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PRELIMINARY STUDIES FOR RISING THE IMMUNE RESPONSE OF CATTLE VACCINATED WITH INACTIVATED RIFT VALLEY FEVER VACCINE USING ZINC AND COPPER.

LILY S. SALAMA, EMAN M. SHLKAMY, T. N. MARCOSS and GIHAN K. MOHAMED

Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

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SUMMARY

In this study, we tried to increase the immunological response in cattle by feeding it with Zinc and or copper added to the ration at the same time of vaccination. Eight cattle were used in this study and divided into four groups two cattle (G1) were vaccinated with binary inactivated RVF vaccine only, two cattle (G2) were fed with zinc at the same time of vaccination and still fed with zinc for three successive days, two cattle (G3) were fed with copper at the same time of vaccination and still feed with copper for three successive days and two cattle (G4) were left as control of this study.

The NI's of cattle to be protective from 2nd week (1.7 and 1.3) for zinc and copper respectively, while cattle vaccinated at the same time with RVF inactivated vaccine only was 1.4. The results of ELISA were in parallel to that obtained by S. N.T.

From the previous results, it was concluded that the addition of zinc to the ration gave higher immune response in vaccinated animals compared with other groups.

INTRODUCTION

Rift Valley Fever (RVF) is an acute viral disease, affecting many species of animals and human. In severe cases in young the deaths are freqently accociated with haemorrhagic symptoms (Peters and Meegan, 1981). The disease appeared for the first time in Egypt during summer of 1977 in an epidemic form and reappeared again after 15 years as the first epidemic 1993 but in milder form (El-Gabery et al., 1994). Trials were done to produce inactivated vaccine (El-Nimr, 1980) which is tissue culture formalin inactivated vaccine. Eman, 1995 prepared RVF vaccine in

cell culture inactivated by binary ethyleneimine which was capable to protect sheep and cattle against the disease.

National attention has been attracted in the last decade of this century towards the possible role played by copper and zinc on the immune response and resistance to infection.

The mechanistic effect of zinc on the immune function has been reviewed by (Sheffy and Schultz, 1979) and (Bettegar et al., 1980).

Zinc is an essential trace element that is required for growth and development, enzyme function, appetite taste, night vision and immune competency (McBean 1991) as well as copper is required only or with iron for haemoglobin formation. It also plays many other important roles in the body. Copper deficiency in cattle and sheep has caused serious losses in several countries. Adding copper sulphate to mineral mixtures or concentrate feeds is effective in preventing the losses.

Committee of diet, nutrition and cancer of nutritional Research Council (1982) reported that pronounced zinc deficiency in animals and human results in depressed immune function. Deficiency of copper in infected lambs lead to more seriously impaired production and may be one of the causes responsible for poor growth as reported by Phillip (1983) as well as zinc deficiency lead also to poor growth, impaired immune response and especially humoral disturbances.

So for practical and economic reasons, efficient immunization may require immunization procedures using immunopotentiating agents which stimulate non-specifically the immune response so that long lasting effective immunity results. The aim of this work is an attempt to increase the immune status of cattle by adding zinc and copper to the ration to compensate the deficiency if present, and to study its effect on the immune response of cattle vaccinated with binary inactivated RVF vaccine.

MATERIAL AND METHODS

Material:

1- Animals:

1-1 Mice:

3-5 days old suckling mice were used for safety test of the binrary inactivated RVF vaccine.

21-30 days old mice were used for testing the potency of the vaccine as well as titration of RVF virus (ZH501)

1-2 Lambs: lambs of 5-10 days old were used for the safety test of the prepared vaccines.

1-3 Cattle:- 8 cattle of about one year old were

used and held in separate places and fed on barseem to study the immune response of the vaccine after feed of zinc and copper drugs to compensate the deficiency of these minerals.

2- Minerals:-

2-1 **Copper:-** It was obtained from "Phizer Egypt" and presented to the animals mixed with ration in a dose of 80 mg/kg feed.

