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Development of a sheep challenge model for Rift Valley fever

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ABSTRACT

Rift Valley fever (RVF) is a zoonotic disease that causes severe epizootics in ruminants, characterized by mass abortion and high mortality rates in younger animals. The development of a reliable challenge model is an important prerequisite for evaluation of existing and novel vaccines. A study aimed at comparing the pathogenesis of RVF virus infection in US sheep using two genetically different wild type strains of the virus (SA01-1322 and Kenya-128B-15) was performed. A group of sheep was inoculated with both strains and all infected sheep manifested early-onset viremia accompanied by a transient increase in temperatures. The Kenya-128B-15 strain manifested higher virulence compared to SA01-1322 by inducing more severe liver damage, and longer and higher viremia. Genome sequence analysis revealed sequence variations between the two isolates, which potentially could account for the observed phenotypic differences. We conclude that Kenya-128B-15 sheep infection represents a good and virulent challenge model for RVF.

Introduction

Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic pathogen within the genus *Phlebovirus*, family *Bunyaviridae*. Although large outbreaks have predominantly occurred in sub-Saharan Africa, recent outbreaks outside of the African continent, in the Arabian Peninsula, have raised concerns about the potential spread of the virus to Europe, Asia and the Americas (Balkhy and Memish, 2003; Bird et al., 2009; Chevalier et al., 2010; Pepin and Tordo, 2010). Human infections with RVFV are associated with acute febrile illness that in some cases can progress to more severe disease, including retinal vasculitis resulting in blindness, encephalitis and hepatitis resulting in fatal hemorrhagic fever (Bird et al., 2009). Case fatality rates as high as 20–50% have been reported (Heald, 2012; Nguku et al., 2010). In sheep, goats and cattle, the disease is characterized by abortion storms and high rates of mortality in young animals (Coetzer, 1977, 1982; Pepin and Tordo, 2010). Due to concerns about its potential use as a biological weapon, RVFV is categorized as a high priority agent by the National Institute for Allergy and Infectious Diseases (NIAID), Centers for Disease Control and Prevention (CDC) and the U.S. Department of Agriculture (USDA).

Because of the presence of experimentally proven competent vectors, there is a high risk for introduction and establishment of RVFV in the US (Iranpour et al., 2011; Turell et al., 2008, 2010). Currently, there are no fully licensed RVFV vaccines in the US for either livestock or human use. Therefore, development of an effective vaccine represents an important area of research and availability of a challenge model is an important prerequisite for the development, evaluation, and licensing of future vaccines. To date, several RVFV animal infection models have been described in non-human primates (Miller et al., 2002; Smith et al., 2012), mice (Busquets et al., 2014; Flick and Bouloy, 2005; Ikegami and Makino, 2011; Linden et al., 2015; Smith et al., 2010; Tomori and Kasali, 1979), hamsters and rats (Easterday, 1965; Findlay, 1932; Flick and Bouloy, 2005; Linden et al., 2015; Miller et al., 2002), and more recently in sheep and goats (Busquets et al., 2010; Weingartl et al., 2014). Also, two types of challenge models have been employed for vaccine efficacy studies: the pregnancy model, which requires effective synchronization of pregnancy in host species (Bird et al., 2011), and the viremia model, which is affected by lack of consistency due to variation in individual host animal responses (Drolet et al., 2012; Fagbami et al., 1975; Kortekaas et al., 2012). Ideally, the development of an RVF vaccine designed for use in livestock should be tested in its natural host species, i.e. in ruminant livestock. Although sheep are the livestock most susceptible to RVFV infection, there is lack of detailed information about the impact of various sheep breed differences on clinical responses to experimental RVFV infection, data which are

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vital for vaccine efficacy study design. A challenge model for both sheep and goats was recently described (Weingartl et al., 2014). In these studies, different breeds of sheep (Suffolk cross, Rideau Arcott cross, Ile-de-France cross with Rideau Arcott) were inoculated with a RVFV strain (ZH501) isolated in 1977 in Egypt. These and other authors found that the clinical and pathological outcome of experimental RVFV infections in ruminants is very much dependent on the strain of RVFV used for inoculation, the species, breed and age of the host animals.

Different strains of RVFV have been responsible for numerous disease outbreaks in Africa and in the Arabian Peninsula. The Kenya-128B-15 (Ken06) strain was isolated from a mosquito during the Kenya 2006/2007 outbreak, which resulted in significant human and livestock mortalities (Sang et al., 2010). The SA01-1322 (SA01) strain was isolated from a mosquito during the Saudi Arabia 2000/2001 outbreak (Miller et al., 2002), which resulted in more than 200 human deaths and significant loss of livestock (Al-Hazmi et al., 2003; Arishi et al., 2000; Madani et al., 2003). These strains represent distinct genetic isolates and we hypothesized that they would have phenotypic differences with different clinical and pathological outcomes in infected susceptible livestock.

The aim of this study was to compare sheep infection with two strains of RVFV, Ken06 and SA01, in order to obtain relevant clinical and pathological data for subsequent evaluation of experimental vaccines. Here we describe the establishment of a small ruminant challenge model for RVF using sheep and two genetically distinct RVFV strains.

