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Research Article
Evaluation and Haematological Profile of an Inactivated RVF Vaccine Locally Produced in Egypt

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Abstract

Background and Objective: The RVF virus cause diseases in newborn puppies, kittens, sheep, goats, cattle, camels, buffaloes and also humans. The RVF disease was first detected among livestock by veterinary officers. The disease causes abortions in animals. The goal of this study was to evaluate the immune response and the haematological profile associated with inactivated RFV vaccine locally produced in Egypt in young puppies and sheep. **Materials and Methods:** Through vaccination, both young puppies and sheep with local produced inactivated RVF vaccine with 2 doses with 2 weeks interval and evaluate the immune response by SNT and ELISA as well as haematological parameters at 0, 7, 14, 21 and 28 days post-vaccination. The variance between vaccinated groups and also non-vaccinated groups were compared by using a one-way Analysis of Variance (ANOVA). **Results:** The findings showed that young puppies had a strong response to antibodies after two doses of the RVF vaccine within the 2 week interval. The neutralization indices (NI) values in young puppies at different periods after RVF vaccination reported the value of 1.08±0.03, 1.23±0.04, 1.30±0.03 and 1.45±0.02 after 7, 14, 21 and 28 days post-vaccination, respectively. Parallel to this the ELISA OP values were 0.30±0.00, 0.39±0.03, 0.52±0.05 and 0.75±0.02 after 7, 14, 21 and 28 days post-vaccination, respectively. Nearly similar immune response was noticed in sheep with NI values of 1.15±0.02, 1.27±0.02, 1.42±0.05 and 1.55±0.03 at 7, 14, 21 and 28 days post-vaccination, respectively. In the same site the ELISA OP values were 0.34±0.00, 0.47±0.01, 0.68±0.00, 0.77±0.00. After 7, 14, 21 and 28 days post-vaccination respectively that are also similar to that in puppies. The haematological profile reported a significant decrease after the 1st week followed by a transient increase after booster dose at 2nd week except for the monocytes that increase after 1st week then decreases after 2nd week post-vaccination. **Conclusion:** Young puppies are similar to sheep in developing antibodies after vaccination with the RFV vaccine with no statistically significant effect within different batches. In addition, ELISA can replace the SNT for evaluation of the immune response. Young puppies are quite equal to sheep for the illustration of neutralizing antibodies for RFV vaccine. Sero-negative puppies can be easily obtained because dogs are not included in the vaccination program of RVF and so they can be used as a good model to determine the efficacy of the RVF vaccine.

Key words: Puppies, RVF, sheep, ELISA, vaccination program

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rift Valley fever (RVF) is an acute or peracute mosquito-borne infection caused by a single-stranded RNA virus named Rift Valley fever virus (RVFV), which belonged to the order Bunyavirales, Family: Phenuiviridae, GenusPhlebovirus[1,2]. The RVF virus causes disease in sheep, goats, cattle, camels, buffaloes, newborn puppies and kittens and also humans. The disease causes explosive abortions in animals. RVF disease was first detected among livestock by veterinary officers in the Rift valley of Kenya around 1915, but the virus was not identified until 1931. Since that time the disease has been reported as a periodic epizootic with a 5-15 year cycle[3]. Great similarities between Rift Valley fever and bovine ephemeral fever in the enzootic process in the Middle East and the transmission by insects and the control of both diseases depend on insect control and vaccination[4]. International trade has been potential role in the introduction of the disease in Egypt through the importation of infected ruminants or camels from Sudan RVF in 1977[5,6] and reappeared in 1993 in Egypt[7]. In Egypt, the first outbreak of RVF started in Belbes and Zagazig region of Shakira Governorate. The disease was first diagnosed in humans as an acute febrile Dengue-like illness followed by multiple outbreaks that have been reported in Egypt (1977, 1978, 1993 and 2003). As a result of nonspecific treatment, the periodical vaccination and insect vector control remain the most appropriate measures to control RFV[8]. Several studies' success to develop RFV vaccines as reported by Smith *et al.*[9], who designed vaccine candidates using reverse genetics to develop deletion mutants of two RVFV virulence factors, the NSs and NSm genes which proved to be protective and immunogenic in rats, mice and sheep, without producing adverse clinical symptoms[10] developed reverse genetics to produce a recombinant RVFV, ΔNSs-ΔNSm rRVFV, which comprises complete gene deletions of the 2 recognized RVFV virulence elements, the NSs and NSm genes. Interestingly, a formalin-inactivated vaccine (ZH501) propagated in BHK-21 cells and inactivated with 0.5% formalin and adsorbed with aluminium hydroxide adjuvant is the only approved vaccine in Egypt[11]. Furthermore, another study[12] used a new adjuvant termed (Montanide IMS 1113) to enhance the immunogenicity of the locally produced inactivated RVF vaccine and to overcome the problems influenced by its short term immunity.

