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Investigating the infection cycle of Rift Valley fever virus in Cameroon and Mauritania and the applicability of MP-12 vaccine for camelids

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To my family

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List of abbreviations

ABTS	2.2′-azino di-ethylbenzothiazoline sulphonic acid
CI	Confidence interval
CO$_2$	Carbon dioxide
Ct value	Cycle threshold value
Cy3	Cyanine 3
DC-Sign	Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
ELISA	Enzyme linked immunosorbent assay
Gc	Glycoprotein c (-terminal)
Gn	Glycoprotein n (-terminal)
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IIFA	Indirect immunofluorescence assay
kGy	Kilo Gray
LANAVET	Laboratoire National Vétérinaire
L Segment	Large segment
M Segment	Medium segment

ND_{50}	Neutralizing dose of 50%
nm	Nanometer
NP	Nucleoprotein
NSm	Nonstructural protein, encoded on the medium segment
NSs	Nonstructural protein, encoded on the small segment
OD	Optical density
OIE	Office International des Epizooties
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
RT-PCR	Reverse transcriptase polymerase chain reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
S/P%	Sample-to-positive-ratio
Sample-ID	Sample identification
SNT	Serum neutralization test
spp.	Species

S Segment   Small segment

TCID$_{50}$ 50% Tissue culture infective dose

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Chapter 1

Introduction

Rift Valley fever (RVF) is a mosquito-borne viral zoonosis, which has caused significant epidemics in Africa and the Arabian Peninsula. The Rift Valley fever virus (RVFV) is a member of the family *Bunyaviridae*, genus *Phlebovirus* and can cause severe diseases in small ruminants, cattle, camels and humans.

The single-stranded RNA virus possesses a tri-segmented genome with a negative and ambisense coding strategy. The S (small) segment is encoding the nucleoprotein NP, which specifically binds viral RNA genome and triggers the RNA encapsidation. NP is not directly involved in the RVFV pathogenesis (Boshra et al., 2011) nor induces neutralizing antibodies, but previous studies found NP to elicit a protective immune response (Lagerqvist et al., 2009). The nonstructural protein NSs is encoded by the ambisense S-segmental RNA and is the main virulence factor, as such primarily responsible for RVFV-pathogenesis by targeting multiple host cell signaling pathways to evade immune responses. It is expressed in the initial phase of the viral infection, typically forming filamentous structures in the nucleus (Mansuroglu et al., 2010). A degradation of the double-stranded RNA-dependent protein kinase R (Habjan et al., 2009), which is associated with a cellular antiviral response and additional antagonistic activities against the antiviral interferon response (Bouloy et al., 2001) are essential for the initial pathogenesis. At later stages a general shutdown of cellular transcription is mediated by an inhibition of the transcription factor TFIIH and its subunit p44 (Le May et al., 2004). Anyhow, NSs has shown to be dispensable for viral replication (Muller et al., 1995). The M (medium) segment is encoding the aminoterminal glycoprotein (Gn) and

the carboxyterminal glycoprotein (Gc). Both glycoproteins are exposed on the virus envelope, being the primary antigenic stimulation of the host immune system and induce potent virus-neutralizing antibodies (Pepin et al., 2010). A further nonstructural protein (NSm) is also encoded on the M-Segment and acts as additional virulence factor, suppressing apoptosis through the caspase pathway (Won et al., 2007). Apparently NSm plays an important role for infection and virus dissemination in mosquitoes (Kading et al., 2014). As described for NSs, absence of NSm also does not alter virus growth in mammalian cells (Gerrard et al., 2007). The L (large) segment finally encodes the RNA-dependent RNA polymerase, which is indispensable for viral RNA transcription and replication by forming the ribonucleoprotein (RNP) complex (Lopez et al., 1995).

The enveloped and spherical virions are approximately 80-120 nm in diameter and their lipid bilayer contains capsomers of glycoprotein heterodimers on an icosahedral lattice with T = 12 quasi-symmetry (Freiberg et al., 2008). A dynamin-dependent caveolae-mediated endocytosis is described as mechanism of cell entry, followed by release of the viral RNP complexes into the cytoplasm (Harmon et al., 2012). After replication in the cytoplasm and budding at the Golgi apparatus, virions are composed of Gn/Gc heterodimers, viral ribonucleoproteins analogous to the three genome segments and copies of the nucleoprotein and the RNA-dependent RNA polymerase (Bouloy and Weber, 2010; Spiegel et al., 2016). Although specific receptors for cell entry are not yet defined, RVFV is binding to DC-SIGN (Lozach et al., 2011) and heparan sulfate (de Boer et al., 2012) for attachment.

RVFV was first described 1931 by *Daubney et al.* (Daubney, 1931), as striking mortalities in lambs were reported after excessive rainfall in the Great Rift Valley in

Kenya. A total of 4,700 lethal cases in sheep, associated with severe necrotic hepatitis were reported. Furthermore, several humans found to be affected with Dengue-like symptoms. In 1950, a devastating epizootic was reported in South Africa with 100,000 lethal cases in sheep and 500,000 abortions (Gerdes, 2004). During the reoccurrence of RVFV in South Africa in 1975, the first RVF-associated fatal human cases were reported (McIntosh et al., 1980). The largest epidemic occurred 1977 in Egypt, with 200,000 human infections, 600 human fatalities and uncounted losses among livestock (Meegan, 1979). Other epidemics of major importance were seen 1987 in Mauritania and Senegal, marking the first description of RVFV in West Africa (Jouan et al., 1988) and 1990 in Madagascar, as the virus was first found beyond African mainland (Morvan et al., 1992). The remarkable transboundary threat of RVFV beyond Africa was furthermore illustrated by the epidemic in Saudi Arabia and Yemen in 2000 (Balkhy and Memish, 2003). Nowadays RVFV is endemic in many parts of Africa and recurrently causes massive outbreaks affecting both human and animal health.

Apart from the virus incursion and various factors, the abundance of vector competent mosquito species is the major determinant for the development of RVFV epidemics. RVFV has been isolated from more than 30 species belonging to 6 genera within the family *Culicidae* (Chevalier et al., 2010). The capability of transovarial transmission of the virus, as described for *Aedes lineatopennis* (Linthicum et al., 1985) is defined as major factor for virus maintenance. RVFV transmission varies between epidemic and enzootic cycles, although both cycles are occasionally overlapping. The enzootic cycle is characterized by only sparse RVFV transmission from primary *Aedes* vectors to susceptible vertebrate hosts like wildlife or livestock. Ongoing transmission events are rare as supportive factors fostering the RVFV spread e.g. environmental conditions are

absent (Bird et al., 2009; Gerdes, 2004). A transition to an epidemic or epizootic cycle occurs as soon as an ecological deviation such as heavy rainfall favors the massive propagation of infectious mosquitoes. Furthermore, infected and dormant eggs of ground pool breeding *Aedes spp.* are inundated and will be able to hatch (Davies et al., 1985b). Consequently the likelihood of transmission to both animals and humans increases significantly. Infected amplification hosts subsequently serve as origin of infection for secondary vectors, such as *Culex* and *Anopheles spp.* (Turell et al., 1984). Animals are primarily infected by bites of infected mosquitoes, whereas most human infections are caused by unsafe handling of viremic animals and only rarely by direct vector to human transmissions.

Infections of humans usually proceed as mild influenza-like illness, characterized by biphasic fever, weakness, headache and nausea (Francis and Magill, 1935). Severe progressions of meningoencephalitis including symptoms of confusion, coma and hallucinations (van Velden et al., 1977) or ocular manifestation in terms of retinitis that can lead to a permanent loss of vision are reported as long term complications of RVFV infections (Al-Hazmi et al., 2005). In some cases acute necrotic hepatitis is followed by severe hemorrhagic fever symptoms that are fatal in 10-20% of the cases (Bird et al., 2009). Overall case fatality rates are ranging between 0.5% and 2% and high viral loads in the blood of patients do significantly correlate with the outcome of infection (Le Roux et al., 2009; Njenga et al., 2009).

Progressions of infection in animals are considerably age-dependent, as lethal cases are more frequent in young animals. Despite of species-specific susceptibility and manifestation of RVF, massive and simultaneous abortions of pregnant ruminants,

which are independent of the stage of gestation, are characteristic for RVFV epizootics (Pepin et al., 2010). Sheep are reported to be the most susceptible species and play an important role in the infection cycle of the virus. Mortality rates of adult sheep after experimental RVFV infection are about 20%, whereat rates of 95% - 100% are observed in newborn lambs. After a short incubation period of 12 – 24 hours, first fatal cases in lambs can be observed within 24 – 72 hours post infection. Although sheep that are older than one week are less susceptible for lethal RVFV infections, symptoms as fever (39 to 40°C), diarrhea, nasal discharge, anorexia and decreased activity do occur (Easterday B.C., 1962b). Breed specific susceptibilities are indicated by several experimental approaches (Busquets et al., 2010; Olaleye et al., 1996). Symptoms observed in infected goats are nearly similar to those in sheep (Easterday B.C., 1962a). Cattle are significantly less susceptible than small ruminants, whereas mortality rates in calves do not exceed 10% - 70% and fatal cases of adult cattle are found only in 5% - 10% (Bird et al., 2009). Most conspicuous features of bovine RVFV infections are lethargy, anorexia and a drop in milk production (Coetzer, 1982). First associations of RVFV and camels were found 1962 in Kenya, as abortions of camels were reported. Although RVFV-specific antibodies were detected in the serum of affected animals, the virus was not found to be causative for the observed pathogenesis (Scott et al., 1963). Although high prevalence was found in this species repeatedly (Britch et al., 2013; Davies et al., 1985a; Swai and Sindato, 2015), only few abortions or perinatal mortalities were reported (Ali and Kamel, 1978; Nabeth et al., 2001). Both unexpected and uncharacteristic manifestations of RVFV infections in camels were observed during an outbreak in Mauritania in 2010. Even adult camels showed severe clinical syndromes, such as ataxia, respiratory distress, icterus, conjunctivitis, hemorrhages, nervous

symptoms and sudden death within 24 hours (El Mamy et al., 2011). Although African buffaloes were proven to be susceptible for RVFV infections in experimental approaches (Davies and Karstad, 1981), clinical manifest infections in the field are not yet reported. However, results of seroepidemiological studies indicate a role of wildlife in the ecology of the virus (Evans et al., 2008).

