



Advances in Diagnosis and Control of Rift Valley Fever in Small Ruminants;

Sheep and Goats - A Review

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THE INTENT of this study was to provide insight on the evaluation and management of Rift Valley fever (RVF) that affect small ruminants; sheep and goats. RVF is a zoonotic disease transmitted by mosquitoes that is acute, febrile, and caused by a virus of the *Bunyaviridae* family, genus *Phlebovirus* that represents a significant threat to humans and livestock. Severe clinical signs usually seen in cattle and small ruminants, where it is characterized by high fever (41 °C), abortions and a high mortality rates in the newborn. ELISA assays can distinguish between past and recent infection, it cannot differentiate between the past infected and vaccinated animals except by paired serum samples examination. A variety of highly sensitive molecular techniques were established for RVF comprising quantitative real-time PCR nested RT-PCR methods, multiplex PCR-based microarray assay, quantitative real-time PCR, recombinase polymerase amplification (RPA) and RT Loop-mediated isothermal amplification (RT-LAMP). The control of the RVF disease depends mainly on two main principles; the first one is the vaccine, and the second is the combating of the arthropod vectors. There are two types of RVF vaccines, the accredited live attenuated and inactivated vaccine preparations. The Clone 13 vaccine depends on the NSs gene, which encodes the primary virulence gene, has a significant loss of naturally attenuated strain, which developed from the Central African strain that was isolated from a human. The vaccine is highly immunogenic in ruminants, save and do not lead to untoward effects in vaccinated animals.

Keywords: Rift Valley fever, Sheep, Goat, Diagnosis, Control.

Introduction

An acute or per-acute, febrile, mosquito-transmitted disease called Rift Valley fever (RVF) is brought on by a virus of the *Bunyaviridae* family, genus *Phlebovirus* that represents a significant threat to humans and livestock. It is responsible for serious economic losses due to abortion and heavy mortalities in newly born animals [1]. The OIE certifies that locally validated techniques like RT-PCR are acceptable for viral RNA detection. Although RVF is less infectious than Foot and mouth disease (FMD) but the losses due to RVF are incomparable to that of FMD [2]. ELISAs and virus neutralization tests have the ability

to detect antibodies to RVF virus. ELISA assays can distinguish between past and recent infection, it cannot differentiate between the past infected and vaccinated animals [3]. Virus neutralization tests depend on the presence of a live virus and this generally not recommended outside endemic regions [4]. Prevention of infection of the disease is the first step in the disease control. Early detection of infection through strict active animal inspection and advised sentinel herd monitoring [5]. Once infected animals are situated, prevention of the further spread can be achieved by mosquito control and animal movement control. Animal vaccination is the most

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significant step in the disease control. Standard precautions diminish transmission risk to healthcare workers and farmers [6]. In Egypt, four epidemics of RVF recorded during 1977, 1978, 1993 and 2003. Furthermore, an epidemic of RVF accompanying with severe abortion rates in sheep and cattle stated in Upper Egypt in 1997. The abortion rate in ewes was roughly 60–70%. The mortality rates were 50– 60% in lambs, and 25– 35% in adult sheep [7]. The disease first reported in Aswan, and then went up to north into eight governorates of the Delta; mainly El-Sharquiya, El- Qualubiya El- Giza and the Nile Valley. The disease was associated with the human cases that were assessed to be 18,000–20,000 individuals sustained from the disease and 599 died in 1977 [8, 9]. Utilizing epidemiological research to evaluate the present RVF situation in Egypt. The used techniques in this study were AGPT, SNT and ELISA [10]. The vaccinated sheep showed lower prevalence of RVF, while vaccinated sheep, goat, cattle and buffaloes revealed a higher prevalence [11]. On the contrary, the non-vaccinated goats, sheep, cattle, camels and buffaloes showed different rates of prevalence. The continual migration of many camels from Sudan poses the biggest danger of