The evaluation of the RVF vaccine was performed in sheep to illustrate the humoral immune response and to carry out the challenge test. Furthermore, another study[13] applied the inhibition ELISA test to screen 977 cattle, 1,549 sheep and 523 goats and the seroprevalence was 42.9, 28.0 and 9.3% in these animals, respectively with a high titer and the seroprevalence increased with age with seasonal pans and in recently vaccinated animals. However, the use of non-human primate models is not practical for large-scale or high-throughput studies. Several studies recorded that all tested inbred mouse models died with high susceptibility to RVFV wild type with severe hepatic disease except the BALB/c mice which remain live for a long time post-infection and are more likely to develop neurological disease[14-16]. The continuous monitoring of the various local produced RFV vaccines particularly in Egypt under field conditions needs an alternative laboratory animal model due to the difficulty to obtain seronegative sheep to evaluate RFV vaccine in Egypt. This is actually due to RVF vaccination program adopted in sheep, besides the circulation of the virus in these animals.

Therefore, the ultimate objective of this study was to demonstrate young puppies as a new alternative experimental modelling RVF vaccine evaluation and test random samples of different batches of the vaccine to confirm the stability of the production process, application of indirect ELISA in a trial to substitute the traditional SNT.

MATERIALS AND METHODS

Study area: The experiment was done at the farm of the Faculty of Veterinary Medicine, University of Sadat City and Menoufiya Governorate from the period May to June, 2019.

RVF virus titration in tissue culture: The original Rift Valley fever (RVF) virus was supplied by NUMRU -3, the virus was passaged in suckling mice intra-cerebral for twice times. It was known as ZH501 with a titer of 107.5. TCID50 mL^{-1}, is the seed virus for vaccine preparation and different batches of RFV vaccine developed in the RVF department of the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The seed virus is also used in the SNT for the evaluation of the vaccine.

The titration of the RFV virus was prepared[17] in BHK_{21} cells. Briefly, serial dilutions from the original stone virus (tenfold) were put in Hank's solution followed by inoculation into BHK_{21} cell culture by the dose of 10 µL per well and 3 wells for each dilution. The titer was determined[18].

Experimental animals

Puppies: Fifteen puppies aged 14-21 days old were selected and housed in a well-ventilated insect-proof in a clean and disinfected room at the faculty of the Veterinary Medicine University of Sadat City under good hygienic conditions, receiving a suitable diet. All the puppies were in good health condition during the time of the experiment. The puppies used for the experiment were free from antibodies against RFV virus through examination of serum samples. The experiment consisted of four groups of puppies:

Group 1: Contains four puppies and injected 1 mL (S/C) by alum adjuvant RFV vaccine (batch 1)

Group 2: Contains four puppies injected 1 mL (S/C) by alum adjuvant RFV vaccine (batch 1)

Group 3: Contains four puppies, injected 1 mL (S/C) of inactivated alum adjuvant RVF vaccine (batch 3)

Group 4: Contains three puppies animals and not received the vaccine as (control negative)

Sheep: Twenty adult local breed sheep aged 3-4 months old were used for the assessment of immune response to the inactivated vaccine. They were supplied by RVF Department Serum and Vaccines Research Institute, Abbasia, Cairo. All of these animals were tested and found to be free from RVF antibodies, free from external and internal parasites. They were housed in insect prove stables receiving balanced ration and adequate water under strict hygienic conditions.