2-2 **Zinc:-** It was obtained from "Phizer Egypt" and presented to the animals mixed with ration in a dose of 300 mg/kg feed.

3- **Virus: Rift Valley Fever (RVF) virus** and had titre of $10^{7.2}$ $TcID_{50}$/ml from RVF Department. Veterinary Serum and Vaccien Research Institute, Abbasia, Cairo.

4- **Antigen for ELISA test:** according to Elian and Botros (1997).

5- **Conjugate:-** Horse reddish perioxidase labeled antispecies (anti-bovine).

Methods:-

1. Titration of virus: according to Walker (1970).

2- Evaluation of the vaccine:- The sterility test to be free from bacteria, fungus and mycoplasma, safety and potency tests were carried according to Walker (1970) and ED50 was calculated according to Randall et al., (1964)

3- Experimental Design: Eight cattle of about one year old which their sera were tested by serum neutralization test and proved to be free from antibodies against RVF virus. The animals were divided into four groups and kept in isolated place

- The first group: consisted of 2 cattle were vaccinated with binary inactivated RVF vaccine only (G1).

- The second group consisted of two cattle were fed with zinc at the same time of vaccination with binary inactivated RVF vaccine and still fed with zinc (300 mg/kg ration) for three successive days (G2).

- The third group consisted of two cattle were fed with copper at the same time of vaccination with binary inactivated RVF vaccine and still fed with the copper (80 mg/kg ration) for three successive days (G3).

- The fourth group consisted of two cattle were as control non vaccinated and non treated (G4).

4- **Serum neutralization test:** Walker et al. (1970).

5- **Enzyme Immunosorbant Assay (ELISA):** Voller et al., 1976.

RESULTS AND DISCUSSION

Rift Valley Fever (RVF) is an acute arthropod-born viral disease causing high morbidity and mortality rates among sheep, cattle, goats, and human (Castro and Heuschele, 1992).

Many studies were performed to improve and increase the immune status of cattle and sheep. Before discussing the effect of diet and nutrition on the immune response and resistance to infection, it is useful to review the effect of some minerals on the immune response. The effect of nutrition on resistance to infection and on immunological capability is of great interest. Scrimshaw et al., 1968 also stated that cell-mediated as well as humoral mechanism of immunity may be severely impaired by nutritional deficiency. So we tried to increase the immune status of cattle by feeding zinc or copper at the same time of vaccination with binary inactivated RVF vaccine.

The results in table (1) showed that the evaluation of the vaccine by testing its sterility, safety and potency revealed that the prepared vaccine was safe and potent where its ED50 was (0.006/ml).

The temperature recorded of all animals within the normal range between 38.5 and 39.5. It was found that the cattle vaccinated with binary inactivated RVF vaccine only and feeding with zinc or copper at 2nd week had protective NI's. Their NI's were 1.4, 1.7 and 1.3 respectively as in table (2).

Table (1) Evaluation of binary inactivated Rift Valley Fever Vaccine.

Sterility	SAFETY		Potency
	I/C In baby mice	I/P & S/C In lambs	ED50/ml
Free from bacteria, fungus and mycoplasma	No signs of the disease or death	No signs of the disease. No elevation of temperature. No death.	0.006/ml

Table (2): Mean of NI's in sera of cattle feeding on zinc or copper at the same time of vaccination with binary inactivated RVF vaccine:-