The liver is known to be primary target for RVFV replication in both animals and humans. Gross pathological examination of infected mammalian hosts reveals large, soft and discolored livers with foci of necrosis that are often accompanied by multifocal hemorrhages (Bird et al., 2009). Histopathology reveals a multifocal necrotic hepatitis with characteristic infiltration of neutrophilic granulocytes and macrophages. Additionally lymphoid necrosis of lymph nodes and white pulp of the spleen are described. Despite of the hepatotropism, the virus also replicates in many other cells and is lytic in nature (Erasmus BJ, 1981). Viral antigens can be found in the wall of small vessels, in spleen and in liver (Van der Lugt et al., 1996). Hematological characteristics of RVFV infections are leucopenia, thrombocytopenia and elevated liver enzymes (Gerdes, 2004).

The combined assessment of virus detection, analysis of differentiated host immune response and in-depth anamnesis informs about the RVFV infection kinetics and helps to draw prognostic conclusions on the disease progression and outcome. Viremia usually persists until a maximum of 14 days post infection with titers ranging from 10^5 to $10^{9.0}$ PFU/ml (Faburay et al., 2016; Niklasson et al., 1983; Weingartl et al., 2014). Seroconversion typically starts from the fourth day post infection (Morrill et al., 1997), whereat IgM antibodies are not detectable beyond the 50th day post infection (Niklasson et al., 1984). Neutralizing antibodies that are essential for antiviral protection are

primarily targeted against Gn and Gc (Pepin et al., 2010). In addition, non-neutralizing antibodies that are found in the serum of infected animals are directed against the major immunogen NP (Jansen van Vuren et al., 2007) and against NSs (McElroy et al., 2009). Due to the brief duration of viremia, an isolation of RVFV or detection of viral RNA is only possible for a short time post infection. For this purpose several quantitative real-time RT-PCR assays have been developed, targeting the S segment (Drosten et al., 2002; Garcia et al., 2001; Weidmann et al., 2008) or the L segment (Bird et al., 2007). Hamsters, suckling mice or mammalian cells like Vero or BHK-21 cells have been employed for virus isolation from peripheral blood, liver, spleen, brain and aborted fetuses (OIE, 2008). A broad range of assays is available for the serological diagnosis of RVFV infections. The serum neutralization test (SNT) is considered as gold standard in serological RVFV diagnostics (OIE, 2008). It is very specific and cross reactions with other phleboviruses are limited (Tesh et al., 1982; Xu et al., 2007). Several approaches of enzyme-linked immunosorbent assays (ELISA) were established (Cetre-Sossah et al., 2009; Kortekaas et al., 2013; Paweska et al., 2003) for a less laborious, safe and time-efficient serological analysis. An indirect ELISA that is applying recombinant NP can be utilized for safe detection of RVFV-specific antibodies in human and animal sera (Paweska et al., 2007). Additionally, an indirect ELISA that applies recombinant Gn as capture antigen is a valuable tool for independent comparable analysis of major and neutralizing antigen reactivity (Jackel et al., 2013a). Subordinate serological assays include indirect immunofluorescence, haemagglutination-inhibition and complement fixation test.

Although comprehensive monitoring and surveillance studies on RVF were carried out in several African countries, there is limited level of awareness and information about this

disease throughout Africa. This includes general questions on RVFV ecology as well as susceptible amplification hosts and the maintenance during enzootic cycles.

Until most recently, there was only vague and/or historic serological evidence for the presence of RVFV in Cameroon such as the first description of RVFV-specific antibodies in sheep from Maroua and Ngaoundéré in 1967 (Maurice, 1967). An involvement of humans in the RVFV infection cycles was demonstrated in 1985-1987 by verifying a specific seroconversion in populations from southwestern (Paix et al., 1988) and northern (Gonzalez et al., 1989; Paix et al., 1988) Cameroon. First comparative serological studies on livestock conducted from 1989-1992, demonstrated sheep and cattle in the south of Cameroon to be RVFV-seropositive with prevalence ranging from 6 – 20% (Zeller et al., 1995b). Recently, evidence for RVFV seropositivity was also found in goats from southern Cameroon (LeBreton et al., 2006). In order to get a more comprehensive picture of the RVFV infection rates in livestock in Cameroon, a systematic perennial and cross-regional study was carried out, which is described in the first manuscript.

For many other countries RVFV is known to be endemic, causing devastating outbreaks frequently, yet without in-depth knowledge of virus ecology during inter-epidemic periods. From 1987 to date, recurrent epidemics and epizootics in Mauritania had significant impact on healthcare and economy. Besides Senegal, Mauritania is known to be an important hotspot for RVFV in West Africa. The 1987 epidemic in Mauritania caused 220 human fatalities and severe losses in livestock. The construction of the Diama Dam in 1986, giving rise to a large artificial lake which has dramatically changed the local micro-climate, is considered to have caused this first noted outbreak (Digoutte

and Peters, 1989; Jouan et al., 1988; Ksiazek et al., 1989; Saluzzo et al., 1987). Thereupon epidemics reoccurred periodically in 1993 (Zeller et al., 1995a), 1998 (Nabeth et al., 2001) and 2003 (Faye et al., 2007). The observed pattern of RVFV epidemics in Mauritania, mainly being associated to the Senegal river was resolved when first cases were observed in northern arid parts of the country in 2010 (El Mamy et al., 2011; Jackel et al., 2013b). Above-average rainfall caused massive abundance of competent mosquito vectors and consequent transmission to susceptible amplification hosts. Repetitive massive precipitation in 2012 led to a further outbreak in southern Mauritania, focusing on the regions Tagant, Brakna, Trarza, Assaba and Hodh-El-Gharbi (Sow et al., 2014). The 2013 epizootic was restricted to Trarza and Brakna nearby the Senegalese border and affected sheep, goats and camels (OIE, World Animal Health Information Database (WAHID), 2014). A recent outbreak from September 2015 to March 2016 was limited temporally and spatially and affected only sheep and goats (OIE, World Animal Health Information Database (WAHID), 2015). This was accompanied by 31 confirmed human cases in Kiffa (Assaba) and Aleg (Brakna) (Boushab et al., 2016). Although recent outbreaks displayed limited impact on national health, the remarkably reduced duration of inter-epidemic periods highlights the predominant importance of RVFV in Mauritania. During those inter-epidemic periods the virus is presumably maintained by infrequent transmission from vertical infected *Aedes spp.* to susceptible amplification hosts, such as wildlife or livestock (Diallo et al., 2005). To date, little is known about characteristics of RVFV ecology and dynamics during inter-epidemic periods in Mauritania. To elucidate the potential of enzootic infections and to compare the immunological status between epidemic and inter-epidemic periods, we analyzed samples of sheep, goats, cattle and camels, collected during inter-epidemic

periods (January to March 2012, January to June 2013) in Mauritania. A multistage serological and molecular analysis was applied for differentiation between acute and chronic infections, revealing important insights into kinetics of RVFV infections.

Especially in Mauritania camels are of major importance in daily routines, agriculture and livelihood. Their particular role in RVFV epidemiology was discussed controversially during the past decades and was mainly influenced by casuistic observations made in Mauritania. As most camels were found to carry RVFV-specific antibodies (Davies et al., 1985a; Scott et al., 1963; Swai and Sindato, 2015) without anamnestic reports of obvious clinical manifestations, their role in the RVFV infection cycle and spread was rather underestimated. Eventually camelids were considered as main drivers of the RVFV introduction to Egypt (Abd el-Rahim et al., 1999; Hoogstraal et al., 1979), which was underlined by the clinical RVF cases in dromedaries during the 2010 epidemic in Mauritania, which were frequently characterized by rapidly progressing diseases and even fatalities within 24 hours. Subsequent human infections demonstrated the high exposure risks to RVF at the human-animal interface especially regarding camelids (El Mamy et al., 2011). Hence there is obviously a strong need for safe and efficacious vaccines for camelids, but corresponding data are currently limited (Daouam et al., 2016). A broad spectrum of RVF vaccines is available for protection of small and large ruminants. The inactivated vaccine TSI-GSD 200 (Pittman et al., 1999) requires for boosting and is therefore rather inappropriate for application in the field. The first live attenuated vaccine, the Smithburn vaccine, is highly immunogenic by a single-shot vaccination but a residual pathogenicity, teratogenicity and abortogenicity limits its practical use (Botros et al., 2006). Other approaches of vaccine development such as recombinant vaccinia viruses (Papin et al., 2011), subunit vaccines (Faburay et al.,

2014), DNA vaccines (Lagerqvist et al., 2009) or VLPs (Liu et al., 2008; Pichlmair et al., 2010) are limited in their immunogenicity and efficacy. Recently characterized live attenuated vaccines like Clone 13 or MP-12 usually provide the best protection against fatal RVFV infections (Kortekaas, 2014). Clone 13 is a naturally attenuated strain with large deletions in the NSs gene (Muller et al., 1995) being highly immunogenic and safe (Dungu et al., 2010). Recently its efficacy was also proven in camelids (Daouam et al., 2016). MP-12 was generated by serial plaque purification of the ZH548 strain in the presence of the chemical mutagen 5-fluorouracil (Caplen et al., 1985). The efficacy of the vaccine was proven in numerous studies in sheep, cattle and macaques (Hubbard et al., 1991; Morrill et al., 1991; Morrill et al., 1987; Morrill et al., 1997; Morrill and Peters, 2003) and recently a phase 2 clinical trial in humans was performed (Pittman et al., 2016). However, when administered during the first trimester of gestation, teratogenic effects and abortions in sheep were observed (Hunter et al., 2002). Studies of *Wilson et al.* additionally suggested the necessity of performing independent and detailed safety testing of veterinary vaccines on target species (Wilson et al., 2014).

For this reason, we analyzed the safety, immunogenicity and pathogenicity of MP-12 in alpacas as model-organism for dromedary camels. Three male alpacas were immunized and acute and subacute immune responses were analyzed. Clinical observations, deviations in hematology and clinical chemistry were used as indicators of tolerability. Furthermore, the genetic stability by resequencing recovered virus was examined. Moreover, virus shedding and replication in tissues of the animals after MP-12 virus challenge was analyzed.

In summary the here described studies deepen the knowledge of distribution and characteristics of RVFV infections in Cameroon and Mauritania, thereby elucidating local RVFV life cycles and dynamics. By analysis of susceptible livestock in Mauritania, first insights into variations and shifts of prevalence and potential virus transmissions during inter-epidemic periods were reached. Eventually, to additionally address the importance of camelid RVFV infections and to investigate potential prevention strategies, an immunization study of alpacas was performed.