Blood and serum samples: Blood samples were collected with ETDA from young puppies and freshly examined for CBC profile to determine the hemogram and leukogram parameters. Blood samples were collected from sheep and puppies, left to clot and then the collection of serum after centrifugation at 3000 rpm was followed. The serum was stored at -20°C ready for use in the serum neutralization test (SNT) and ELISA.

Hematological examination: All the haematological examinations involving RBCs, Hb, PCV, MCV, MCH, MCHC, WBCs and differential leukocytes count were conducted[19].

Serum neutralization test (SNT): The serum neutralization test was carried out according to the method of constant serum-virus dilution procedure[18]. In summary, Hanks solution, serial tenfold dilutions of the virus were prepared with a fixed volume of each dilution and an equivalent volume of undiluted serum previously inactivated at 56°C for 30 min before use. In another series, normal negative control inactivated serum as control was mixed with an equal volume of each virus dilution, the plates were then incubated for 1 h at 37°C followed by the addition of maintenance medium. The plates were incubated for 5-7 days with daily examination for the appearance of CPE. The serum neutralization index was calculated as the variance between the virus titer in the presence of negative serum and that in the presence of suspected positive serum and expressed[18] as NI_{50}.

ELISA: Indirect ELISA has been conducted by Kim *et al.*[20]. In summary, the antigen on a solid plate was adsorptive. The tested serum was incubated with the antigen on the solid plate. Washing the plate is followed to remove the unreacted serum components, then the addition of an enzyme conjugate and incubated. After washing again to remove the non-reacted material, the enzyme-substrate was added. Colour change will determine the amount of the conjugate fixed, which is relative related to the antibody level in the test sera with other modifications of ELISA. The cut-off value of the positive samples though the determination of the optical density was calculated[21].

Statistical analysis: The collected data were presented as the variances between the vaccinated groups and the control group. The means for each group was estimated and compared with the different groups by using one-way analysis of variance (ANOVA)[22].

RESULTS

Immune response of puppies vaccinated with different batches of inactivated RVF vaccine via SNT: Table 1 showed the neutralization indices (NI) of young puppies after vaccination. The Rift Valley fever inactivated vaccine. The NI began to appear on the 7th day after vaccination (1.35±0.0) in comparison to the control group (0.00±0.04). After 14, 21 and 28 days after vaccination, the mean neutralization indices were elevated and reached the protective level at 1.35±0.09, 1.43±0.08 and 1.20±0.12, respectively. The statistical analysis confirmed the substantial difference of NIs in different periods after vaccination between the groups. There was a significant difference between the vaccinated and non-vaccinated group and the period before vaccination at ($p \leq 0.05$). Statistical analysis showed also a significant difference of NIs in different times post-vaccination between the different batches ($p \leq 0.05$).

Table 1: Immune response of puppies vaccinated with different batches of inactivated Rift Valley fever vaccine estimated by SNT and ELISA

Inoculum	Batch	Anti-RVFV IgG Mean±SE SNT	Anti-RVFV IgG Mean±SE ELISA
Day 0 post-vaccination	Batch 1	0.00±0.04[a]	0.29±0.00[a]
	Batch 2	0.00±0.04[a]	0.23±0.00[b]
	Batch 3	0.00±0.04[a]	0.22±0.01[b]
	Control	0.00±0.00[a]	0.23±0.00[b]
Day 7 post-vaccination	Batch 1	1.05±0.09[b]	0.30±0.00[a]
	Batch 2	1.35±0.09[a]	0.29±0.01[a]
	Batch 3	1.05±0.09[b]	0.30±0.01[a]
	Control	0.00±0.00[c]	0.23±0.01[b]
Day 14 post-vaccination	Batch 1	1.20±0.00[a]	0.43±0.02[a]
	Batch 2	1.35±0.09[a]	0.39±0.03[a]
	Batch 3	1.20±0.00[a]	0.44±0.02[a]
	Control	0.00±0.00[b]	0.24±0.02[b]
Day 28 post-vaccination	Batch 1	1.43±0.08[a]	0.75±0.02[a]
	Batch 2	1.20±0.12[a]	0.72±0.01[a]
	Batch 3	1.35±0.09[a]	0.71±0.02[a]
	Control	0.00±0.00[b]	0.23±0.00[b]