RVFV introduction. The Nile River's surroundings are the only places where there is a danger of RVFV transmission by vectors, and this risk does not change greatly over the course of the year [12]. Imported camels placed in quarantines, where there is typically little risk of vector-borne RVFV transmission. Then, they are sent to slaughterhouses or animal markets, many of which are found in populated areas, where there is a considerably higher danger of transmitting RVFV to humans or animals [13]. Other (risk-based) monitoring tactics suggested because the current measures (quarantines, vaccine, or testing) appear to have a limited impact in reducing the likelihood of RVFV introduction [14]. Consequently, the purpose of the current study was to shed light on the diagnosis and control of the Rift Valley fever that affect small ruminants; sheep and goats.

Etiology

Rift Valley Fever (RVF) is mosquito-borne, zoonotic disease, causing acute fever viral disease of livestock and humans people. It brought by a virus of the genus *Phlebovirus*, family *Bunyaviridae* (Fig. 1, a & b) [15].

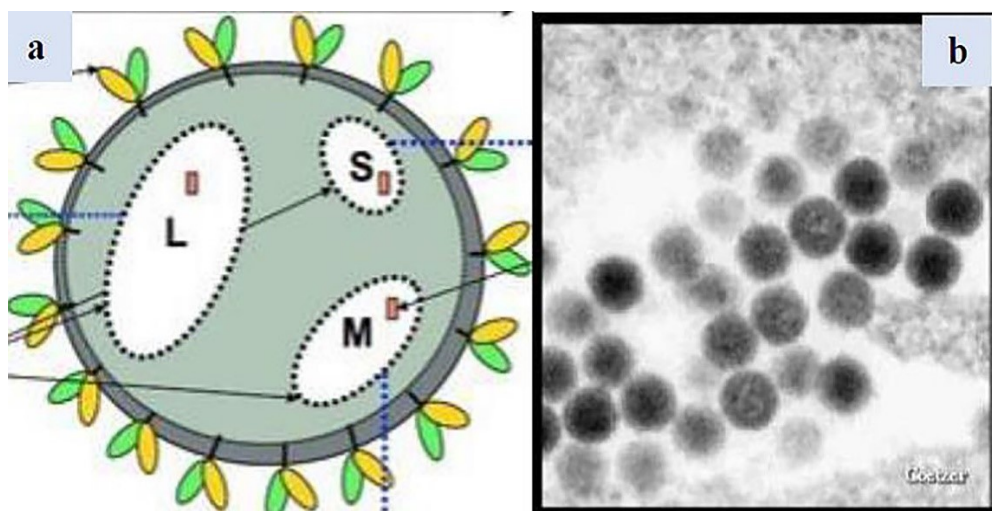


Fig. 1. Rift-Valley-Fever-virus schematic-diagram (a) and electron micrograph (b) [15].

Transmission

A mosquito *Aedes*, *Culex*, *Anopheles*, *Erethmapodites*, *Monsosmia*, primarily transmits RVF from animal to animal (Fig. 2 a, b & c) [16].