Results

Rectal temperatures

In the first study in Dorper x Katahdin sheep inoculated with 1×10^6 PFU of SA01 or Ken06 rectal temperatures were measured taken daily on 0–10, 14 and 21 days post infection (dpi). Rectal temperatures showed transient increases from baseline with the highest increase for both experimental groups (Ken06 and SA01) occurring at 2 dpi (Fig. 1A). There were significant differences in mean rectal temperatures between baseline, 0 dpi (SA01 = 39.8 °C, Ken06 = 39.7 °C, $n=6$) and 2 dpi (SA01 = 40.8 °C, Ken06 = 41.1 °C, $n=6$) for animals inoculated with either RVFV strains ($P<0.05$). No significant change from baseline occurred in the mock-inoculated control group ($n=2$) during the length of the study. Also the observed mean temperature differences between the virus inoculated and mock- inoculated groups on 2 dpi were statistically significant ($P<0.01$). There was no significant difference in mean rectal temperatures between SA01 and Ken06 inoculated groups at 2 dpi ($P>0.05$).

Polypay sheep ($n=5$) inoculated with 2×10^6 PFU of Ken06 in the second study showed variable temperature responses (Fig. 1B). Peak temperatures were observed on 1 or 2 dpi (range = 40.1–41.9 °C), with an increase in all sheep ($n=4$), except for sheep #45, which showed an unusual decline in temperature from 1 to 3 dpi then increase at 4 dpi. Three sheep, #41, #43 and #44, maintained high temperatures (41.2 °C, 41.9 °C, 41.4 °C, respectively) at 2 dpi, whereas sheep #42 had a lower temperature at 2 dpi. Thereafter, animals showed transient increase and decrease in temperature until 10 dpi, the study endpoint (Fig. 1B).

Mortality

There was no mortality in the first study in the Dorper x Katahdin sheep. However, of the 5 Polypay sheep inoculated with the higher dose of Ken06 virus, 3 animals (#41, #45, #44) died at 3, 4 and 5 dpi of acute RVFV infection.

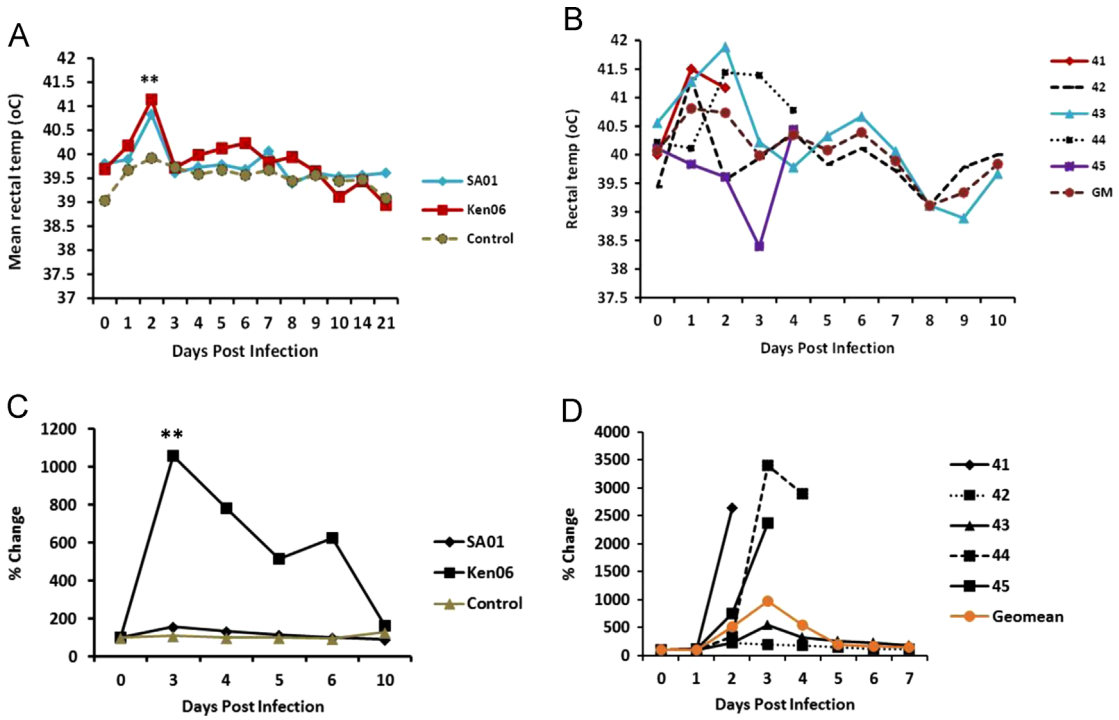

Fig. 1. Kinetics of rectal temperatures of sheep inoculated with RVFV SA01 and Ken06 (A) and sheep inoculated with a higher dose of Ken06; ** denotes mean rectal temperatures of SA01 and Ken06 inoculated sheep are significantly higher than mock inoculated sheep ($P<0.01$), (B) GM = group mean, (C) shows percentage change of the geometric mean AST values for the different groups of sheep inoculated with the two different wild type strains of RVFV; **denote AST values of Ken06 inoculated sheep are significantly different from SA01 or mock-inoculated sheep ($P<0.001$), (D) shows percentage change in individual AST and geometric mean (Geomean) AST values for sheep ($n=5$) inoculated with 2×10^6 pfu of Ken06;