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# Serological Survey of Rift Valley Fever among Sacrifice Animals in Holy Mecca During Pilgrimage Season

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**Abstract:** The current research aimed to evaluate prevalence of RVF exposure among sacrifice animals during pilgrimage season in Saudi Arabia and to determine the vulnerability of the sacrifice herds based on its immune status. A total of 580 sacrifice animals including 120 local and 460 imported animals were randomly selected from the sacrifice herds at Al-Kaakiah slaughter houses, Mecca during the 2009 pilgrimage season. Blood samples were collected from all investigated animals for serum separation and conduction of capture and sandwich ELISA techniques for detection of anti-RVF virus IgM and IgG immunoglobulins, respectively. The study revealed an overall rate of recent RVF exposure, manifested by positive IgM sera, of 2.59% among investigated sacrifice animals (0.83% among local animals and 3.04% among imported ones). On the other hand, the overall herd rate of immunized animals based on the positive IgG cases were 47.06% (55% among local animals and 45% among imported ones). In spite of the low prevalence of recent RVF exposure among sacrifice animals, the low level of immunity among those animals increase the potential risk of eruption of another outbreak among sacrifice animals during pilgrimage with the subsequent socio-economic and public health consequences. The study clearly denotes the importance of applying an effective and controlled vaccination program for local animals and verification of the immune status of imported herds.

**Key words:** Rift valley fever, sacrifice animals, serological survey, IgM and IgG immunoglobulins, pilgrimage, Saudi Arabia

## INTRODUCTION

Rift Valley Fever (RVF) is an acute vector-borne zoonotic disease that affects both humans and domestic animals. In humans, it can cause a fatal hemorrhagic fever disease (Davoust *et al*., 2008). In animals, particularly sheep and goats, the disease is usually accompanied with characteristic clinical symptoms ranged from high mortalities among young animals to severe abortion among adult female animals (Munyua *et al*., 2010). However, asymptomatic infection has been described among these animals as well. Both clinically and sub-clinically affected animals represent a potential source of human infection. Humans are susceptible to infection by handling infected material and through transmission by mosquito vector (Isaacson, 2001; Woods *et al*., 2002; Gerdes, 2004). At the beginning of the third millennium (September 2000 to April 2001), an outbreak of RVF has been reported in Saudi Arabia with many humans and animals victims. The outbreak marks the first appearance of Rift valley fever outside Africa (Madani *et al*., 2003). In South-West Saudi Arabia, the Jazan region was the hardest hit by the outbreak with an infection rate of 23% followed by 8.7% in Asir and 2% in Mecca based on sero-testing (Elfadil *et al*., 2004). The mass vaccination of livestock (primarily sheep and goats) with the live attenuated RVF Smithburn strain vaccine that were implemented in the South-West immediately after the outbreak has resulted in remarkable control of the disease in this region manifested by the

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absence serological evidence of recent RVF infection in 2003 (Elfadil *et al*., 2004). However, a more recent serological survey conducted in 2006 revealed the persistence of RVF in the Jazan region of Saudi Arabia (Elfadil *et al*., 2006).

In holy Mecca, around 10-15 million small ruminants are sacrificed annually during the pilgrimage season. Although, some of these animals come from the Arabian Peninsula itself, most are imported across the Red sea, from countries in East Africa where RVF is known to be enzootic and can be greatly amplified during periods of epizootic virus activity (Jupp *et al*., 2002; Davies, 2006). These animals may be transported to and arrive in Mecca within the incubation period of the disease (Elfadil *et al*., 2004).

Epidemiologic surveillance of RVF disease at potentially threatened areas is an essential practice that should be carried out periodically to avoid another possible outbreak (Davoust *et al*., 2008; LaBeaud *et al*., 2008). Serological survey is an effective tool of active surveillance and control of diseases. It is usually practiced to determine the distribution of infection, monitor herd immunity and also to follow up the course of the disease in different seasons (Scott *et al*., 1986; Paweska *et al*., 2005b; Elfadil *et al*., 2006). Sandwich and capture ELISAs for detection of anti RVF virus-IgG and IgM, respectively in bovine, caprine, ovine and human sera has been developed and validated (Paweska *et al*., 2003, 2005a, b; LaBeaud *et al*., 2008). Compared to virus neutralization and haemagglutination-inhibition tests, ELISAs have showed superior sensitivity in detecting earliest immunological responses to infection or vaccination with Rift valley fever virus (Soliman *et al*., 1988; Zaki *et al*., 2006). These assays are reported to be useful for epidemiological surveillance and control programmes, import/export veterinary certification, early diagnosis of infection and for monitoring of immune response in vaccinated animals (Paweska *et al*., 2003).