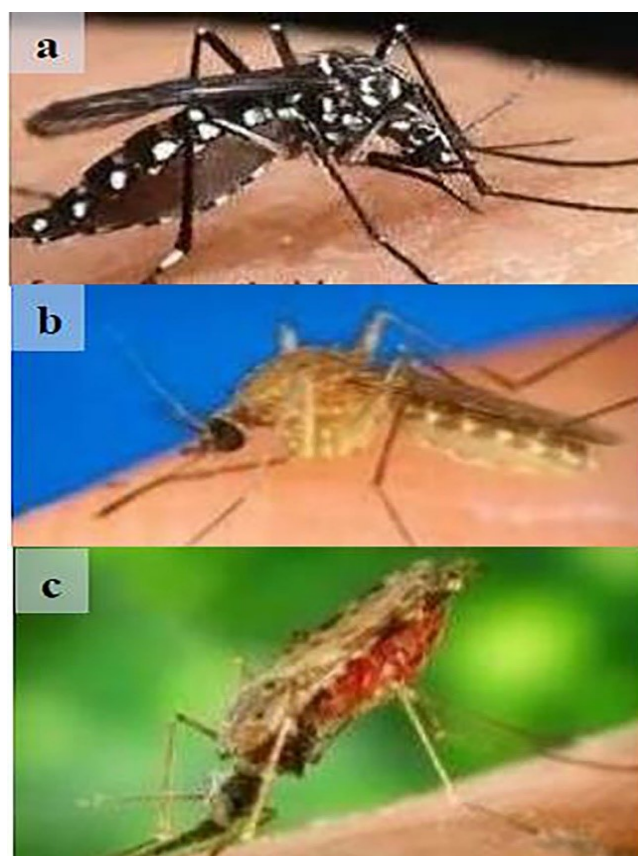


Fig. 2. RVF; transmitting mosquitoes, *Aedes* (a), *Culex*, (b) and *Anopheles* (c) [16].

Diagnosis of Rift valley fever

Clinical Picture

Rift valley fever typically exhibits severe clinical symptoms in cattle and small ruminants, where it characterized by high fever (41°C), In Sheep, Loss of appetite, jaundice and weakness. Nearly all lambs under two weeks of age will die and in older sheep, mortality reaches to 30% with

abortions approaching to 100%. Cattle are less susceptible than sheep, some are subclinical; anorexia, excessive salivation, fetid diarrhea, weakness, fall in milk yield and mortality averages 5% with some abortions. Water buffalo up to 50% abortion rate camels (in Egypt) in-apparent disease except abortions (Table 1. and Fig. 3 a & b) [17].

TABLE 1. Rift Valley fever host range and disease severity

Mortality ~100%	Severe Illness Abortion, Low Mortality	Severe Illness Viremia Abortion	Infection Viremia	Refractive to infection
- Calves	- Cattle	- Camels	- Horses	- Guinea pigs
- Lambs	- Sheep	- Monkey	- Dogs	- Pigs
- Kids	- Water buffalo	- Rats	- Cats	- Rabbits
- Kittens	- Goats	- Gray squirrels	- Monkeys	- Tortoises
- Puppies	- Humans			- Hedgehogs
- Hamster				- Frogs
- White mice				- Canaries
- Door mice				- Chickens
- Field mice				- Pigeons
- Field voles				- Parakeets



Fig. 3. Clinical signs of RVF; weakness and diarrhea in sheep (a), anorexia and emaciation in cattle (b) [17].

Post-mortem findings

The postmortem findings associated with RVF included enlarged friable liver with generalized or focal necrosis of liver, enlargement, congestion, and liver discoloration with sub capsular and petechial hemorrhages in the cutaneous and serosal surfaces. Brown-yellowish color of liver in aborted

fetuses. Other gross lesions are generalized jaundice, petechial and ecchymotic hemorrhages, subcutaneous hemorrhages on both surfaces of the internal organs, hemorrhages, edema and necrosis of lymph nodes, kidney and gallbladder congestion and cortical hemorrhages (Fig. 4 a, b, c & d) [18].

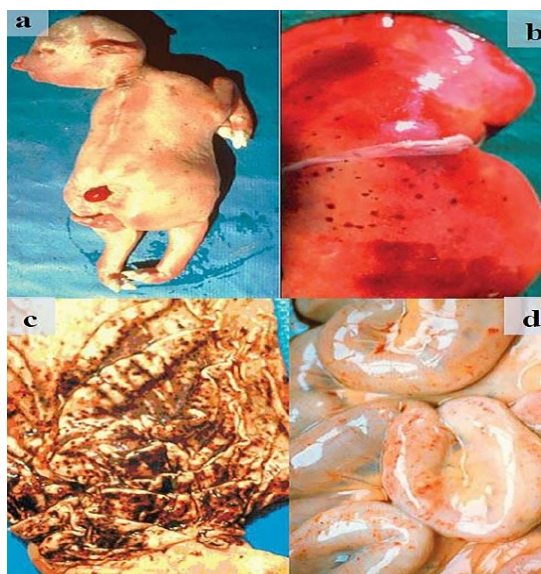


Fig. 4. Sheep affected by RVF; aborted fetus (a), necrotic liver (b), abomasum mucosal hemorrhage (c) and petechial hemorrhages on the intestinal serosa (d) [18].