Chapter 2

Manuscript

Evidence for enzootic circulation of Rift Valley fever virus among livestock in Cameroon

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2.1 Abstract

Rift Valley fever virus (RVFV) is an arthropod-borne pathogen, causing serious epidemics in Africa and the Arabian Peninsula. In Cameroon serological data indicate the presence of RVFV, but active circulation of RVFV, causing clinical infections has not been proven yet. For this purpose we carried out a comprehensive serological and molecular study on a total of 1,953 serum samples of small ruminants and cattle, which were collected in years 2013 and 2014 in Cameroon. In a first step, sera were screened serologically using a variety of assay formats to reveal RVFV specific antibodies. At the second stage, seropositive specimen were assessed for acute RVFV infections via IgM-specific ELISA and quantitative real-time RT-PCR. Our data show a significant difference in the antibody prevalence in cattle (13.5% [95% confidence interval: 11.4 – 15.7]) and small ruminants (3.4% [95% confidence interval: 2.3 - 4.7]), with indications for annual fluctuations and significant regional differences of seropositivity. One small ruminant and three bovines were eventually found to be positive in IgM ELISA and an ongoing viremia was verified in one bovine by RVFV genome detection using quantitative real-time RT-PCR. The results of this study therefore corroborate the presence of acute RVFV-infection and its circulation in Cameroon.

2.2 Introduction

Rift Valley fever (RVF) is causing severe diseases among small ruminants, cattle, camels and humans. The disease is caused by Rift Valley fever virus (RVFV), a RNA virus of the family *Bunyaviridae* (Bouloy and Weber, 2010). It was first described in 1931, as massive abortions accompanied by necrotic hepatitis were observed in sheep

in the Rift Valley, Kenya (Daubney, 1931). Nowadays, RVFV is endemic throughout Africa and Arabia and substantial epidemics have been observed in South Africa, Egypt, Kenya, Saudi Arabia and Yemen (Nanyingi et al., 2015). RVFV is transmitted by a broad range of mosquitoes, whereat transovarial transmission of the virus in *Aedes spp.* is a major factor of virus maintenance (Pepin et al., 2010). The virus is transmitted either during enzootic or epizootic cycles, albeit both cycles are partly overlapping. In the epizootic cycle serious ecological changes, such as heavy rainfalls and flooding of ponds, are leading to abundance of vector population with subsequent massive infections of susceptible species. In contrast, the enzootic cycle is characterized by low-level transmissions from RVFV-infected mosquitoes to susceptible wildlife and livestock (Bird et al., 2009; Pfeffer and Dobler, 2010). Clinical manifestations in animals are multifaceted and directly depend on infected species and the age of the affected animals. Characteristic abortions storms accompanied by increased mortality rates in adult animals are indicative for RVFV. Clinically apparent infections are mainly observed in sheep, goats, cattle and camels. Most human infections proceed as mild flu-like illnesses, but severe manifestations such as retinitis and blindness, meningoencephalitis or hemorrhagic fever can also occur infrequently. In addition to mosquito bites, humans mainly get infected through contact with viremic animals during slaughtering, necropsy or veterinary activities (Chevalier et al., 2010).

Verification of RVFV-specific antibodies in Cameroon were reported sporadically in humans. Initially, RVFV specific antibodies were detected 1985 in urban populations from southwestern (Paix et al., 1988) and northern (Gonzalez et al., 1989; Paix et al., 1988) Cameroon. In livestock, serological evidence for RVFV was first reported in 1967, as specific antibodies were detected in sheep from Maroua and Ngaoundéré (Maurice,

1967). Comparative serological studies of susceptible livestock were conducted from 1989-1992 in the south of Cameroon. The prevalence was ranging from 6 – 20% in sheep and cattle (Zeller et al., 1995). Latest studies also found RVFV related seroconversions in goats from southern Cameroon (LeBreton et al., 2006). Despite of these serological findings revealed in the past, acute infections in livestock and circulation of virus were never shown to date.

The here presented data were obtained by analyzing a total of 1,953 ruminant sera collected in 2013 and 2014 in 9 out of the 10 regions of Cameroon, using serological and molecular methods. Seroprevalence and the demonstration of IgM positive sera as well as the finding of a low viremic bovine, prove past and ongoing RVFV infections. Regional, annual and species-dependent variations help understanding the RVFV ecology and should prompt the implementation of public health protection measures.

2.3 Materials and methods

Study area and sampling

The regions Far North, North, Adamawa, Northwest, West, Centre, Southwest, Littoral and South were covered in this study (Figure 1). Within these regions, 27 different departments with 1-5 different sampling locations were included. A total of 1,953 sera of productive livestock, in detail 921 sera of sheep and goats and 1,032 sera of cattle were collected under the direction of the National Veterinary Laboratory (LANAVET) Cameroon in 2013 and 2014. Blood was collected through puncture of the *vena jugularis* by trained personnel according to good veterinary practice. Regarding safety protocols all sera were irradiated before handling (gamma radiation, Synergyhealth, Radeberg,

Germany). Additionally all sera from small ruminants were pretreated by heating at 56°C for 1 hour as described before (van Vuren and Paweska, 2010).

Serological and molecular investigation

Initially, all serum samples were analyzed in the ID Vet competition ELISA and samples of small ruminants were additionally tested in the indirect IgG ΔGn ELISA. As the ID Vet competition ELISA is utilizing the nucleoprotein (NP) and the indirect ELISA the glycoprotein (ΔGn) as capture antigen, an independent comparison of reactivity against the two antigens is achieved. Positive results of ELISA were confirmed with the serum neutralization test (SNT), which is known as the gold standard in serological diagnostic of RVFV. In case of negative SNT, samples were tested in indirect immunofluorescence. In those cases the indirect immunofluorescence was determining the final assessment of the sample. All sera which were positive or inconclusive in ID Vet competition ELISA were additionally tested in ID Vet IgM capture ELISA for specific presence of IgM. Finally RNA was extracted of all IgM positive sera and a quantitative real-time RT-PCR was run. Genetic analyses by multiple RT-PCR were performed for those samples with positive real-time RT-PCR.

Indirect IgG ΔGn ELISA

Sera from small ruminants were tested in the indirect IgG ΔGn ELISA as described previously (Jackel et al., 2013a). Briefly, plates coated with recombinant ΔGn-protein were blocked and incubated with sera [1:25]. A serum of an immunized rabbit was used as positive control [1:20,000]. A serum of a German sheep from quarantine facilities of Friedrich-Loeffler-Institut was used as negative control [1:25]. An 1:5,000 Protein G conjuagte was visualized with 2.2′-azino di-ethylbenzothiazoline sulphonic acid (ABTS, Roche, Mannheim, Germany). After stopping the reaction with 1%Sodiumdodecyl-

Sulfate, plates were read at 405nm. All samples with a percentage positive (OD value of sample/Median of positive control x 100) higher than 20.75 were assessed as positive.

ID Vet Competition ELISA

Sera were tested with the ID Screen® RVFV Competition Multi-species ELISA (ID Vet, Montpellier, France) according to the manufacturer's instructions. Both IgG and IgM are detected indistinguishably because competitive reactions are detected. Samples with a percentage of inhibition lower than 40% were defined as positive. Samples with a percentage of inhibition between 40-50% were defined as inconclusive and those with a percentage of inhibition higher than 50% as negative.

ID Vet IgM Capture ELISA

Sera were tested in the ID Screen® Rift Valley Fever IgM Capture ELISA (ID Vet) for specific occurrence of IgM. According to manufacturer's instructions all samples with a sample-to-positive-ratio higher than 50% were considered to be positive. Sample-to-positive-ratios between 40% and 50% were identified as inconclusive and those lower than 40% as negative.

Serum Neutralization Test

The serum neutralization test was performed as described in the OIE Terrestrial Manual 2014 (OIE, 2008). Briefly, 100 $TCID_{50}$ of RVFV (MP-12 vaccine strain) were added to serial two-fold diluted and heat inactivated sera. Following an incubation of 30 minutes at 37°C and 5%CO_2, 3×10^5 Vero 76 cells (Collection of Cell Lines in Veterinary Medicine, Friedrich-Loeffler-Institut, Germany) diluted in minimal essential medium with penicillin, streptomycin and 5% fetal calve serum were added to each well. Plates were incubated at 37°C, 5% CO_2 for 6 days. Neutralizing doses of 50% (ND_{50}) were expressed as the reciprocal of the serum dilution that still inhibited >50% of

cytopathogenic effect. A serum sample was considered as positive with a ND_{50} of 10 or higher. Positive and negative control sera as well as cell-growth controls were always included. Additionally the $TCID_{50}$ of the challenge virus was checked via titration in each run.

Indirect Immunofluorescence

Sera were tested with a commercial kit for Rift Valley fever virus indirect immunofluorescence (Euroimmun, Lübeck, Germany) with adaptations as described previously (Jackel et al., 2013b). Sera were diluted 1:100. The detection of antibodies was realized with species-specific Cy3 labeled secondary antibodies (donkey anti-sheep; donkey anti-goat; goat anti-bovine) in a 1:200 dilution. Species-specific positive and negative controls were included at each slide.

Real time reverse transcriptase (RT) PCR and genetic analysis

RNA extraction was performed using the QIAmp® Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufactures' instructions. As internal extraction control a MS2 bacteriophage was added to each serum before the extraction process (Ninove et al., 2011). Quantitative real-time RT-PCR, which targets the L segment at nucleotide position 2912-3001 (Bird et al., 2007) was carried out using the QuantiTect Probe RT-PCR Kit (Qiagen). For each reaction 5µl of RNA, 10 pmol of both forward and reverse primer and 1.25 pmol of the probe were used in a total volume of 25µl. RNA of the vaccine strain MP-12 in a 10^{-4} concentration was used as positive control. For quantification a synthetic RNA was used as described before (Jackel et al., 2013b). PCR reaction conditions were used as follows: 50°C for 30 min; 95°C for 15 min and 45 cycles at 95°C for 10 s, 55°C for 25 s and 72°C for 25 s. Of those samples positive in real-time RT-PCR, sequences were amplified using the Superscript III One Step RT-

PCR Kit (Invitrogen, Carlsbad, USA). A total of six different primer pairs were applied, targeting various regions of the S-, M- and L-Segment (Table 1). PCR reaction conditions were used as follows: 15 minutes for 45°C, 3 minutes at 95°C, 40 cycles at 95°C for 20 s, 55°C for 30 s, 72°C for 60s and finally 7 minutes at 72°C .

Statistical analysis

Seroprevalence and 95% confidence intervals were calculated with R version 2.14.0. For comparison of regional, annual and species-dependent seroprevalence Pearson´s Chi-Square test with a significance level of 5% was applied, using SAS Enterprise Guide 7.1.