[a,b,c]Values within the same Rift Valley fever inoculation day category within the same column with different superscripts differ significantly at $p \leq 0.05$

Table 2: Immune response of sheep vaccinated with different batches of inactivated Rift Valley fever vaccine estimated by SNT and ELISA

Inoculum	Batch	Anti-RVFV IgG Mean±SE SNT	Anti-RVFV IgG Mean±SE ELISA
Day 0 post-vaccination	Batch 1	0.59±0.08[a]	0.28±0.00[a]
	Batch 2	0.53±0.01[a]	0.23±0.01[b]
	Batch 3	0.67±0.06[a]	0.25±0.01[ab]
	Control	0.00±0.02[a]	0.23±0.01[b]
Day 7 post-vaccination	Batch 1	1.10±0.10[a]	0.34±0.00[a]
	Batch 2	1.10±0.10[a]	0.33±0.00[a]
	Batch 3	1.20±0.00[a]	0.25±0.09[b]
	Control	0.00±0.02[b]	0.25±0.01[b]
Day 14 post-vaccination	Batch 1	1.30±0.10[a]	0.47±0.01[a]
	Batch 2	1.20±0.00[a]	0.47±0.00[a]
	Batch 3	1.30±0.10[a]	0.46±0.00[a]
	Control	0.00±0.00[b]	0.25±0.01[b]
Day 21 post-vaccination	Batch 1	1.40±0.10[a]	0.68±0.00[a]
	Batch 2	1.50±0.00[a]	0.67±0.00[a]
	Batch 3	1.30±0.10[a]	0.68±0.01[a]
	Control	0.00±0.00[b]	0.25±0.01[b]
Day 28 post-vaccination	Batch 1	1.60±0.10[a]	0.77±0.00[a]
	Batch 2	1.50±0.00[a]	0.77±0.00[a]
	Batch 3	1.50±0.00[a]	0.75±0.00[b]
	Control	0.00±0.00[b]	0.24±0.01[c]

[a,b,c]Values within the same Rift Valley fever inoculation day category within the same column with different superscripts differ significantly at $p \leq 0.05$

Immune response of sheep vaccinated with different batches of inactivated RVF vaccine via SNT: The neutralization indices (NIs) of sheep after vaccination with fever inactivated vaccine is illustrated in Table 2. The neutralization indices reached the protective level on the 7th day after vaccination to 1.10±0.10 compared to the control group 0.00±0.02. The mean neutralization indices were steadily raised after 14, 21 and 28 days after vaccination at 1.20±0.00, 1.50±0.00 and 1.50±0.00, respectively. The statistical analysis showed that there was a significant difference of NIs in different times post-vaccination between the groups and there was a significant difference between vaccinated and non-vaccinated groups and time before vaccination at ($p \leq 0.05$). Statistical analysis showed also that there is a significant difference of NIs in different times post-vaccination between the different batches ($p \leq 0.05$).

Comparative evaluation of the immune response in sheep and young puppies vaccinated with the inactivated vaccine of RFV via SNT and ELISA: Table 3 showed that, both sheep and young puppies vaccinated with inactivated RVF vaccine did not significantly differ ($p > 0.05$) by SNT test at 7, 14, 21 and 28 days post vaccination that reach 1.08±0.03, 1.23±0.04, 1.30±0.03 and 1.45±0.02, respectively in young puppies. However, sheep showed slightly greater effect ($p < 0.05$) than young puppies with a booster dose of vaccine at 3rd and 4th week with neutralization indices at 1.15±0.02, 1.27±0.02, 1.42±0.05 and 1.55±0.03, respectively. In a related context, the use of the ELISA test to determine the immune response in sheep and dogs vaccinated with inactivated RVF vaccine and the results demonstrated a substantial difference ($p < 0.05$) or ($p < 0.01$) between sheep and dogs. Sheep vaccinated with the RVF vaccine displayed a substantially higher immune response than a dog from zero-day till the 4th week of vaccination. In addition, sheep also displayed a higher response to inactivated RVF vaccine than dogs.

