 03/10/2006 with history of no clinical symptoms of RVF.

Blood samples were collected from jugular vein using a sterile needle for each animal in a sterile labeled venoject tube. All tubes were left in a slant position to separate amount of serum and transported as early as possible on an ice packed thermos to laboratory at Mit-ghamer Veterinary Medicine district. All serum samples were centrifuged at 2000 rpm for 10 minutes to obtain clear serum samples. The sera were separated in sterile capped rails and inactivated at 56°C for 30 minutes to remove non- specific inhibitors. All serum samples were placed in a cool chamber container and stored at -20°C at the laboratory for virological investigation at Department of Virology, Animal Health Research Institute, Dokki – Giza.

2. Virus

The virus used in this study was RVFv Zagazig strain 504, kindly supplied by the Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. It was stored at -70°c until used.

3.Tissue culture

Baby Hamster Kindly cells (BHK – 21 Clone 13) was lined by National Veterinary Disease Laboratory (NVDL) in USA and maintained in animal Health Research Institute, Dokki – Giza.

4.Media

a. Growth media

Minimum essential media (modified Eagle's) with Earle's salts plus L-glutamine and sodium bicarbonate (flow laboratories, UK) was prepared

b. Maintenance media

Minimum essential media with Hank's salt (flow laboratories, UK) was prepared.

5. Stock antibiotic solution

A stock solution of antibiotics (penicillin and streptomycin) was prepared in Hank's solution and sterilized by filtration.

6.Trypsin versen Solution (0.25%): It was used in preparation of tissue culture (BHK 21) cell line.

7.Sodium bicarbonate solution (7.4%)

Dispensed into tightly stoppered bottles and autoclaved at 115°C for 15 minutes.

8. Inverted microscope

Olympus ix61 inverted microscope (Olympus, Chicago-USA) was used for detection of the cytopathic effect of the inoculated virus in BHK cells.

9. Micro titer plates

It was supplied from Nunclon TM surface Cat.No.167008, batch No. 064568 made in Denmark.

Serum neutralization test

It was used for detection of antibodies against RVF in the collected- serum samples. This technique was applied as previously described *(16,17).*

Experiment (2) Evaluation herd immunity against RVF by using Capture Enzyme linked immunosorbant assay (ELISA) IgG

1.Serum samples

The prepared serum samples previously tested in experiment (1) was used for evaluation herd immunity against RVF using Capture Enzyme linked immunosorbant assay (ELISA) IgG.

2.Solution and buffers of solid phase ELISA

It was used for detection of anti-RVF IgG antibody in collected serum samples according to method described by National Institute for Communicable Diseases, Special Pathogens Unit, PrivateBag X4, Sandringham 2131, and Johannesburg.

Experiment (3): Evaluate virucidal efficacy of available disinfectants on RVF Virus:

This experiment was carried out as previously described *(18)* .

1.Virus: The virus used in this study was RVFv antigen: lyophilized RVFcell lysate antigen (CLA). Titration of the virus was expressed as 100TCID50/ml of the origin virus *(19).*

2.Disinfectants: 3% sodium hypochlorite, VirkonS (potassium peroxymonosulfate, 4-5% acetic acid (Vinegar) and Phenole 0.5%) was used in this experiment.

3. Cell culture: Baby Hamster Kidney cell (BHK21) and virus titration using standard method *(20)* were carried out.

RESULT AND DISCUSSION

As shown in Table 1 the presence of neutralizing antibodies against RVFv was detected in collected serum samples from Dakahlia governorate with a percentage of 77.2% in cattle,77.3% in buffaloes, 77.7% in sheep and 78.8% in goats, while tested serum samples were detected in Sharkia governorate with a percentage of 73% in cattle, 63% in buffaloes, 60.2% in sheep and 52% in goats. These results were different from those previously recorded which reported that SNT was detected neutralizing antibodies against RVF V in 74.02% of cattle, 64.4 of buffaloes, 84.6% of sheep, and 57.2% of goats in Sharkia governorate *(21)* .This may be attributed to the differentiation of localities, from which serum samples were collected and time of vaccination .