2.4 Results

Serological screening for RVFV-specific antibodies

Testing of small ruminant sera (n=921) in both ID Vet competition ELISA and the indirect IgG ∆Gn ELISA resulted in 41 seropositive sera. A total of 16 sera were found to be positive in only one of the screening ELISAs and four samples gave inconclusive results in the ID Vet competition ELISA (Table 2). Accordingly, 21 samples were detected as positive in both tests. After verification with SNT, 31 sera were confirmed as positive. The remaining 10 samples without neutralizing antibodies proved to be negative in indirect immunofluorescence too. In summary, a total of 31 samples were confirmed to carry RVFV-specific antibodies, leading to a seroprevalence of 3.4% (95% confidence interval: 2.3 - 4.7) for small ruminants from Cameroon in years 2013 and 2014. Antibodies were found in all regions without significant differences of regional

prevalence (p = 0.76) (Table 3, Figure 2). Comparing results of the years 2013 and 2014, neither total annual (p = 0.083) nor regional annual differences were observed.

In cattle (n=1,032) 145 sera were found positive or inconclusive after screening with the ID Vet competition ELISA (Table 2), whereat 121 sera found to carry RVFV-specific neutralizing antibodies. Additionally, 18 sera that were either negative or showed cytotoxic effects in SNT could be confirmed as positive by IIFA. Comprehensive analyses finally resulted in a total prevalence of 13.5% (95% confidence interval: 11.4 – 15.7) with detection of antibodies in every sampled region (Table 3, Figure 2), yet without significant regional differences (p = 0.057). Anyhow, a north-south divide of prevalence was observed for cattle, being most prominent in 2013. The total prevalence was significantly higher in 2014 (p = 0.003), which is mainly caused by an increased prevalence in Adamawa.

Comparing total and species-independent prevalence, significant regional differences became apparent (p < 0.0001) (Figure 3). As already suggested by analysis of cattle samples, a clear north-south division is noticeable with highest prevalence in Far-North and lowest in Littoral. Furthermore, species-specific differences of seropositivity were observed (p < 0.0001) (Figure 4), displaying significant higher regional-independent prevalence in cattle than in small ruminants.

Serological screening for RVFV-IgM

Thirty-four sera from small ruminants and 145 sera from cattle were tested with the ID Vet IgM capture ELISA for the specific occurrence of IgM. In four sera RVFV-IgM antibodies were detected. Three of these IgM positive sere were collected in 2013 and one in 2014. One serum originated from a small ruminant from the northwestern region and three cattle sera from the northern parts of Cameroon (Table 4).

Molecular analysis

All IgM-positive sera were analyzed by quantitative real-time RT-PCR. One out of the four sera was weakly positive in quantitative real-time PCR, harboring a Ct value of about 38 (Table 4). Unfortunately no partial sequences could be generated by applied RT-PCRs and the virus isolation was not possible, as all samples were irradiated prior to analysis as biosafety measure.

2.5 Discussion

Previous studies demonstrated the serological evidence of RVFV in Cameroon, yet lacking information for the entire country. Therefore samples of small ruminants and cattle collected in 2013 and 2014 were analyzed and for the first time serological and to a certain degree, molecular evidence for acute RVFV infection of livestock in Cameroon was found. As samples covered almost the entire country, regional, annual and species-specific characteristics of RVFV ecology in Cameroon can now be assessed.

As RVFV was isolated in Nigeria in 1958, the potential for inapparent RVFV infections in Cameroon was examined by serological screenings of sheep subsequently, whereat 33% of tested specimen were found to carry RVFV-antibodies (Maurice, 1967). Although samples originated only from two locations and were limited in quantity, a virus circulation in the country or transboundary transmission from neighboring countries was indicated for the first time. Similarly, 1.06% of humans living in the urban population from Nkongsamba displayed RVFV specific antibodies in 1985 (Paix et al., 1988), being concordant with findings of investigations from 1985-1987, displaying that the majority of

positive human RVFV cases originated from Nkongsamba too. Nkongsamba is an important area where cattle are gathered before being send to Yaoundé (Gonzalez et al., 1989). Thus a correlation of livestock-density and the likelihood of human infections was assumed. Following the RVF outbreak 1987 in Mauritania, comparative analyses of cattle and sheep sera were conducted in Cameroon and other West African countries (Zeller et al., 1995). Seroprevalence for southern parts of Cameroon was ranging from 6 – 20%, being slightly elevated in small ruminants. Most recently, *LeBreton et al.* also found goats to carry RVFV-specific antibodies (LeBreton et al., 2006).

Serological results of this study generally verify a species-specific difference in prevalence, as the likelihood for cattle to carry RVFV-specific antibodies is significantly higher than for small ruminants ($p < 0.0001$). This ratio can also be observed for all regions, where both cattle and small ruminants were sampled. Detected species-dependent prevalence was often observed during inter-epidemic periods in other countries (Boussini et al., 2014; Thiongane et al., 1994). Various susceptibilities and husbandry forms might be causative for this observation. Although *Zeller et al.* reported the ratio to be vice versa, regional and temporal characteristics need to be considered. Assessing regional prevalence, a considerable north-south division can be noted for Cameroon. Promoting factors for sporadic transmission seem to exist predominantly in the north, yet not being strong enough to lead to a severe epidemic or epizootic. Favoring factors may be presence of competent arthropod vectors, the existence of ponds, regional weather conditions or even increased incursion risks from neighboring countries, where RVFV has been reported before (Meunier et al., 1988; Ringot et al., 2004) . As the seropositivity was higher in 2014 as compared to 2013, a retrospective analysis might help to reveal these promoting factors. No recent investigations were

conducted in terms of broad species sampling and pathogen analyses of mosquitoes in the RVFV hotspot areas in Cameroon. Data of this study indicate the strong perspective need for such investigations.

Specific analysis of small ruminant population resulted in a general low prevalence of 3.4% without significant annual and regional variations. A regional and temporal stability of prevalence of these highly susceptible species is indicative for the absence of substantial RVFV infections in the country. Sporadic and non-fatal infections were demonstrated by IgM detection in one small ruminant and in three bovines, one of which giving also a low positive qPCR result indicative for a still ongoing viremia. While IgM positive cattle mainly originated from northern parts of Cameroon (Far North, North, Adamawa), the IgM positive small ruminant sample originated from the Northwest region. The bovine sample containing the RVFV-derived genome derived from Adamawa in the North. Taken together, results indicate a higher RVFV circulation in northern regions of Cameroon. Indeed Ct-values were high and copy numbers/µl RNA relatively low, which is probably caused by gamma-irradiation (30 kGy) of the samples, leading to multiple strand breaks. It is therefore very likely that the viral load was substantially higher in the samples before treatment. The failure of genomic recovery in the other three IgM-positive sera may also be caused by irradiation or by the fact that these animals were sampled after viremia which persists only 14 days post infection (Pepin et al., 2010). In contrast IgM antibodies can be detected up to 2 month post infection (Morvan et al., 1992; Paweska et al., 2003). Therefore the results are the first proof of low-level circulation of RVFV in livestock in Cameroon. This sporadic RVFV transmission is characteristic for the enzootic transmission cycle of RVFV (Bird et al., 2009). Although an above normal precipitation was recorded in some parts of Cameroon

in 2012 (ACMAD, 2012), a transfer to an epidemic cycle did not occur. Thus, the absence of further specific ecologic and climatic factors prevented abundance of competent vectors and a massive outbreak.

In summary the results demonstrate the active circulation of RVFV in cattle and small ruminants in Cameroon. In fact it is the first time that a molecular evidence of RVFV in Cameroon was generated. As the molecular prevalence is relatively low, presumably findings of RVFV are representative for the existence of enzootic cycles with low level transmissions of the virus to susceptible livestock without epizootic manifestation.

However, data are verifying the presence of RVFV within the country as basis for severe epidemics. As soon as requirements for massive amplification of the virus are given, human infections are likely to occur. To date, these specific requirements seem to be absent but it has to be a primary objective to define the promoting factors through screening of mosquitoes, analyses of human samples and extended surveillance of animal population. Knowing these factors, forecasts can be developed, preparedness and awareness can be raised and serious effects for public and animal health can be minimized.