Effect of inactivated RVF vaccine on blood picture in young puppies: Table 4 showed RBCs, HB and WBCs values in young puppies vaccinated with different batches of RFV inactivated vaccine. The value of red blood cells (RBCs) was significantly decreased to 4.89 after 1 week post-vaccination, then

Table 3: Comparative evaluation of the immune response in sheep and young puppies vaccinated with inactivated RFV vaccine by SNT and ELISA

	SNT					ELISA			
Test	0 days	7 days	14 days	21 days	28 days	0 days	7 days	14 days	21 days
Sheep	0.60±0.03*	1.15±0.02*	1.27±0.02*	1.42±0.05**	1.55±0.03**	0.29±0.02[d]	0.29±0.02[d]	0.29±0.02[d]	0.29±0.02[d]
Dogs	0.00±0.02*	1.08±0.03*	1.23±0.04*	1.30±0.03**	1.45±0.02**	0.28± 0.00[d]	0.28± 0.00[d]	0.28± 0.00[d]	0.28± 0.00[d]

*Non-significant, **Significant with $p < 0.05$ and [a,b,c] Values within the same column with different superscripts differ significantly at $p \leq 0.05$

Table 4: RBCs, HB and WBCs values in young puppies vaccinated with different batches of RFV inactivated vaccine

	Red blood cell (RBCs) value				Hemoglobin (Hb) value				White blood cells (WBCs) values			
Time of sera collection	Gr. 1 Bt. 1	Gr. 2 Bt. 2	Gr. 3 Bt. 3	Gr. 4 Ctrl	Gr. 1 Bt. 1	Gr. 2 Bt. 2	Gr. 3 Bt. 3	Gr. 4 Ctrl	Gr. 1 Bt. 1	Gr. 2 Bt. 2	Gr. 3 Bt. 3	Gr. 4 Ctrl
0 day	6.78±0.77^{a}	6.6±0.54^{a}	6.78±0.77^{a}	6.78±0.77^{a}	13.46±1.57^{a}	13.56±1.56^{a}	13.46±1.57^{a}	11.46±1.23^{a}	16.76±0.87^{b}	9.16±1.06	9.16±1.06	9.16±1.06
1st week after vaccination	4.89±0.12^{b}	4.89±0.72^{b}	4.21±0.58^{b}	6.78±0.77^{a}	8.8±0.99^{b}	7.97±1.01^{bc}	7.65±0.56^{c}	14.13±1.56^{a}	8.175±0.98^{d}	1.42±0.11	1.42±0.11	1.42±0.11
2nd week after vaccination	5.18±0.05^{b}	4.93±0.45^{b}	6.03±0.61^{a}	6.17±0.46^{a}	9.67±0.99^{c}	9.3±1.05^{c}	11.22±1.23^{b}	13.46±1.57^{a}	17.15±0.87^{a}	13.97±1.01	13.97±1.01	13.97±1.01
1st week after booster dose	5.05±0.63^{b}	4.43±0.66^{b}	4.94±0.32^{b}	6.6±0.54^{a}	9.25±1.02^{b}	7.65±0.78^{d}	8.35±1.00^{c}	13.93±1.56^{a}	15.75±1.05^{b}	11.7±1.02	11.7±1.02	11.7±1.02
Reference	5.5-8.5× 10^{6}/µL	5.5-8.5× 10^{6}/µL	5.5-8.5× 10^{6}/µL	5.5-8.5× 10^{6}/µL	11-19/dL	11-19/dL	11-19/dL	11-19/dL	6-17×103	4-12.6×103	4-12.6×103	4-12.6×103

transient increase to 5.18 at 2nd week post-vaccination and minor fluctuation in the mean of Red blood cells (RBCs) after booster dose to 5.05. Haemoglobin (Hb) count was significantly decreased to 8.8 after 1 week post-vaccination, then transient increase to 9.67 at 2nd week post-vaccination and minor fluctuation in the mean of Hemoglobin (Hb) after booster dose to 9.25. The WBCs value was significantly decreased to after 8.17after 1 week post-vaccination, then transient increase to 17.15 at 2nd week post-vaccination and minor fluctuation in the mean of White Blood Cells (WBCs) after booster dose to 15.75.