Differential diagnosis

To distinguish between the other illnesses that can be misdiagnosed with RVF, a differential diagnosis must be performed. These diseases causing fever and abortion in small ruminants. The following; Abortifacient agents, agents causing hepatitis and agents that cause hemorrhages (Wesselsbron disease, Bluetongue, Ephemeral fever, Enterotoxemia of sheep, Vibriosis, Brucellosis, Nairobi sheep disease

Rinderpest, Peste des petits ruminants and bacterial septicemias) [19]. Signs of diarrhea, pneumonia, can avoid Peste des Petits Ruminants disease (PPR) and signs of nasal ulceration and facial edema can remedy bluetongue. Pock lesions differentiate capripox, Vesicular stomatitis excluded pneumonic pasteurellosis and contagious ecthyma, and caprine pleuropneumonia characterized by respiratory manifestation alone (Fig. 5) [20].

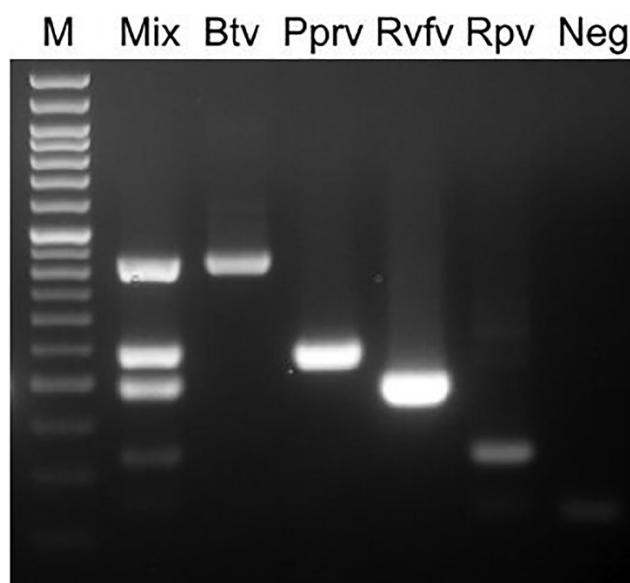


Fig 5. Multiplex RT-PCR for Rift Valley Fever virus (RVFV), rinderpest virus (RPV), bluetongue virus (BTV), and Peste des petits ruminant's virus (PPRV). M, 50-bp DNA molecular weight marker and Mix, four viruses in a single-tube [20].

Laboratory diagnosis

Virus isolation

Although virus isolation is also a possibility, lab workers are at risk from this virus. Although hamsters, adult or suckling mice, embryonated chicken eggs, or two-day-old lambs can also be

utilized, animal inoculation should avoided wherever possible. Rift Valley fever virus can be cultivated on BHK 21, chicken embryo-related (CER) cells, Vero cells, AP61 mosquito cell lines and *LT cell line* (Fig. 6 a & b) [21].