Given the potential risk for eruption of an outbreak among sacrifice animals with the subsequent public health concern during the pilgrimage season, three crucial questions need to be answered. These questions are wetherthere are infected cases of RVF among sacrifice animals what is the immune status due to previous exposure or vaccination of these animals and if there is a risk of re-occurrence of RVF outbreak during pilgrimage. Therefore, the aim of the current study was to conduct serological survey of RVF among both local and imported sacrifice animals to determine the prevelance of recent RVF exposure and to determine the vulnerability of sacrifice herd based on its immune status in order to evaluate the potential risk of other outbreak during pilgrimage season.

## MATERIALS AND METHODS

**Study population and sampling:** During the pilgrimage season of 2009 in November, a total number of 580 animals were randomly selected from the yards of Al-Kaakeeya slaughterhouse, one of the main collection zones and slaughterhouses for sacrifice animals in Mecca, KSA. Investigated population included 120 local and 460 imported animals. The local animals included 30 sheep from Mecca and 50 sheep and 40 goats from Jazan. On the other hand, imported animals included 160 sheep from Sudan and 120 sheep and 180 goats from Somalia. Whole blood sample were collected from the jugular vein of all investigated animals and were used for serum separation at the same day. Produced sera were kept at -80°C freezer till time of serological analysis.

**Detection of RVF virus-specific IgM and IgG antibodies:** Capture and sandwich ELISA techniques were adopted for detecting IgM and IgG antibodies, respectively in the serum of sheep and goats as previously described (Paweska *et al*., 2003). A commercial capture Enzyme-Linked Immunoassay (ELISA) for the detection of anti-RVF Virus (RVFV) IgM antibody in sheep and goat sera was used in the current study for this purpose (National Institute for Communicable Diseases, Johannesburg, South Africa). It is based on a capture format in which the plates are coated with rabbit anti-sheep IgM capture antibody and then reacted with test sera. The captured IgM antibody is reacted with RVFV antigen and the bounded antigen is then detected with mouse anti-RVFV antibody and anti-mouse HRPO conjugate plus ABTS substrate. Sandwich ELISA based on indirect format in which the plates are coated with a bacterially expressed recombinant RVFV antigen were used for detection of specific anti-RVFV IgG antibodies. Specific anti-RVFV IgG antibody is detected with recombinant Protein G HPRO conjugate and ABTS substrate. Commercial ELISA kits including high positive ($C^{++}$), low positive ($C^{+}$) and negative ($C^{-}$) control sera were used as instructed by the manufacturer (National Institute for Communicable Diseases, Johannesburg, South Africa). The amount of developed color in both capture and sandwich ELISAs is proportional to the amount of the captured anti-RVF virus IgM and the amount of anti-RVFV IgG antibody that has been bound to RVFV recN, respectively. Optical Density (OD) of the developed color was read at 405 nm. For detection of IgM, net OD values were first calculated for each test or control sera as the OD value with RVFV antigen minus the OD value with control antigen. The net OD was then used in the calculation of Percentage Positivity (PP) of $C^{+}$, $C^{-}$ and test sera as (Net OD of $C^{+}$, $C^{-}$ or test sera)/(Net OD of $C^{++}$)

X 100. Goat and sheep sera that produced PP values ≥8 PP and 9.5 PP, respectively were considered positive. For detection of IgG, PP of $C^+$, $C^-$ and test sera were calculated as (Mean OD of $C^+$, $C^-$ or test sera)/(Mean OD of $C^{++}$ ) X 100. Goat and sheep sera that produced PP values ≥25 PP were considered positive.