Granulocytes, monocytes and lymphocytes values in young puppies vaccinated with different batches of RFV inactivated vaccine: Table 5 showed granulocytes monocytes and lymphocytes values in young puppies vaccinated with different batches of RFV inactivated vaccine. Granulocytes count was significantly decreased to 1.42 after 1 week post-vaccination, then transient increase to 13.9 at 2nd week post-vaccination and minor fluctuation in the mean of Granulocytes after booster dose to 11.7 and monocytes count was significantly increased to 1.07 after 1 week post-vaccination, then transient decrease to 0.85 at 2nd week post-vaccination and the mean of monocytes after booster dose to 3.27. Lymphocytes count was significantly decreased to 5.87 after 1 week post-vaccination, then transient increase to 7.30 at 2nd week post-vaccination and minor fluctuation in the mean of Lymphocytes after booster dose to 2 after 1 week after booster injection.

DISCUSSION

In Egypt since the first outbreak of RVF in 1977, the Egyptian veterinary organization implemented strict quarantine measures and vaccination programs using locally produced tissue culture inactivated RVF vaccine[9]. This was followed by many trials by using different adjuvants with or without lyophilization to improve the immunogenicity of the vaccine[23,24]. Estimation of the immune response of sheep after vaccination is the main criteria to evaluate the efficacy of the vaccine. Application of the gold standard SNT to determine the level of protection of the inoculated vaccine confirmed the strong correlation between the neutralization indices and the protection level[25].

In this study, the neutralizing indices (NIs) of sheep after vaccination with inactivated RVF vaccine illustrated the protective level at 7th day post-vaccination in compared to the control group and the mean of (NIs) which increased

Table 5: Granulocytes, monocytes and Lymphocytes values, values in young puppies vaccinated with different batches of RFV inactivated vaccine

	Granulocytes values					Monocytes value					Lymphocytes value				
Time of sera collection	G. 1 Bt. 1	G. 2 Bt. 2	G. 3 Bt. 3	G. 4 Ctrl	G. 1 Bt. 1	G. 2 Bt. 2	G. 3 Bt. 3	G. 4 Ctrl	G. 1 Bt. 1	G. 2 Bt. 2	G. 3 Bt. 3	G. 4 Ctrl
0 day	9.16±1.06	0.60±0.00^{b}	0.60±0.00^{b}	0.60±0.00^{b}	0.60±0.00^{b}	0.60±0.00^{b}	0.60±0.00^{b}	0.60±0.00^{b}	2.13±0.09^{a}	2.27±0.34^{a}	2.13±0.09a	2.1±0.06a
1st week after vaccination	1.42±0.11	0.55±0.00^{b}	0.90±0.00^{a}	0.93±0.03^{a}	1.07±0.06^{a}	0.55±0.00^{b}	0.90±0.00^{a}	0.93±0.03^{a}	5.87±0.22^{c}	7.30±0.33^{a}	4.75±0.06^{d}	6.93±0.33^{b}
2nd week after vaccination	13.97±1.01	0.90±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.85±0.00^{a}	0.90±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	7.30±0.33^{a}	4.75±0.06^{d}	6.93±0.33^{b}	2.13±0.09^{a}
1st week after booster dose	11.7±1.02	3.27±0.12^{a}	0.75±0.1^{b}	0.56±0.00^{b}	3.27±0.40^{a}	3.27±0.12^{a}	0.75±0.1^{b}	0.56±0.00b	2.00±0.33^{a}	2.95±0.23^{a}	2.13±0.09^{a}	2.56±0.11^{b}
Reference	4-12.6×103	0.8-5.1×103	0.8-5.1×103	0.8-5.1×103	0.8-5.1×103	0.8-5.1×103	0.8-5.1×103	0.8-5.1×103	0.8-5.1× 103/µL	0.8-5.1× 103/µL	0.8-5.1 × 103/µL	0.8-5.1× 103/µL