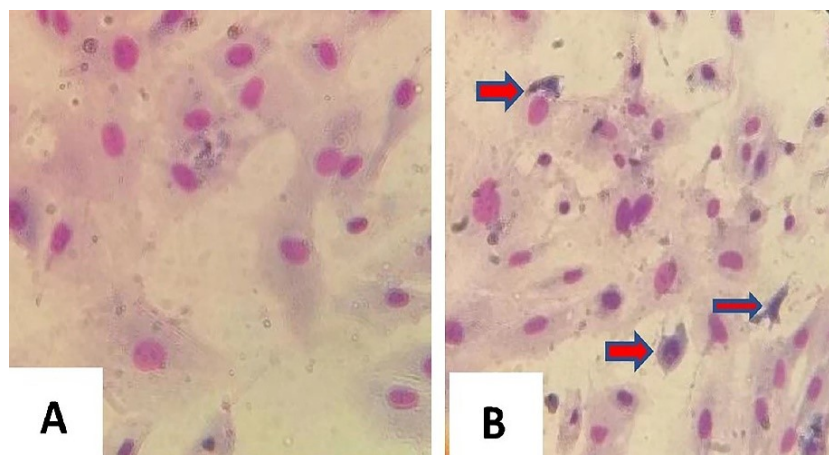


Fig. 6. Giemsa staining of RVFV for infection of cultured LT cell ($\times 400$). (A) Mock (control) cells. (B) cytopathic effect of RVFV, necrotic foci (red arrows) [21].

Molecular detection

A variety of highly sensitive molecular techniques were established for RVFV that include quantitative real-time PCR, nested RT-PCR, RT Loop-mediated isothermal amplification (RT-LAMP), multiplex PCR-based microarray assay, and

recombinase polymerase amplification (RPA). Using a set of six primers, the RVF RT-LAMP amplifies specific nucleic acid sequences. Amplification results in the development of a DNA precipitation that is visible to the naked eye. The assays are costless, easy and practical (Fig. 7) [22].

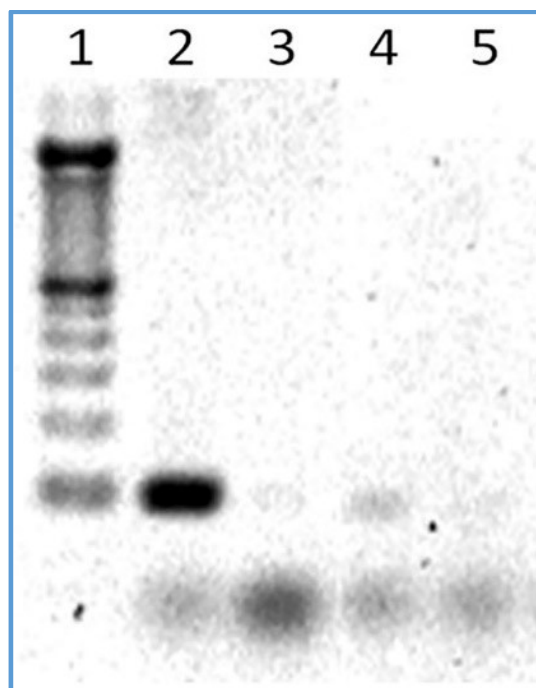


Fig. 7. Rift Valley fever virus positive bands on PCR gel (90 bp). Rift Valley fever virus MP-12 positive control, lane 2, molecular mass ladder, lane 3, negative control, lane 4, pool 103 (positive), and lane 5, pool 86 (negative) [22].

The OIE confirms that locally validated techniques like RT-PCR or real-time RT-PCR are acceptable for viral RNA detection. A blind portion of RVFV analyzed by thirty professional laboratories from sixteen different nations. Both positive and negative samples are included in this section. Laboratories employed their regional RVFV test that

molecularly based. The findings revealed that 28/30 laboratories (93%) used qRT-PCR because to its high specificity and sensitivity. RVFV kit used for qualitative detection of infection in human serum or plasma sample by using Real Time PCR system (Fig. 8) [23].



Fig. 8. Real Time PCR Rift valley fever virus detection kit [23].

Serological diagnosis

Virus neutralization assay (VNA)

There are several analyses available for identifying RVFV antibodies in various animal species. Despite the fact that ELISAs and VNAs are quite specific, cross-reactivity between RVFV and the other members of phleboviruses can occur [24].

The Virus neutralization assay for RVFV recorded as the gold standard test and it used for vaccine potency evaluation and international trade assessment. Although the test highly specific and could be used on serum from many different host animals, it is limited to special laboratories that have a proper biosecurity facility [25].