## RESULTS AND DISCUSSION

**Rate of recent infection and herd immunity among investigated sacrifice animals:** The study of the rate of recent RVF infection, represented by the rate of IgM-positive cases and the herd immunity, represented by the rate of IgG-positive cases among investigated sacrifice animals revealed that out of 580 investigated sacrifice animals, 15 (2.59%) and 273 (47.06%) were positive for IgM and IgG, respectively (Table 1).

**Rate of IgM and IgG-positive cases among local sacrifice animals:** With regard to local animals, one case (0.83%) and 66 cases (55%) out of 120 investigated ones were positive for IgM and IgG, respectively. The results (Table 2) showed that out of 30 investigated Mecca sheep, no positive IgM cases were detected while 12 (40%) cases were positive for IgG. On the other hand, out of 50 investigated Jazan sheep, one case (2%) and 36 cases (72%) were positive for IgM and IgG immunoglobulins, respectively. Regarding Jazan goats, the results revealed that out of 40 investigated cases, no IgM positive cases were detected while 18 cases (45%) were positive for IgG immunoglobulin.

**Rate of IgM and IgG positive cases among imported sacrifice animals:** The current study revealed that 14 cases (3.04%) and 207 cases (45%) out of 460 investigated imported animals were positive for IgM and IgG, respectively. Out of 160 investigated Sudanese sheep, 3 (1.8%) IgM-positive cases and 101 (63.1%) IgG-positive cases were detected. On the other hand, out of 120 investigated Somalia sheep, 4 (2%) and 52 (43.4%) cases were positive for IgM and IgG immunoglobulins, respectively. Regarding Somalia goats, 7 (3.88%) IgM-positive cases and 54 (30%) IgG-positive cases were detected out of the 180 investigated cases (Table 3).

In holy Mecca, around millions of small ruminants, mostly imported from RVF risk areas are sacrificed annually during pilgrimage season. During the traditional sacrificial slaughtering practices, aerosols of infected blood may be generated which intensify the risk of infection among slaughterhouse workers. Moreover, the increased population of mosquitoes, the main RVF vector, among the overcrowded pilgrims is another potential risk factor of human infection. In order to detect any potential risk for eruption of new RVF outbreak among sacrifice animals during pilgrimage season, the current study aimed to conduct serological survey where the presence of RVFV-specific IgM and IgG imunoglobulins were evaluated in a representative sample of both local and imported sacrifice sheep and goat during pilgrimage season.

Regarding the detection of recent RVF exposure, represented by anti RVF-specific IgM, the results of the current study revealed an overall prevalence of 2.59% among both local and imported animals. The currently detected prevalence would be considered highly accurate based on the previous report of 100% sensitivity of the capture ELISA for detecting RVFV-specific IgM immunoglobulins (Paweska *et al*., 2003). However, other studies have reported less sensitivity rate (51%) of the RVFV-specific IgM capture ELISA (Madani *et al*., 2003). Based on these reports, the currently recorded rate of RVF exposure could be very much lower than the actual rate which could be at least doubled.

The rate of recent RVF exposure as reported in the current study was lower among local animals (0.38%) as compared to imported ones (3.04%). This finding is consistent with the fact that Saudi Arabia imported

Table 1: Overall rate of IgM and IgG positive cases among local and imported animals

| Animal source | Investigated numbers | Positive IgM | | Positive IgG | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| Local | 120 | 1 | 0.83 | 66 | 55.00 |
| Imported | 460 | 14 | 3.04 | 207 | 45.00 |
| Total | 580 | 15 | 2.59 | 273 | 47.06 |

Table 2: Rate of IgM and IgG positive cases among local sacrifice animals

| Source of animals | Animal species | Total | Positive IgM | | Positive IgG | |
|---|---|---|---|---|---|---|
| | | | No. | % | No. | % |
| Makkah | Sheep | 30 | - | - | 12 | 40 |
| | Goat | 0 | - | - | - | - |
| | Sub-total | 30 | - | - | 12 | 40 |
| Jazan | Sheep | 50 | 1 | 2.00 | 36 | 72 |
| | Goat | 40 | - | - | 18 | 45 |
| | Sub-total | 90 | 1 | 1.10 | 54 | 60 |
| Total | | 120 | 1 | 0.83 | 66 | 55 |