Type of vaccine	No. of animals	NI's before vacc	NI's Post vaccination and feeding																
			1st D	2nd D	5th D	1st W	2nd W	3rd W	4th W	6th W	8th W	10th W	12th W	14th W	16th W	18th W	20th W	22nd W	24th W
RVF Only (G_1)	2	0.6	0.5	0.5	0.6	0.8	1.4	1.5	2.1	2.5	2.5	2.7	2.5	2.3	2.1	2.1	1.6	1.3	1.3
RVF+ feeding with zinc (G_2)	2	0.7	0.6	0.5	0.9	0.9	1.7	1.9	2.5	2.7	2.9	3.0	3.0	3.3	3.5	3.0	2.1	1.9	1.6
RVF+ Feeding with copper (G_3)	2	0.5	0.3	0.6	0.6	0.8	1.3	1.7	2.3	2.7	2.7	2.8	2.7	2.9	3.0	2.7	1.8	1.6	1.5
Control non vaccinated non feeding with trace mineral (G_4)	2	0.5	0.6	0.4	0.6	0.5	0.7	0.6	0.4	0.5	0.4	0.6	0.7	0.6	0.6	0.5	0.4	0.6	0.7

NI's = neutralizing indices D= Days W = weeks

Table (3): Mean of ELISA reading in sera of cattle feeding on zinc or copper at the same time of vaccination with binary inactivated RVF vaccine:-

Type of vaccine	No. of animals	O D before vacc	O D reading Post vaccination and feeding																
			1st D	2nd D	5th D	1st W	2nd W	3rd W	4th W	6th W	8th W	10th W	12th W	14th W	16th W	18th W	20th W	22nd W	24th W
RVF Only (G_1)	2	0.24	0.29	0.36	0.42	0.46	0.60	0.61	0.63	0.69	0.74	0.80	0.84	0.98	1.10	1.21	1.35	1.48	1.56
RVF+ feeding with zinc (G_2)	2	0.26	0.36	0.41	0.49	0.51	0.63	0.66	0.79	0.85	0.94	0.99	1.20	1.26	1.46	1.59	1.71	1.85	1.97
RVF+ Feeding with copper (G_3)	2	0.21	0.32	0.34	0.44	0.50	0.61	0.64	0.69	0.75	0.80	0.87	0.97	1.06	1.26	1.35	1.46	1.58	1.76
Control non vaccinated non feeding with trace mineral (G_4)	2	0.23	0.25	0.28	0.30	0.30	0.34	0.31	0.33	0.34	0.36	0.37	0.39	0.38	0.36	0.34	0.35	0.33	0.34

O D = Optical Density D= Days W = weeks N. B. Cut off = 0.56.

NI's of animals started to be protective from 2nd till 24th week vaccine (1.3, 1.6 and 1.5) for RVF only or with zinc ffeding and copper feeding respectively while non vaccinated non treated was (0.7) Walker et al., 1970.

ELISA test was in parallel coorelation with SNT test as in table (3).

This mean that the addition of zinc or copper to the diet for feeding cattle during vaccination with binary inactivated RVF vaccine plays an important role to increase the immune response of cattle with respect to the cattle vaccinated RVF only.

These results agreed with Betteger et al., 1980 who mentioned that zinc has a stabilizing effect on biomembranes and that erythrocytes from zinc deficient rate exhibits increased fragility and that extracellular zinc improve membrane integrity. This may explain the better resistance of zinc supplemented chickens

Immune defects associated with zinc deficiency and their possible biochemical losses has been reviewed by Gershwin et al., 1987 and Hambidge et al., 1986. As well as deficiency of copper in infected lambs lead to more seriously impaired production and may be one of the causes responsible for poor growth as reported by Phillip, 1983. Also Tanaseseu et al., 1996 found that of these two metal ions zinc and copper were the most important influencing the interdependence between the immunoglobulins inactive forms of disease. While Virgili et al., 1998 said that zinc supplementation imporoved cell mediated immune response where zinc increase the number of CD4+DR+T-cells and T-lymphocytes.

Finally, Zinc regulates nucleic acid and metabolism by its role in DNA and RNA polymerase enzyme thus affecting the production of various key proteins such as albumin and globulin Payne et al., 1987.

We concluded from our study the importance of imporving the humoral immune response of the animals after adding zinc or copper to the diet at the same time of vaccination.

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