2.6 Figures
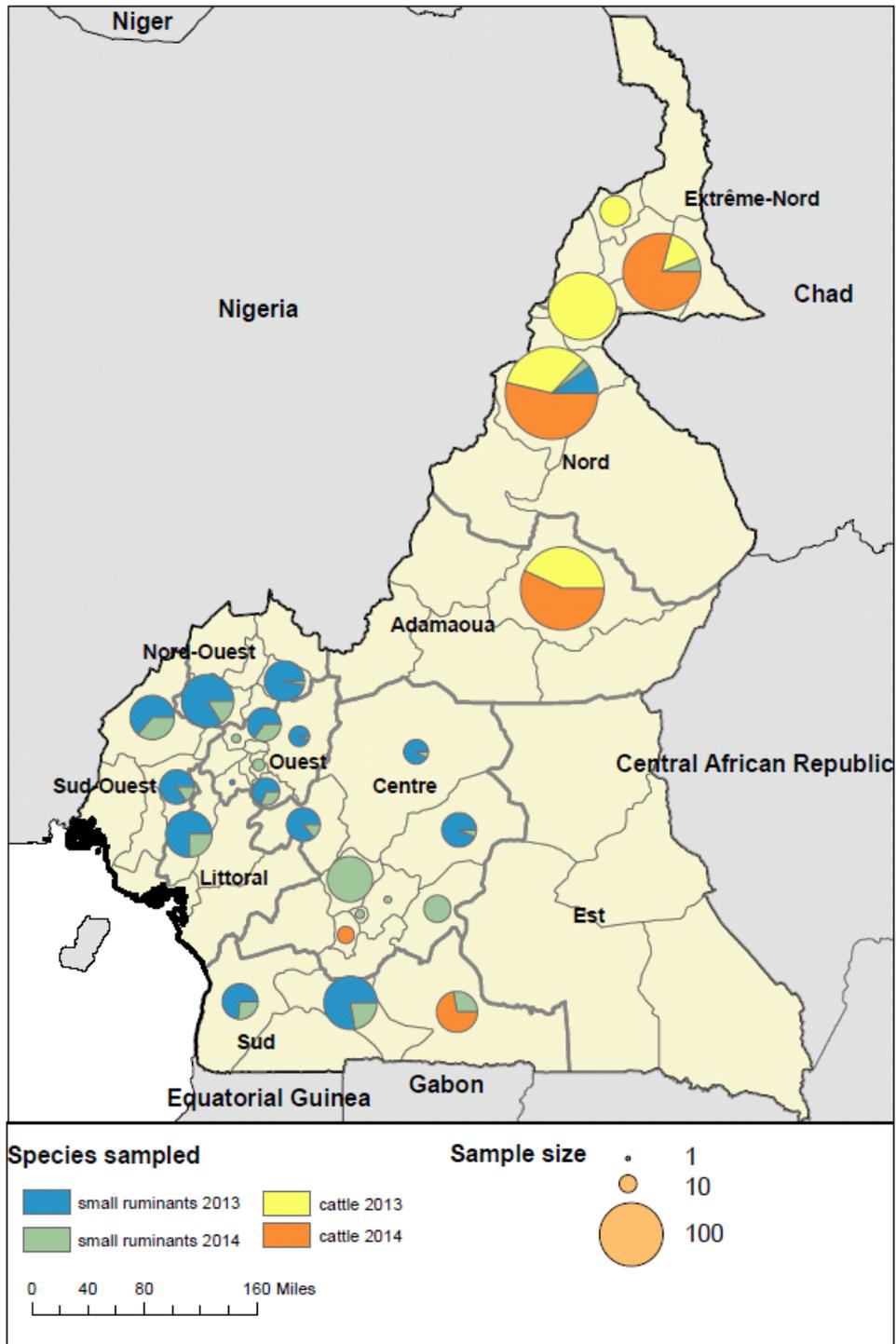
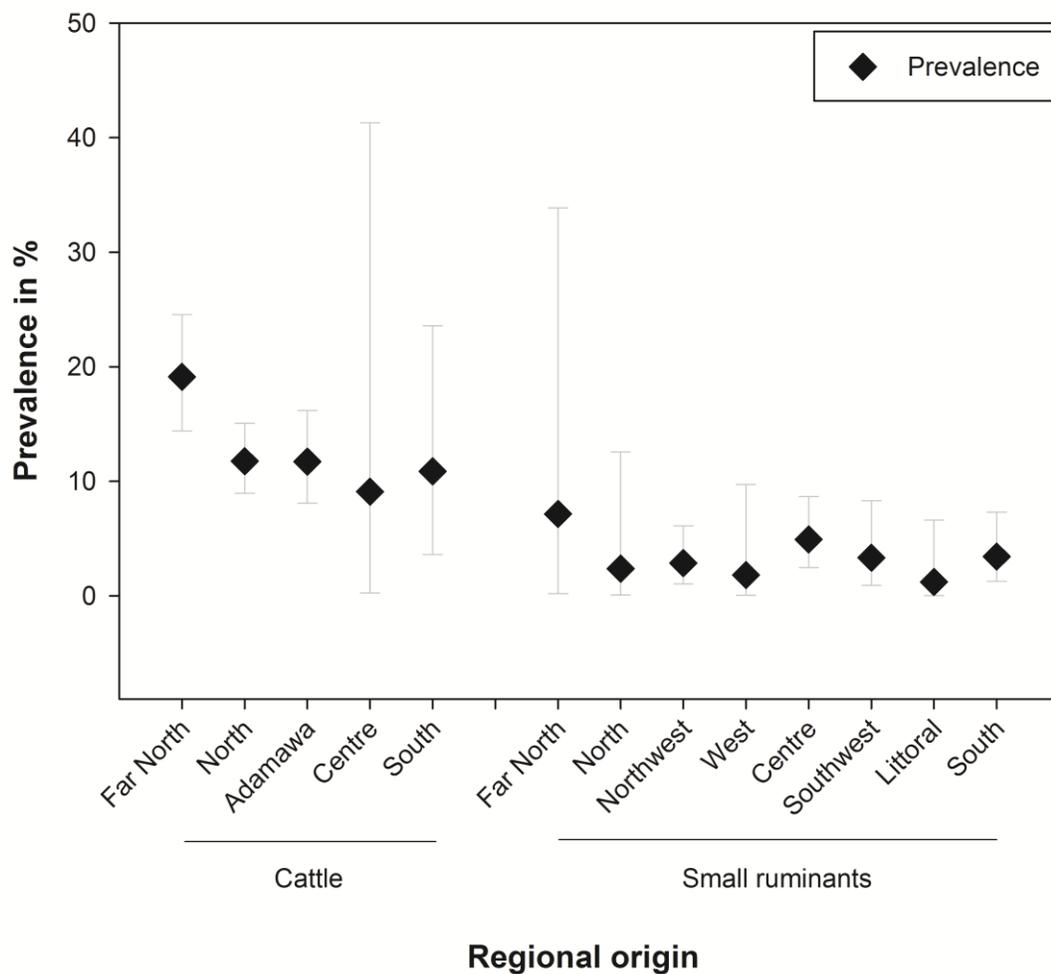Figure 1 Sampling locations 2013 and 2014

Figure 2 Species-dependent regional prevalence

The regional prevalence for 2013-2014 is separately illustrated for cattle and small ruminants. Error bars are indicating 95% confidence interval.

Figure 3 Regional prevalence
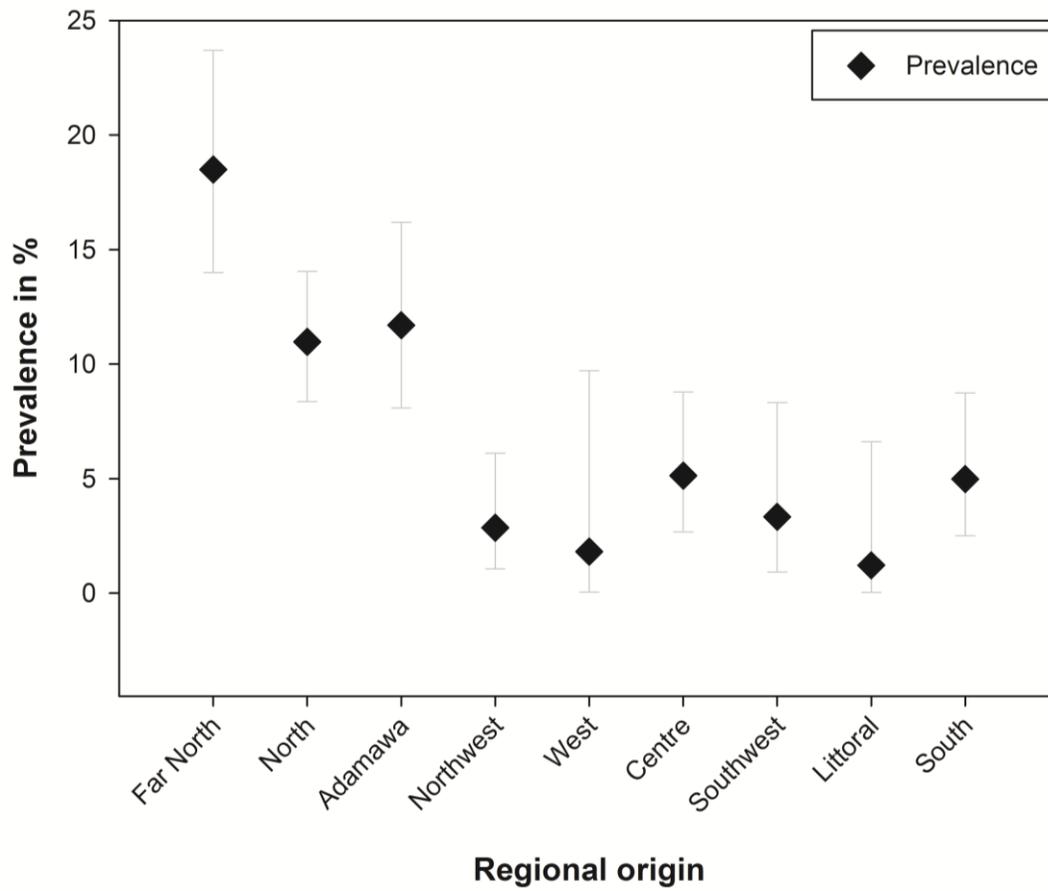
Total regional, species-independent prevalence is illustrated from years 2013-2014. Error bars are indicating 95% confidence interval.

Figure 4 Species-dependent total prevalence
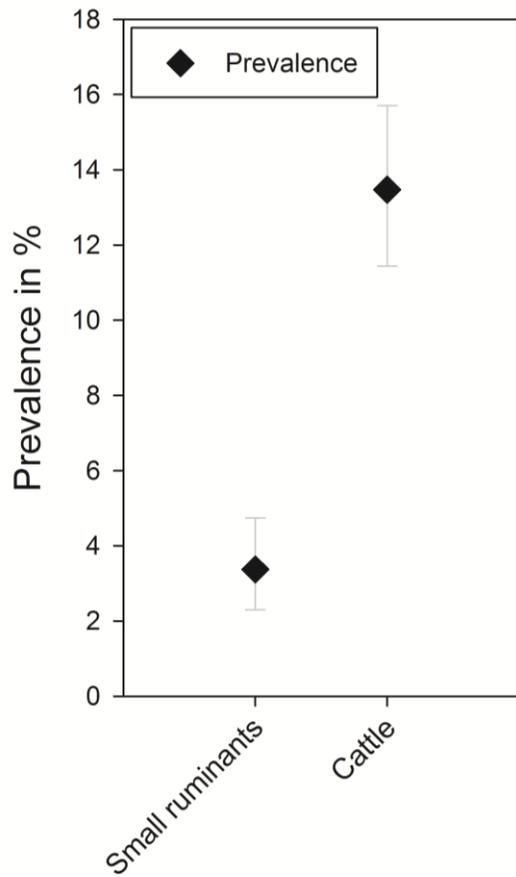
Species-dependent total prevalence for 2013-2014 is illustrated. Error bars are indicating 95% confidence interval.