As shown in Table 2 the presence of neutralizing antibodies against RVF v was detected in collected serum samples from Dakahlia governorate with a percentage of 78.5% in cattle,80.2% in sheep and 81.4% in goats, while tested serum samples were detected in Sharkia governorate with a percentage of 75% in cattle, 59.6% in sheep and 55.5% in goats. These results were nearly similar to those previously recorded *(22, 23),* which reported that 81.01% for RVF inactivated vaccine in sheep, 82.26%in goats, and 76.7% in cow at Dakahlia Governorate by IgG ELISA, while at Sharkia Governorate were 59.5% in sheep, 50% in goat and 79.4 in cow. The percentages of IgG among the vaccinated cattle and buffaloes in East Egypt (Sharkia Governorate) were 88.9 and 88.1,respectively *(24).* This may be due to collection of samples from sporadic animals and the possibilities escaping of animals from vaccination or exposure of vaccinated animals to stress factors (nutritional or parasitic infestation) which inhibiting the immune response.

Table 1. Results of Serum neutralization test (SNT) in farm animals at Dakahlia and Sharkia Provinces

Animal species	Dakahlia province			Sharkia province		
	No. of examined Serum samples	positive samples		No. of examined Serum samples	positive samples	
		No.	%		No.	%
Cattle	695	537	77.2	200	146	73
Buffaloes	525	406	77.3	148	93	63
Sheep	503	397	77.7	186	112	60.2
Goats	408	321	78.8	157	83	52

Positive sample =*Protective level (titer $\leq$ 40

Table 2. Results of Enzyme Linked Immuno Sorbent Assay (ELISA) IgG and in farm animals at Dakahlia and Sharkia Provinces .

Animal species	Dakahlia province			Sharkia province		
	No. of examined Serum samples	positive samples		No. of examined Serum samples	positive samples	
		No.	%		No.	%
Cattle	695	545	78.5	200	150	75
Sheep	503	403	80.2	186	111	59.6
Goats	408	332	81.4	157	87	55.5

Percentage positivity (pp)= $\frac{\text{Net optical density test serum}}{\text{Net mean optical density Control (++)}} \times 100$

(PP Values $\leq$ 16.4)

Correlation between the results of ELISA IgG and SNT at Dakahlia and Sharkia provinces was estimated. The results of tested serum samples obtained by SNT were lower to that obtained by ELISA Table (3). The percent of positivity in Dakahlia province was 77.9% by SNT and 79.7% by ELISA. The percent of positivity at Sharkia province was 62.8% by SNT and 64% by ELISA.

The statistical analysis confirmed the presence of no significance between ELISA and SNT (P<1) for the sera collected from Dakahlia and Sharkia provinces, that recorded in Table 3. This can be explain as follow; SNT has been successfully used for many years and it is the accepted test for the quantification of antibodies against RVFv. The test was considered sensitive, specific and relatively simple to perform, but required tissue culture cells which often vary in sensitivity. On the other hand ELISA is rapid and relatively simple to perform. It is economic of reagents and results may be reported after one day and that ELISA is an accurate for monitoring of immune response in vaccinated animals *(7)*.

The residual virus titers by tissue culture infective dose 50 ($TCID_{50}$) following exposure to disinfectants and dialysis are shown in Table 4. Exposure of the virus to distilled water (the negative control) had no detectable effect on the virus titer, indicating that the experimental design itself did not affect virus viability. Mean while, 3% Sodium hypochlorite, VirkonS (potassium peroxy monosulfate) and 4-5% acetic acid completely inactivated RVF virus. On the other hand phenol 0.5% had no detectable effect on the virus titer. These results substantiate the previously reported by others *(25)*, which indicated that disinfectants can aid in controlling transmission of virus with less deleterious effects of sodium hypo-chlorite.