Table 3: Rate of IgM and IgG-positive cases among imported sacrifice animals in relation to source of animals

| Source of animals | Animal species | Total | Positive IgM | | Positive IgG | |
|---|---|---|---|---|---|---|
| | | | No. | % | No. | % |
| Sudan | Sheep | 160 | 3 | 1.87 | 101 | 63.12 |
| | Goat | 0 | 0 | 0.00 | 0 | 0.00 |
| | Sub-total | 160 | 3 | 1.87 | 101 | 63.12 |
| Somalia | Sheep | 120 | 4 | 3.30 | 52 | 43.40 |
| | Goat | 180 | 7 | 3.88 | 54 | 30.00 |
| | Sub-total | 300 | 11 | 3.66 | 106 | 35.33 |
| Total | | 460 | 14 | 3.04 | 207 | 45.00 |

live sheep and goats from African countries where RVF is assumed to be endemic including Sudan and Somalia (Davies, 2006). Moreover, the current results revealed that all recorded recent infections among local animals were among sheep of Jazan and no cases were recorded in Mecca local animals. This finding could be explained in the light of the fact that RVF is enzootic in Jazan region where the continuous diagnosis of IgM antibodies in clinically suspected herds suggests the persistence of RVF infection in the Jazan region (Elfadil *et al*., 2006). On the other hand, regarding imported animals, the current results revealed lower prevalence of recent exposure among Sudanese sacrifice animals (1.87%) as compared to that among imported sacrifice animals from Somalia (3.66%). Although, both countries are from the African nations where the disease is endemic, the current results indicated relative lower rate of RVF infection and better control measurements in Sudan as compared to Somalia. This is consistent with the recent reports of detecting 114 RVF cases including 51 deaths in Somalia. The majority of cases (64%) were reported from areas with difficult security situation (WHO, 2007).

With regard to vaccination/old exposure-based immune status of sacrifice animals, the current study, based on positive IgG cases among investigated animals, revealed low level of herd immunity with an overall rate of 47.06%. In other words, >50% of investigated sacrifice animals are vulnerable to the RVF infection. The rates of herd immunity were relatively comparable between local (55%) and imported (45%) sacrifice animals. However, with regard to local animals, the rate of immunized animals as reveled in the current study was higher among those of Jazan (60%) as compared to Mecca ones (40%). This could be explained in the light of the situation that more strict vaccination program is practiced in Jazan as compared to Mecca region for couple of reasons first, Jazan is considered high risk area (suspected RVF enzootic area) as compared to Mecca, second, practicing vaccination programs among large scales animal population as in Jazan is easier and more efficient than that among small scales animal population as the case in Mecca (Madani *et al*., 2003; Davies, 2006; Elfadil *et al*., 2006). On the other hand with regard to imported animals, the current results showed that the level of herd immunity among Sudanese sacrifice imported animals was higher (63.12%) as compared to that (35.33%) of Somalia animals. These findings clearly denote that better and more efficient vaccination programs were practiced by the Sudanese authority as compared to that in Somalia. This is obviously attributed to the fact that animal vaccination which would be the most efficient control measure for RVF is not considered feasible under the current conditions and security situation in Somalia in addition to the absence of an efficient central government (Davies, 1998; WHO, 2007).

## CONCLUSION

The current study obviously revealed no major RVF disease incident, manifested by the relatively low rate of recent infection (2.59%) among sacrifice animals. However, the overall low herd immunity of investigated animals as manifested in the current study is a potential risk for eruption of new RVF outbreak among those animals during pilgrimage seasons with the expected socio-economic and public health consequences. In addition based on the current findings that suggest the persistence of RVF infection in Jazan region, the current study confirm previous reports that described the status of RVF in Jazan as being more or less similar that in enzootic areas of Africa. This would suggest classification of Jazan region as highly risk area for RVF infection which could be a source of imminent outbreak in Saudi Arabia. The recognition of pre-epizootic conditions may be the signal for the need for strict vaccination programs adopted by the local authorities in order to limit the chance for catastrophic outbreak during pilgrimage season. Finally, the current study strongly recommends the assessment of the relative risk presented by RVF in the area from which the animals originate and regulation of animal trade based on specific disease information (IgM and IgG levels in risk areas). Such information can be systematically gathered and reported in a network with good information flow coordinated and validated by international organizations such as the FAO, WHO and OIE.

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