Enzyme-linked immunosorbent test (ELISA)

ELISA used to approve the existence of RVF specific IgM and IgG antibodies, which indicate recent infection or old infection, respectively [26]. The appearance of the antibodies is in a reversal relationship to the virus titer in the blood i.e. the antibodies appears with the gradual disappearance of the virus. Therefore, the collected serum or blood samples may contain live virus. These samples should be inactivated before testing the detection of IgM indicated a recent infection. ELISA assays can distinguish between past and recent infection, it cannot differentiate between the past infected and vaccinated animals except by paired serum samples examination [27]. Special indirect ELISA was established to differentiate the infected animals from the vaccinated (DIVA) ELISAs and virus

neutralization have the ability to detect antibodies to RVF virus. Virus neutralization tests depend on the presence of a live virus and this generally not recommended outside endemic regions. Commercial ELISA testing is far more beneficial in low- and middle-income settings due to its advantages of speed, reduced cost, and lack of containment requirements when compared to plaque reduction neutralization testing [28].

Other serological methods

Other serological methods like the immunohistochemistry (Fig. 9), Indirect Immunofluorescence, agar gel immunodiffusion in addition to radioimmunoassay and complement fixation, hemagglutination inhibition assay (HIA) are now a limited to be used for the serological diagnosis of RVF in suspected animals [29].

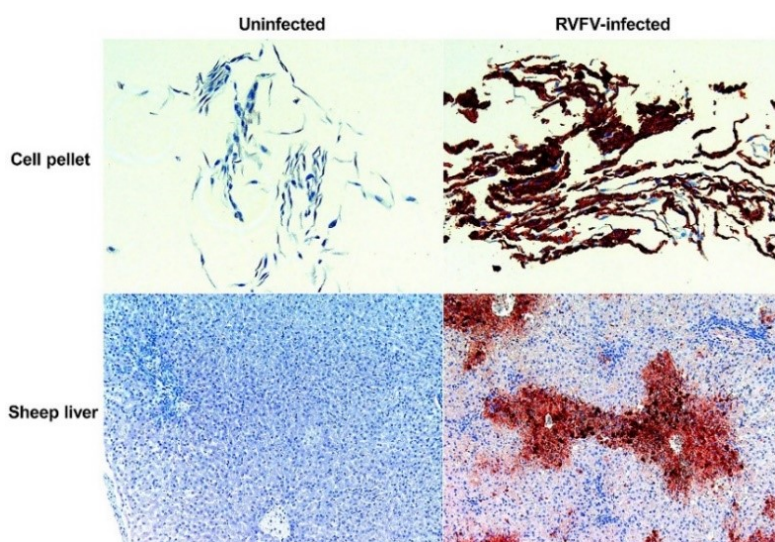


Fig. 9. Immunocytochemistry and immunohistochemistry for RVFV, sheep liver sections (lower images) Vero cells (upper images) tested for RVFV antigen. Slices from infected cells or liver from an infected sheep showed extensive intra-cytoplasmic antigen staining in hepatocytes (right photos), but slices from uninfected cells or liver showed no antigen [22].

Field test

It was successful in developing and validating a pen-side test for the diagnosis of Rift Valley fever using a lateral flow immunochromatographic strip test (LFT) that relies on finding the RVF virus nucleoprotein (N) in serum samples (Fig. 10), [30].

Prevention and control of Rift valley fever

The control of the RVF disease depends mainly on two main principles; first, one is the combating of the arthropod vectors, and the second is the vaccination [31].