The virus survived for a variable time in various animal objects forming a rather hazard of infection. So, farms must be cleaned firstly then effective disinfectant is applied ***(15)***. Virus destruction by acetic acid and its resistance to phenol was reported , and the virus was resistant to alkaline pH, but inactivated by pH <6.8. Furthermore, virus was inactivated by ether and chloroform, strong solutions of sodium or calcium hypochlorite and Can survive contact with 0.5% phenol at 4°C for 6 months ***(25)***.

Table 3. Correlation between results of Enzyme Linked Immuno Sorbent Assay (ELISA)IgG and Serum neutralization test (SNT) in farm animals at Dakahlia and Sharkia Provinces.

province	NO. Of examined Serum samples		positive serum samples				X^2
	SNT	ELISA	SNT		ELISA		
			No.	%	No	%	
Dakahlia	2131	1606	1661	77.9	1280	79.7	0**
Sharkia	691	543	434	62.8	348	64	

Non significant at (p < 1).**

Table 4. Residual virus titers (TCID50/ml) after exposure of virus to the disinfectants and control solutions

Disinfectant	Residual RVF Virus titer (TCID50/ml)
3% Sodium hypochlorite	0
VirkonS	0
4-5% acetic acid (Vinegar)	0
Phenole 0.5%	$10^{4/}$mL

*The residual RVF virus titer was10^5/ml for control solution (distilled water).

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الملخص العربي

دراسة وبائية حول مرض حمى الوادى المتصدع

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**مركز بحوث صحة الحيوان بالدقى –جيزة- قسم الفيروسات

احتل هذا المرض مساحة واسعة من الاهتمام خلال الأعوام القليلة الماضية وحذرت منظمة الصحة العالمية من خطورته حيث ينتشر بواسطة البعوض مما قد يسبب كوارث وبائية تشمل مناطق بأكملها.يجرى التحصين الوقائي المنتظم باستعمال العترة المحلية للفيروس من العديد من البلدان بغرض السيطرة الأساسية علي المرض ، لذلك تم إجراء هذه الدراسة لتحديد نسبة تواجد الأجسام المناعية الخاصة بهذا الفيروس في قطعان الأبقار والجاموس والأغنام والماعز في محافظة الدقهلية والشرقية باستخدام اختبار الأاليزا واختبار المصل المتعادل والمقارنة بين نتائج الاختبارين لقياس مستوي الأجسام المناعية وقد اسفرت نتائج المسح السيرولوجي علي حيوانات مزارع محافظة الدقهلية والشرقية)باستخدام اختبار الأاليزا (بأن نسبة الحيوانات المحصنة والقادرة علي صد المرض في مزارع محافظة الدقهلية كانت ٧٩% أعلي منها في مزارع محافظة الشرقية ٧٧,٩ % ؛ اما باستخدام اختبار المصل المتعادل فكانت النسبة في مزارع محافظة الدقهلية ٦٤% أعلي منها في مزارع محافظة الشرقية ٦٢,٨% . علما بانه لم يكن هناك فاعلية حدوث وباء أثناء فترة الدراسة بسبب برامج التحصين الإجبارية .

وللسيطرة على المرض تم اختبار بعض المطهرات المتاحة والمتداولة في السوق لتقييم كفاءة المطهرات ضد فيروس حمي الوادي المتصدع وقد اسفرت نتائج التجربة المعملية بان :الصوديوم هيبوكلوريت ٣%، هيدروكسيد صوديوم ٢% ، حامض خليك ٤-٥% ، فيركون اس، بوتاسيوم بيروكسى مونوسالفات قضي علي الفيروس نهائيا ، لكن الفينول ٥% كان تأثيره ضعيف علي الفيروس .وعلى هذا الاساس اتضحت أهميةِ التحصين كل ٤ شهور ،بالإضافة إلى برنامج التطهير للمزارع بهذه المطهرات السيطرة على هذا المرض.