2.7 Tables

Table 1 Primers applied for sequencing

Name	Genomic segment	Amplicon size in bp	Sequence	Reference
RVF1	S	390	AAG CCA TAT CCT GGC CTC TT	(Aradaib et al., 2013)
RVF2	S		TCC AGT TGT TTC TCC CCA TC	
NS3a	S	669	ATGCTGGGAAGTGATGAGCG	(Faye et al., 2007)
NS2g	S		TGATTTGCAGAGTGGTCGTC	
MRV1a	M	809	CAAATGACTACCAGTCAGC	(Faye et al., 2007)
MRV2g	M		GGTGGAAGGACTCTGCGA	
M-F675	M	970	ACCATCATTGCAAAGGCTGA	(Andriamandimby et al., 2010)
M-R1645	M		GCCATGTGAACCCCTATGTC	
mR-1580	M	255	GGTGGAAGGACTCTGCGA	(Soumare et al., 2012)
mR-1342	M		CCTGACCCATTAGCATG	
Wag	L	212	ATTCTTATTCCCGAATAT	(Faye et al., 2007)
Xg	L		TTGTTTTGCCTATCCTAC	

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Table 2 Methodical comparison and serological results

			ID Vet competition ELISA[a]	Indirect ΔGn ELISA[a]	SNT[b]	IIFA[c]	Final assessment (%)
2013		n					
	Small ruminants	612	*positive*				
			18 (7)	18 (7)	16	0	**16 (2,6)**
			negative 594	594	9	9	**596 (97,4)**
			inconclusive 0	-	-	-	**-**
	Cattle	468	*positive* 39	-	35	12	**47 (10)**
			negative 421	-	7	0	**421 (90)**
			inconclusive 8	-	5[d]	-	**-**
2014							
	Small ruminants	309	*positive* 12 (2)	10 (0)	15	0	**15 (4,9)**
			negative 293	299	1	1	**294 (95,1)**
			inconclusive 4	-	-	-	**-**
	Cattle	564	*positive* 79	-	86	6	**92 (16,3)**
			negative 466	-	12	6	**472 (83,7)**
			inconclusive 19	-	-	-	**-**
Total							
	Small ruminants	921	*positive* 30 (9)	28 (7)	31	0	**31 (3,4)**
			negative 887	893	10	10	**890 (96,6)**
			inconclusive 4	-	-	-	**-**
	Cattle	1032	*positive* 118	-	121	18	**139 (13,5)**
			negative 887	-	19	6	**893 (86,5)**
			inconclusive 27	-	5[d]	-	**-**

[a] total result (number of sera being positive in corresponding ELISA only)

[b] confirmation test for ELISA positive samples only

[c] confirmation test for SNT negative samples only

[d] cytotoxic effects of serum

Table 3 Regional prevalence

	Department (Region)	No. 2013 /No. 2014	No. total	No. positive	Prevalence in %	95% CI
Small ruminants	Diamaré (Far North)	0/14	14	1	7.14	0.18 - 33.87
	Bénoué (North)	32/10	42	1	2.38	0.06 - 12.57
	Bui (Northwest)	59/2	61	0	0	0 - 5.87
	Momo (Northwest)	89/17	106	2	1.89	0.23 - 6.65
	Ngo-Ketunjia (Northwest)	28/15	43	4	9.3	2.59 - 22.14
	Haut Nkam (West)	1/0	1	0	0	0 - 97.5
	Ndé (West)	20/9	29	1	3.45	0.09 - 17.76
	Noun (West)	15/1	16	0	0	0 - 20.59
	Bamboutos (West)	0/3	3	0	0	0 - 70.76
	Koung-Khi (West)	0/6	6	0	0	0 - 45.93
	Haute sanaga (Centre)	42/3	45	2	4.44	0.54 - 15.15
	Lekié (Centre)	0/79	79	1	1.27	0.03 - 6.85
	Mbam et Inoubou (Centre)	37/6	43	1	2.33	0.06 - 12.29
	Mbam et Kim (Centre)	21/2	23	0	0	0 - 14.82
	Méfou-et-Afamba (Centre)	0/2	2	0	0	0 - 84.19
	Mfoundi (Centre)	0/3	3	0	0	0 - 70.76
	Nyong-et-Mfoumou (Centre)	0/28	28	7	25	10.69 - 44.87
	Koupé et Manengouba (Southwest)	38/7	45	3	6.67	1.4 - 18.27
	Manyu (Southwest)	48/27	75	1	1.33	0.03 - 7.21
	Mungo (Littoral)	62/20	82	1	1.22	0.03 - 6.61
	Mvila (South)	84/24	108	3	2.78	0.58 - 7.9
	Océan (South)	36/13	49	1	2.04	0.05 - 10.85
	Dja-et-Lobo (South)	0/18	18	2	11.11	1.38 - 34.71
	Total	**612/309**	**921**	**31**	**3.4**	**2.3 - 4.7**
Cattle	Diamaré (Far North)	34/181	215	43	20	14.87 - 25.98
	Mayo Sava (Far North)	36/0	36	5	13.89	4.67 - 29.5
	Bénoué (North)	109/175	284	28	9.96	6.65 - 13.93
	Mayo Louti (North)	175/0	175	26	14.86	9.94 - 21
	Vina (Adamawa)	114/151	265	31	11.7	8.09 - 16.19
	Méfou-et-Akono (Centre)	0/11	11	1	9.09	0.23 - 41.28
	Dja-et-Lobo (South)	0/46	46	5	10.87	3.62 - 23.57
	Total	**468/564**	**1032**	**139**	**13.5**	**11.4 – 15.7**

Table 4 Characterization of IgM positive samples

	Sample-ID	Origin[1]	Year	IgM (S/P%)	Neutralizing antibodies (ND_{50})	Ct-value (copies/µl)
Small ruminants	523	Babessi, Ngo-Ketunjia, Northwest	2013	+ (41.5%)	+ (>30)	N/A
Cattle	80	Lougguere, Mayo Louti, North	2013	+ (50.9%)	+ (>30)	N/A
	166	Wakwa,Vina, Adamawa	2013	+ (73.2%)	+ (20)	38 (3)
	329	Maroua, Diamare,Far North	2014	+ (63.2%)	+(>30)	N/A

[1] sampling location, department, region

ND_{50} - neutralizing dose of 50%

S/P% - sample-to-positve ratio

N/A - no detectable Ct-value

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Chapter 3

Serological and genomic evidence of Rift Valley fever virus during inter-epidemic periods in Mauritania

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Abstract

Rift Valley fever virus (RVFV) is an emerging pathogen of major concern throughout Africa and the Arabian Peninsula, affecting both livestock and humans. In the past recurrent epidemics were reported in Mauritania and studies focused on the analysis of samples from affected populations during acute outbreaks. To verify characteristics and presence of RVFV during non-epidemic periods we implemented a multistage serological and molecular analysis. Serum samples of small ruminants, cattle and camels were obtained from Mauritania during an inter-epidemic period in 2012 / 2013. This paper presents a comparative analysis of potential variations and shifts of antibody presence and the capability of inter-epidemic infections in Mauritanian livestock. We observed distinct serological differences between tested species (seroprevalence: small ruminants = 3.8%; cattle = 15.4%; camels = 32.0%). In one single bovine from Nouakchott, a recent RVF infection could be identified by the simultaneous detection of IgM antibodies and viral RNA. This study indicates the occurrence of a low-level enzootic RVFV circulation in livestock in Mauritania. Moreover, results indicate that small ruminants can preferably act as sentinels for RVF surveillance.

Chapter 4

Vaccination of alpacas against Rift Valley fever virus: safety, immunogenicity and pathogenicity of MP-12 vaccine

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Abstract

Rift Valley fever (RVF) is an emerging zoonosis of major public health concern in Africa and Arabia. Previous outbreaks attributed camelids a significant role in the epidemiology of Rift Valley fever virus (RVFV), making them an important target species for vaccination. Using three alpacas as model-organisms for dromedary camels, the safety, immunogenicity and pathogenicity of the MP-12 vaccine were evaluated in this study. To compare both acute and subacute effects, animals were euthanized at 3 and 31 days post infection (dpi). Clinical monitoring, analysis of liver enzymes and hematological parameters demonstrated the tolerability of the vaccine, as no significant adverse effects were observed. Comprehensive analysis of serological parameters illustrated the immunogenicity of the vaccine, eliciting high neutralizing antibody titers and antibodies targeting different viral antigens. RVFV was detected in serum and liver of the alpaca euthanized 3 dpi, whereas no virus was detectable at 31 dpi. Viral replication was confirmed by detection of various RVFV-antigens in hepatocytes by immunohistochemistry and the presence of mild multifocal necrotizing hepatitis. In conclusion, results indicate that MP-12 is a promising vaccine candidate but still has a residual pathogenicity, which requires further investigation.

Chapter 5

Discussion

Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic RNA-virus, causing devastating epidemics in Africa and Arabia. Clinical manifestations are variable and range from mild to lethal progression in humans, ruminants, camels and wildlife.

The particular importance of RVFV infections is highlighted in various respects: Macroeconomic impacts are illustrated by the 2007 RVFV outbreak in Kenya that has caused a loss of more than US$32 million on Kenyan economy. The Saudi Arabian outbreak even led to losses of US$132 million (Rich and Wanyoike, 2010). On the other hand outbreaks of RVF do raise public health concerns illustrated by 600,000 infected animals during the 1950 epizootic in South Africa (Gerdes, 2004) or 200,000 infected humans during the 1977 epidemic in Egypt (Meegan, 1979). Recurrent epidemics emerged in additional countries such as Kenya (Jost et al., 2010; Woods et al., 2002), South Africa (Gerdes, 2004; Pienaar and Thompson, 2013) or Mauritania (El Mamy et al., 2011; Jouan et al., 1988; Nabeth et al., 2001), leading to a consistent or even increasing number of severe infections. In some cases, serious outbreaks such as 2007 in Sudan (Hassan et al., 2011) or 2008 in Madagascar (WHO, 2008) were observed, despite of pre-existing RVF circulation in livestock as demonstrated by serological and molecular studies (Findlay GJ, 1936; Mathiot et al., 1984). Finally, due to spatial proximity and a naïve livestock population, Europe is increasingly exposed to the risk of RVF introduction (Chevalier et al., 2010).

Therefore it is of utmost importance to recognize potential risk factors for RVFV

epidemics at an early stage in order to initiate preventive precautions or rather restrict adverse effects as early and best as possible. Whereas vaccination is classified as most effective preventive measure, a close and strategic monitoring of potential amplification hosts is a further important strategy for pathogen control. Comprehensive knowledge of the country-specific RVFV ecology, dynamics and epidemiology is therefore essential for adequate reaction in case of initial indications of a RVF (re-)occurrence.

To extend the in-depth knowledge about RVFV specifically for Cameroon, a biennial nationwide monitoring of small ruminants and cattle was implemented in accordance with aforementioned motivation.

First signs of RVFV circulation in Cameroon appeared in 1967, as 33% of tested sheep found to carry RVFV-specific antibodies (Maurice, 1967). Later on, seroconversion was found in a small number of humans during investigations from 1985-1987 (Meunier et al., 1987; Paix et al., 1988). Serological evidence was also found in Cameroonian cattle by first comparative studies in 1989 (Zeller et al., 1995b) and in a recent study goats were also found to carry RVFV-specific antibodies (LeBreton et al., 2006).