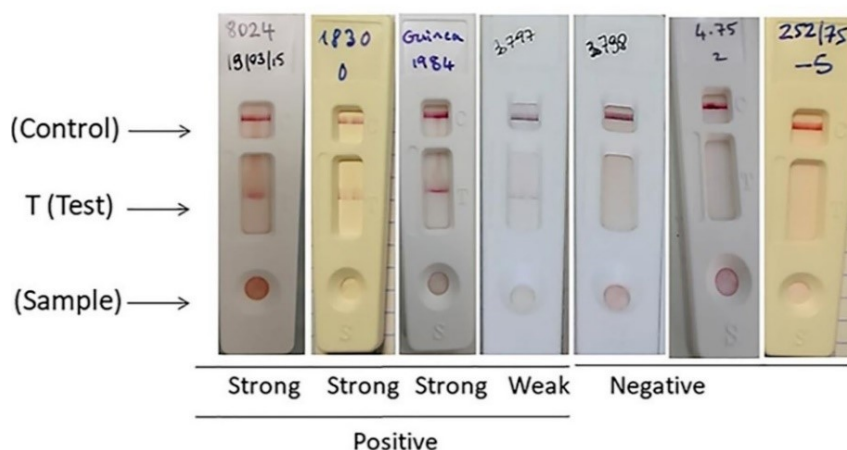


Fig. 10. Lateral flow immunochromatographic (LFT) strip test for the detection of Rift Valley Fever (RVF)infection using the two selected monoclonal antibodies [30].

Prevention of animal and human outbreaks

Once diseased RVF animals are located, further transmission can be stopped by controlling animal movement and mosquitoes. It is advised to limit the slaughter of cattle or, at the very least, to use protective gear (gloves, masks, and gowns) when handling carcasses or aborted fetuses. To stop the further transmission of animal diseases to humans, public awareness and education about the warning signals and risk factors are essential. The most important step in the prevention of disease is vaccination of animals. Standard precautions reduce the danger of transmission to farmers and healthcare professionals [32].

Combating of the vector

Mosquito repellents in the animal houses, trousers and long shirts for the workers and other arthropod control techniques exclusively recommended to stop mosquitoes and other potential insect vectors from spreading disease to human and animal. Decreasing the green covering will help in the reduction of the mosquitoes densities. All the water ponds must be buried or covered with nets to prevent the multiplication of mosquitoes [33]. The use of biological combating by growing the Gambusia fish in water resources and the huge areas of the stagnant water to get rid of the developmental stages of mosquitoes recommended [34].

Vaccination

Although there is no approved vaccine for practice in humans, three licensed veterinary vaccines are present to protect livestock [35]. The approved live attenuated (Smithburn vaccine) and inactivated vaccine formulations are the two forms of

RVF vaccines. In endemic areas, usage of the two vaccinations is restricted. In the US, the live attenuated MP-12 vaccination has a conditional authorization. Zimbabwe and South Africa have also granted licenses for the Clone-13 vaccination. The potential for co-infection and assortment between the vaccination and the field virulent viruses suggests that a new generation of efficacious and safe veterinary RVFV vaccines still urgently needed [36].

RVF in Egypt

Four epidemics of RVF were recorded in Egypt; during 1977, 1978, 1993 and 2003 (Jun–Oct) [37]. Furthermore, an epidemic of RVF accompanying with severe abortion rates in sheep and cattle stated in Upper Egypt in 1997. The abortion rate in ewes was roughly 60–70%. The mortality rates were 50–60% in lambs, and 25–35% in adult sheep [38]. RVF has documented in man, cattle, and buffaloes in Aswan Governorate. Similar epidemic, El-Faiyum Oasis and the majority of the governorates in the Nile Delta both saw infection spread. After this outbreak, RVF reappeared in intervals between 1993 and 2003 [39]. Moreover, the Ministry of Health distinguished 745 RVF cases (IgM positive) throughout 1993–2004 in some governorates, which suggested recent infections in humans. Following these outbreaks, many instances (5306) were suspected to develop between 2004 and 2008 [40]. The RVF vaccine available in Egypt is inactivated killed RVF vaccine, produced by Veterinary Serum and Vaccine Research Institute, prepared from Zagazig H501 strain. Clone13 live attenuated virus vaccine was also available in Egypt [41].