gradually after 14, 21 and 28 days post-vaccination. Statistical analysis showed that there was a substantial difference of NIs in different times post-vaccination between the groups and there was a significant difference between vaccinated and non-vaccinated groups and time before vaccination at ($p \leq 0.05$), while non-significant variance between different batches was recorded. These findings are close to that recorded[24], they recorded that the protective level with one dose of the Montanide Gel 01TM inactivated RVF vaccine administration was obtained at the 7th day post-vaccination and remain for 11 months post-vaccination and recommended it in emergency vaccination. In the early studies reported[9,26] the protective NI level of the inactivated RVF virus vaccines was reached to the maximum level at 2 weeks after vaccination.

Many other studies support and are nearly similar to the result of the present studies[27-29]. In a comparative study adopted by parallel to these studies[24] who reported that the booster dose from inactivated RVF vaccine is needed to achieved protection and high NI(1.7) at the 2nd week post-vaccination then reach the peak at the 4th week post-vaccination. The early protection after 1 week of vaccination reported in the study of the present work[24] is really due to the improvement of the inactivated RVF vaccine by using different types of adjuvants and the inactivation process.

Statistical analysis of the present studies showed no significant effect between the different batches of the inactivated RVF vaccine. A point which is important that conclude the stability of the vaccine production.

The current serum neutralization test (SNT), need the handling of live RVFV during the preparation of antigen and/or the demonstration of tests. Outsides of endemic locations, the laboratory works involve a biohazard and limit to strong restraint abilities. The recent occurrence of RVF in France[30] poses a global medical and veterinary risk resulted in a surge in the global need for safe, standardized and proven diagnostic immune reagents. To date, only inactivated virus has been utilized as antigen in ELISA detecting RFV antibodies. one of the most basic immunoassays techniques for the detection of antibodies is the I-ELISA. However, the unspecific signal resulting from the use of semi-purified or unpurified antigens is one of the key issues impeding its wider, routine diagnostic applications[31,32]. Purification of equal amounts of recombinant antigen can take less time and cost compared to the production of whole viral antigen. Furthermore, recombinant antigens have no interestingly and are extremely stable[33].

In the present study, the application of indirect ELISA following the immune response after 2 doses of sheep vaccination with aluminium hydroxide inactivated RVF vaccine revealed the optical density values of 0.29±0.02, 0.41±0.02, 0.57±0.02 and 0.64±0.02 after 1, 2, 3 and 4 weeks' post-vaccination. Such results are in accordance with Alsaid *et al*.[24], they assessed the humoral immune response by ELISA with nearly similar values of the present work. They added that ELISA is a sensitive and accurate test to evaluate the immune status of RVF in animals. They also reported the ELISA values of 2.1 and 3.28 and 1.8 after 2, 4 weeks and 10 months post-vaccination of lyophilized inactivated RVF vaccine, respectively. While vaccination by the aluminium hydroxide inactivated RVF vaccine recorded 1.7, 3.13 and 1.7 values after 2, 4 weeks and 10 months of post-vaccination. In the present study, the ELISA results come in parallel to results obtained by SNT. Such results are supported that obtained by another researcher[24] as they concluded that ELISA titers take the same plateau of the SNT. Moreover, in experimentally infected sheep, the titer of the IgG I-ELISA was more sensitive than virus neutralization and haemagglutination-inhibition assays in detecting the early immune response[34].