These studies clearly imply the potential of RVFV infections in Cameroon. However, analyzed samples were limited in quantity and representative nationwide origin. Additionally no evidence of acute infections has succeeded yet. For that reason a comprehensive and comparative study to assess the serological and molecular incidence of RVF in livestock from Cameroon was required. A total of 1,953 sera (1,032 cattle, 921 small ruminants) were collected from 2013-2014 and covered almost the entire country (Far North, North, Adamawa, Northwest, West, Centre, Southwest, Littoral and South). A commercial competition NP based ELISA, an indirect IgG ΔGn ELISA and

an IgM capture ELISA were applied to determine infection kinetics and to acquire data about antigen-specific reactivity. The serum neutralization test, defined as gold standard for serological diagnostics (OIE, 2008), was used as confirmatory test and an indirect immunofluorescence assay adapted to corresponding species, was used in case of doubtful results. In case of IgM positive samples, the presence of RVFV-derived RNA was examined by quantitative RT real-time PCR (Bird et al., 2007). Regional, annual and species-specific characteristics of RVFV ecology in Cameroon were assessed with obtained data and considerable species-specific variations in the likelihood to carry RVFV-specific antibodies were detected. A total prevalence of only 3.4% was determined in the highly susceptible small ruminant population that was accompanied by a lack of annual or regional variations. In contrast, a significantly higher ($p < 0.0001$) seroprevalence was found in cattle (13.5%). These species related differences in seroprevalence were found consistently in both years and all regions where both cattle and small ruminants were sampled. The same species-dependent prevalence could also be observed during inter-epidemic periods in other countries, such as Burkina Faso or Senegal (Boussini et al., 2014; Thiongane et al., 1994). Additionally, the total, species-independent prevalence significantly ($p < 0.0001$) decreases from north to south, indicating a considerable north-south division. Based on these findings geo-ecological investigations would be helpful to elucidate promoting factors of RVFV epidemics, such as presence of competent vectors, amplification hosts or regional climates. Although *Ae.aegypti* displays higher infestation rates in northern Cameroon (Simard et al., 2005), which correlates with the findings of our study, specific studies of their virus competency and dissemination capacity were not conducted so far. Furthermore an annual increase of total seropositivity (2013: 5.8%; 2014: 12.3%; $p = 0.003$) was observed. In the course

of the countrywide surveillance, evidence for acute RVFV infections was found for the first time. Acute non-fatal infections were demonstrated by IgM detection in one small ruminant and three bovines, one of which displayed a low-level viremia as revealed by a positive RT-qPCR result. A main area of RVFV circulation could therefore exist in the north of Cameroon, as IgM positive cattle originated from Far North, North and Adamawa and the IgM positive small ruminant from Northwest region. The molecular prevalence of RVFV, being found in Cameroon for the first time, is relatively low but it is the first proof for the existence of an enzootic cycle within livestock in Cameroon, characterized by low level transmission of RVFV to susceptible livestock without epizootic manifestation. Although no epizootic was observed, the molecular and serological evidence of RVFV in the country implies a possible transition to an epizootic cycle, as soon as specific ecological and climatic factors will facilitate an abundance of competent vectors. Although *Bunyaviridae* such as Simbu virus, Tataguine virus or Bunyamwera virus have been isolated from mosquitoes caught in Yaoundé 1964 (Brottes et al., 1966; Salaun et al., 1969), current studies about the presence of RVFV in mosquitoes from Cameroon are absent. Therefore, the findings of this study indicate the strong need for such investigations. A broad screening of different mosquito species, human samples and potential amplification hosts will be of major importance to characterize supportive factors for RVFV epidemics and thus to create a basis for effective preventive measures and emergency preparedness.

Contrary to Cameroon, where specific data about RVFV infections are sparse and noticeable or substantial outbreaks did not occur yet, Mauritania is a hotspot of significant RVFV epidemics and recurrent epizootics. Even for countries in which RVFV is endemic, a continuous monitoring will enhance knowledge of infection ecology,

especially by characterizing inter-epidemic periods and potential reservoir hosts.

The first RVFV epidemic in Mauritania and in West Africa in particular in 1987, caused 220 human deaths and was associated with the construction of the Diama Dam (Digoutte and Peters, 1989; Jouan et al., 1988; Ksiazek et al., 1989; Saluzzo et al., 1987). Further outbreaks in 1993 (Zeller et al., 1995a), 1998 (Nabeth et al., 2001), 2003 (Faye et al., 2007), 2010 (El Mamy et al., 2011), 2012 (Sow et al., 2014), 2013 (OIE, World Animal Health Information Database (WAHID), 2014) and recently in 2015 (OIE, World Animal Health Information Database (WAHID), 2015) illustrated the disease burden of RVFV for Mauritania as high mortalities in both livestock and humans were reported repeatedly. The temporal succession of these outbreaks does further demonstrate the remarkably reduced duration of inter-epidemic periods. To date, investigations of infection dynamics of RVFV in Mauritania mainly focused on the analysis during epidemic periods, while the characterization of inter-epidemic periods was barely addressed. However, knowledge of potential mechanisms of pathogen persistence and transmission cycles during these periods is of equal significance for the global understanding of pathogen interaction.

To analyze the serological evidence and distribution of RVFV infections during inter-epidemic periods, 1,066 samples of small ruminants, camels and cattle were collected from January to March 2012 and from January to June 2013. The already described sequential serological screening, including ELISA, serum neutralization test and immunofluorescence was applied correspondingly. The presence of an acute infection was verified by IgM specific ELISA and by quantitative real-time RT-PCR.

In summary it was demonstrated that RVFV-specific antibodies persist in Mauritanian

livestock during inter-epidemic periods with species-specific patterns and new infections occur only at a low level. Results obtained in Cameroon were equally confirmed by the results of the serological analysis of Mauritanian livestock, as significant species-specific seroprevalence (Fisher´s exact test, p = 0.03) was observed. The investigation revealed that small ruminants had lowest prevalence rates (3.8%), followed by cattle (15.4%) and camels (32.0%). The obtained data were compared to those reported during epidemic periods: In 2010, 54% (El Mamy et al., 2011) or even up to 69% (Jackel et al., 2013b) of small ruminants carried RVFV-specific antibodies. Although quantity and location of samples were largely comparable between epidemic and inter-epidemic periods, a significant decrease by more than 50% of seroprevalence was observed for small ruminants. In contrast, a seroprevalence of 33% (El Mamy et al., 2011) respectively 45% (Jackel et al., 2013b) was detected in camels in 2010, which is approximately equal to the here reported prevalence of 32% during the inter-epidemic period. Similar results were obtained for RVFV positive cattle, exhibiting a prevalence of 13% during the 2010 epidemic (Jackel et al., 2013b) compared to 15.4% positives during inter-epidemic periods. Data indicate that seroprevalence of small ruminants seems to decrease substantially during inter-epidemic periods, but only marginally for cattle and camels. An increase of seroconversion in a given small ruminant population is therefore an adequate indicator for the onset of RVFV epizootics, making small ruminants the most appropriate sentinel for RVFV. Species-specific differences of prevalence can again be explained by higher susceptibility and shorter lifespan of small ruminants. In general it can be postulated that a decreased susceptibility against RVFV enhances the probability for detection of RVFV-specific antibodies during inter-epidemic periods. Observations are in accordance with previous studies from other countries where a low prevalence in

small ruminants (Chevalier et al., 2005; Kifaro et al., 2014; Sumaye et al., 2013) and a higher prevalence in cattle (Boussini et al., 2014; Thiongane et al., 1994) were reported during inter-epidemic periods.

Regarding regional distribution of RVFV-specific antibodies, positive sheep and goat sera were mainly detected in Tagant and Gorgol in central and southern Mauritania, where RVF cases were reported before (El Mamy et al., 2011; Sow et al., 2014). This indicates the presence of persisting antibodies from former non-fatal infections. The same observations were made for camels, as most previous RVFV infections of camelids were detected in Adrar in central Mauritania (49% seropositivity). No significant regional pattern can be detected for cattle, since only few previous sampling data are available. Seropositive specimen are often characterized by high neutralizing antibody titers of up to 1:10,240 but a significant regional pattern or a correlation with the age of animals was not observed. In Mauritanian samples only one bovine was found to carry RVFV-specific IgM antibodies as well as viral RNA, indicating a recent infection and a low-level virus transmission during inter-epidemic periods. The generally low rate of IgM in tested specimen supports the hypothesis that detected antibodies in this study are persisting from former infections and that there is no broader epizootic circulating in the herds.

In conclusion, results highlight the pivotal role of small ruminants as most sensitive indicators of acute infections, thus being best choice as sentinels and important component of active epizootic prevention. In contrast, samples of cattle or camels are suitable to elucidate past outbreaks and to reveal even non-recognized RVFV infections, as prolonged persistence of antibodies is caused by their restricted susceptibility

towards RVFV. The implied potential of inter-epidemic RVFV transmission to susceptible livestock needs to be further analyzed. Regarding the applied methods, both ELISA and SNT demonstrated a high correlation with only few exceptions. The indirect ΔNP IgM ELISA for camelids was applied for the first time in a broad screening of camel sera. Although no IgM positive camel sera were detected, the indirect ΔNP IgM ELISA offers high potential for future monitoring and should be evaluated through integration in further screenings of camelid sera.

Prospectively further and extended studies for the analysis of potential known and unknown amplification hosts and competent indigenous vectors during inter-epidemic periods are needed to fully understand the enzootic circulation and maintenance mechanisms of this virus.