Conclusions

Special indirect ELISA established to differentiate the infected from the vaccinated animals (DIVA). A variety of highly sensitive molecular techniques were established for RVFV involving quantitative real-time PCR, nested RT-PCR methods, multiplex RT Loop-mediated isothermal amplification (RT-LAMP), PCR-based microarray assay, and recombinase polymerase amplification (RPA). The control of the RVF disease depends mainly on two main principles; the first one is the vaccine, and the second is the combating of the arthropod vectors. There are two types of RVF vaccines, the accredited live attenuated (Smithburn vaccine) and inactivated vaccine preparations. The Clone 13 vaccine depends on the primary virulence gene (NSs gene) having a significant deletion of naturally attenuated strain, which developed from the Central African strain that was isolated from a human. The vaccine is highly immunogenic in ruminants, save and do not lead to untoward effects in vaccinated animals. Another recombinant vaccine developed that utilizes Lumpy Skin Disease virus (LSDV) as a vector or carrier for RVFV glycoprotein, resulting in a bivalent RVFV vaccination with the added benefit of defending animals from Lumpy Skin Disease.

Conflicts of interest

The authors declared no competing interests.

Funding statement

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الاتجاهات الحديثة في تشخيص ومقاومة حمى الوادي المتصدع في المجترات الصغيرة: الأغنام والماعز – بحث مرجعي

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الهدف من هذه الدراسة هو إلقاء الضوء على تشخيص ومكافحة حمى الوادي المتصدع (RVF) التي تصيب المجترات الصغيرة؛ الأغنام والماعز. وحمى الوادي المتصدع مرض حمى حاد، مصحوب بارتفاع شديد للحرارة وهو حيواني المنشأ (ينتقل بين الإنسان والحيوان)، ينقله البعوض ويسببه فيروس من عائلة الفيروسات البنيوية، جنس الفيروسات الوريدية الذي يمثل تهديداً كبيراً للبشر والماشية. والأعراض المرضية تتمثل في انها علامات سريرية حادة تظهر عادة في الأبقار والمجترات الصغيرة، حيث تتميز بارتفاع درجة الحرارة (41 درجة مئوية)، مع حدوث الإجهاض بالإضافة إلى ارتفاع معدلات الوفيات عند الأطفال حديثي الولادة. ويمكن باستخدام اختبارات الإليزا (ELISA) التمييز بين العدوى السابقة والحديثة، ولا يمكنها التمييز بين الحيوانات المصابة والمحصنة سابقاً إلا عن طريق فحص عينات المصل المقترنة. لذلك يمكن استخدام مجموعة اختبارات متنوعة من التقنيات الجزيئية شديدة الحساسية لحمى الوادي المتصدع التي تتضمن طرق وتفاعل البلمرة المتسلسل المتداخلة، وتفاعل البلمرة المتسلسل الكمي، ومقاييس المصفوفة الدقيقة المستندة إلى تفاعل البلمرة المتسلسل التسلسلي والتضخيم متساوي الحرارة الحلقي وتعتمد طرق المكافحة والسيطرة على مرض حمى الوادي المتصدع بشكل أساسي على مبدئين رئيسيين؛ الأول هو استخدام التحصين (اللقاح)، والثاني هو مكافحة ناقلات الأمراض الحشرات. فهناك نوعان من لقاحات حمى الوادي المتصدع، مستحضرات اللقاح الحية المضعفة ومستحضرات اللقاح الميتة المعطلة. ويعتمد لقاح Clone 13 على وجود حذف كبير في جين الفوعة الرئيسي (NSs) للسلالة الموهونة طبيعياً، والتي تطورت من سلالة أفريقية الوسطى التي تم عزلها من الإنسان. واللقاح يعطى مناعة عالية في الحيوانات المجتررة، ولا يؤدي إلى آثار غير مرغوب فيها في الحيوانات المحصنة.

الكلمات الدالة: حمى الوادي المتصدع، الأغنام، الماعز، التشخيص، المكافحة.