In the present work vaccinated puppies with inactivated RVF vaccine 1mL S/C followed by a booster dose after 2 weeks showed neutralization indices detected at 7th day post-vaccination in comparison to the control group and then increased gradually to reach 1.20± 0.03, 1.25± 0.01 and 1.35±0.02 after 14, 21 and 28 days post-vaccination. The statistical analysis showed a significant difference of NIs in different times post-vaccination between the groups and there was a significant difference between vaccinated and non-vaccinated groups and the time before vaccination. Statistical analysis showed no significant effect between the different batches of the RVF inactivated vaccine used for dog vaccination. On the other hand, there was a significant difference ($p \leq 0.05$) between the vaccinated and the non-vaccinated group. These results are pioneer and novel in this field as it is the first time to use puppies as an experimental model for the evaluation in the inactivated RVF vaccine. To our knowledge, there is no available literature on this concern. The results of the immune response of puppies to the aluminium hydroxide inactivated RVF vaccine tested by SNT and ELISA is similar to that recorded in sheep in this study. This finding suggests the use of puppies to substitute sheep for the evaluation of the inactivated RVF vaccine. This is due to, it is easy to obtain seronegative puppies than sheep because dogs are not included in the annual program of RVF vaccination.

Concerning the changes in the haematological pictures in puppies vaccinated with inactivated RVF vaccine, the results reported a significant decrease in all hemogram and leukogram parameters after the 1st week followed by an increase after booster dose at 2nd week except for the monocytes that increase after 1st week then decreases after 2nd week post-vaccination. These results are parallel that Fakour *et al*.[35] reported an increase in leucocyte count after vaccination of sheep with inactivated RVF vaccine. This increase in total leucocytic count post-vaccination might clear the picture of antibody formation which is produced by B lymphocytes. In comparative studies between life attenuated and inactivated RVF vaccine reported[28], a significant increase of lymphocytic count after vaccination of sheep with inactivated RVF vaccine which supports the results in this study concerning the changes of blood picture of puppies after vaccination of inactivated RVF.

In continuation the present results agree with other study by Bahgat[36], who found that sheep vaccinated with Montanide IMS3015 inactivated RVF vaccine had higher lymphocyte count compared with control and higher than sheep vaccinated with aluminium hydroxide inactivated RVF vaccine. These results indicated no conclusive effect on the various haematological parameters of the vaccinated puppies with local produced inactivated RVF vaccine in Egypt and the safety effect of the local prepared RFV vaccine on the haematological profile. In a comparative study in India, Kumar *et al*.[37] reported that granulocytes, monocytes and lymphocytes were the most haematological biomarkers that correlate with the evaluation of the *Brucella melitensis* vaccine safety in India. In a similar context, Starita *et al*.[38] reported a minimal change in total protein, globulins fractions, IgM and IgG and mild increase of IFAT titers with Leishmania infantum vaccine (CaniLeish) and its safety administration in dogs.

CONCLUSION

This study reflects the high antibody response of puppies after vaccination with RVF inactivated vaccine. Puppies are quite equal to sheep for the illustration of neutralizing antibodies following vaccination with inactivated RVF vaccine. Statistical analysis showed that there was no significant effect of different batches of inactivated RVF vaccine in both puppies and sheep. The haematological pictures in puppies vaccinated with RFV inactivated vaccine reported a significant decrease in almost blood parameters after the 1st week followed by a transient increase after booster dose at 2nd week. This study also, concluded that the use of puppies as a model

instead of sheep is good for the evaluation of RVF inactivated vaccine. The ELISA can replace the SNT for evaluation of the immune response. Further studies are needed to discover more recent methods for evaluating of inactivated RVF vaccine.

SIGNIFICANCE STATEMENT

This study revealed that young puppies are similar to sheep in developing antibodies after vaccination with the inactivated RFV vaccine with no statistically significant effect within different batches. In addition, ELISA can replace the SNT for evaluation of the immune response. Young puppies are quite equal to sheep for the illustration of neutralizing antibodies for RFV vaccine. Sero-negative puppies can be easily obtained because dogs are not included in the vaccination program of RVF and so they can be used as a good model to determine the efficacy of the RVF vaccine. Moreover, the haematological profile reported a significant decrease after the 1st week followed by a transient increase after booster dose at 2nd week except for the monocytes that increase after 1st week then decreases after 2nd week post-vaccination.

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