Besides of prevention and containment of devastating impacts of RVFV epidemics through targeted and coordinated surveillance measures, the spread of RVFV can currently be prevented most sufficiently by deployment of effective vaccines. Therefore numerous promising vaccine candidates have been designed, among them the well characterized live attenuated vaccine MP-12, which has a conditional license for veterinary application in the U.S. and was evaluated in a phase 2 trial in humans (Pittman et al., 2016). MP-12 has been already applied successfully in livestock like sheep (Miller et al., 2015; Morrill et al., 1991) and cattle (Morrill et al., 1997) but not yet in camelids although dromedary camels play an important, but yet not completely understood role in the virus ecology as demonstrated by the 2010 epizootic in Mauritania (El Mamy et al., 2011). For this purpose, the immune response of alpacas that were chosen as model-organisms for camelids, was analyzed in terms of safety,

immunogenicity and potential pathogenicity. Three male alpacas were immunized subcutaneously with 2 x 10^6 $TCID_{50}$/ml of RVFV MP-12. Serum, whole blood, nasal swabs, conjunctival swabs and feces were taken regularly after inoculation. Animal #A1 was euthanized three days post infection (dpi), while animals #A2 and #A3 were sacrificed 31 dpi. To evaluate the safety and tolerability of MP-12 for camelids, all animals were monitored daily for clinical anomalies. The MP-12 was well tolerated as no adverse effects were observed and neither persisting deviations of liver enzyme levels nor hematological parameters were recorded. No virus shedding, as assessed by real-time RT-PCR and subsequent virus isolation, was observed for any of the nasal and conjunctival swabs or feces. The immunogenicity of the vaccine is of particular importance as it primarily influences the efficacy of an immunization. The MP-12 induced a strong immune response and the generation of neutralizing antibodies as verified by competition ELISA and serum neutralization test respectively. The exact course of immune response was determined with different indirect in-house ELISAs, targeting antibodies against structural and non-structural proteins Gn, Gc, NP, NSm and NSs correspondingly. Detection of IgM antibodies was possible by using isotype specific secondary antibodies. Furthermore, an indirect immunofluorescence assay was developed that allowed the differentiation of whole antibodies, IgG and IgM by isotype-specific secondary antibodies. Seroconversion started from 7 dpi and both animals elicited stable titers of neutralizing antibodies of up to 1:1,280. The required minimal acceptable titer for at-risk personnel of 1:40 (Pittman et al., 1999) was clearly achieved and maintained for the entire experiment. However, it still needs to be investigated whether a single MP-12 vaccination is sufficient to stimulate a long-term antibody response in camelids, as already demonstrated for sheep (Miller et al., 2015). Regarding

antigen-specific reactions, RVFV-specific antibodies were primarily directed against NSs, NP and Gn, which was equally demonstrated in other studies for sheep and cattle (Hossain et al., 2016). However, the observed strong formation of NSs derived antibodies in alpacas remains unique. In general IgM response, mainly directed against NP, occurred for a shorter period and was less intense compared to IgG. The overall results are the first presentation of a complex acute humoral immune response of camelids against RVFV in an experimental approach. Despite of highly promising safety and immunogenicity of this live attenuated vaccine, an evaluation of remaining pathogenicity in camelids is strongly needed, as already indicated by previous studies of *Wilson et al.* (Wilson et al., 2014). This concerns especially the capability of vaccine virus replication in tissues and blood. RVFV-derived RNA was detected in the serum of two animals (#A1 and #A2) three days post infection, however subsequent virus replication was observed for #A1 derived serum only. A transient viremia was observed in humans, cattle, macaques and sheep before (Morrill et al., 1987; Morrill et al., 1997; Morrill and Peters, 2003; Pittman et al., 2016), which can be explained by residual replicative potential of the live attenuated vaccine. Although the risk of MP-12 shedding was estimated to be very low, findings of transient viremia raise concerns for vector transmission. However, the capability of mosquito-borne MP-12 transmission was proven to be very low, as high viral loads were needed to efficiently infect mosquitoes (Miller et al., 2015; Turell and Rossi, 1991). RVFV-derived genomes were also detected in liver, spleen and lung of #A1, but virus could only be isolated from liver specimen. The efficient clearance of the virus is indicated by the absence of RVFV genomes in all tissues of #A2 and #A3. Because a major concern of live attenuated vaccines is the possibility of reversion to parental virulence (Miller et al., 2015), the genetic stability of

MP-12 was examined by next generation sequencing of recovered virus from liver propagations. Indeed mutations were found in the S- and M segment, but they were limited in quantity and sustainability. Results of recent studies, demonstrated that the attenuation of MP-12 is caused independently by every segment (Ikegami et al., 2015; Nishiyama et al., 2016), which makes a reversion to virulence by single mutations rather unlikely. For further analysis of potential pathogenicity, all tissues were examined pathohistologically and tissues with suspicious lesions were further analyzed for specific antigen presence by immunohistochemistry. Most prominent alterations were found in the liver of animal #A1 and are characterized by a mild multifocal necrotizing hepatitis. The onset of immunological activation and acute innate and resorptive reactions were represented by the presence of macrophages and neutrophilic granulocytes. Lesion-associated RVFV antigens were detected by immunohistochemistry. While NP, Gn, Gc and NSm were consistently present, NSs was not detectable in the liver of #A1. Temporal variations of expression of NSs as described previously (Ikegami et al., 2005) or a low expression level might be causative for the absence of NSs antigen in liver lesions. Compared to animal #A1, fewer and smaller lesions were observed in the livers of animal #A2 and #A3. Hepatic lesions were mainly infiltrated by lymphocytes and histiocytes, indicating a reactive phagocytosis and an active adaptive immune system. Additionally the absence of any virus derived antigen, demonstrates the efficacy of immunological clearance and the absence of persisting restrictions for the organism.

Overall, these results demonstrate the suitability of live attenuated vaccine MP-12 for camelids in general. However, remaining pathogenicity should be considered when pregnant or immunocompromised animals are immunized. These findings only became available in their entirety by the establishment of camelid-specific diagnostics. Alpacas

were proven to be a valuable model for studying camelid-associated pathogens as they were both susceptible to RVFV-strains and all camelid-specific diagnostics were applicable.

Recurring and emerging outbreaks of RVF demonstrated the devastating potential of this viral infectious disease. To be prepared it is essential to define the infection status of ruminants and hotspots in relevant regions. However, a rapid spread and clinical manifestation of this highly pathogenic infectious disease can only be prevented effectively and reliably by using well-characterized vaccines.

For this purpose, both serological and molecular investigations of the presence and distribution of RVFV in Cameroon and Mauritania and an immunization study with camelids have been conducted. Through a perennial cross-regional seroepidemiological screening of livestock from Cameroon, characteristics of regional and species-specific prevalence were elucidated and acute infections were verified for the first time. Studying inter-epidemic periods in Mauritania illustrated that small ruminants are most significant indicators for RVFV epizootics. Finally, the immunization study of alpacas did clarify the applicability and limitations of MP-12 vaccination for camelids and the usefulness of newly developed camelid-specific diagnostics. Further analysis of indigenous mosquito populations, potential reservoirs such as wildlife or small mammals and humans will be needed to clearly define the interplay of virus circulation between humans, animals and environment. Results and established diagnostics of the immunization trial represent a basis for further vaccination and pathogenesis studies in camelids addressing the long-term immunity, efficacy and potential teratogenic effects in particular.

Chapter 6 Summary

Investigating the infection cycle of Rift Valley fever virus in Cameroon and Mauritania and the applicability of MP-12 vaccine for camelids

Melanie Rissmann

Rift Valley fever virus (RVFV) is a member of the family *Bunyaviridae* and can lead to severe diseases in humans and livestock. Although most human infections proceed as mild flu-like illness, severe manifestations as retinitis, meningoencephalitis or even hemorrhagic fever syndromes due to fulminant hepatitis do occur in about 1-2% of the cases. Infections of adult ruminants and camels rarely lead to manifest and lethal hepatitis, but are rather observed as febrile diseases. However, so called 'abortion storms' are characteristic for RVFV infections of pregnant ruminants, leading to an up to 100% mortality rate in new borne animals. While human infections are mostly caused by contact to viremic animals, the transmission through RVFV-infected mosquitoes is of major importance for livestock and wildlife. To date RVFV was found in more than 30 mosquito species. Currently RVFV is widely endemic in Africa, recurrently causing substantial outbreaks. Significant losses in human and animal populations highlight the major impact of the pathogen for both healthcare and animal husbandry. For mitigation and monitoring of these impacts, knowledge of the specific infection ecology is of particular importance.

To address these issues, a cross-regional serological and molecular screening of livestock sera in Cameroon and Mauritania was implemented. The findings in Cameroon

demonstrated considerable inter-species differences, reflected by a significantly higher seroprevalence of cattle compared to small ruminants. Additionally, striking regional variabilities of seropositivity were observed, implicating a decline from north to south Cameroon. Apart from general seroconversion, acute infections were detected for the first time in three cattle and one small ruminant, harboring RVFV-specific IgM antibodies. Moreover, virus derived RNA was detected in one IgM positive cattle, indicating the existence of low-level circulation of RVFV. By providing first evidence of acute infections, both the existence of an ongoing enzootic cycle and the potential for severe outbreaks in future was demonstrated.

Although recurrent RVFV outbreaks in Mauritania led to massive losses in the past, serological and molecular investigations during inter-epidemic periods are absent to date. Therefore, samples of small ruminants, cattle and camels that were collected during inter-epidemic periods from 2012-2013, were analyzed. Comparative analyses demonstrated a significant difference in small ruminants, showing a strong decline of seroprevalence during inter-epidemic periods. In contrast, the rate of seropositivity in camels and cattle was almost identical to those detected during epidemics. Obtained data do therefore clarify the significant role of small ruminants as important sentinels for RVFV, as a remarkable increase of seroconversion will indicate a possible introduction of RVFV into the herds. Furthermore the evidence of an IgM positive cattle harboring viral RNA illustrated the presence of an enzootic cycle.

Camelids play a yet neglected but pivotal role in transmission and spread of RVFV and associations with human infections highlight the eminent need for effective vaccines for this species. For this purpose alpacas were chosen as model organisms for camelids

and were immunized with the live attenuated MP-12 vaccine, evaluating its safety, immunogenicity and pathogenicity. The application of MP-12 proved to be safe as no shedding of vaccine virus was recorded and no persisting alterations in hematology and clinical chemistry were observed. Additionally the vaccine was highly immunogenic, as stable neutralizing antibody titers were generated by a single application. A detailed investigation of antigen-specific reactivity demonstrated a significant generation of antibodies directed against NSs, NP and Gn proteins. A minimal residual pathogenicity was demonstrated in alpacas 3 dpi as a replicative potential was verified in serum and liver. In addition pathological examinations revealed a mild, multifocal, acute necrotizing hepatitis with antigenic presence of NP, Gn, Gc and NSm. In contrast, hepatic lesions 31 dpi displayed a lymphohistiocytic character, indicating the efficient immunological clearance and absence of sequelae. Furthermore, next generation sequencing of recovered MP-12 confirmed the genetic stability of the vaccine. Therefore MP-12 is a safe and immunogenic vaccine for camelids, yet with considerable residual pathogenicity.

In summary, the here presented results elucidate characteristics of the RVFV infection ecology in Cameroon, present comparative analyses during inter-epidemic periods in Mauritania and evaluate the suitability of the RVFV vaccine MP-12 for camelids.

The obtained data can be used for awareness raising and risk assessment of Rift Valley fever as well as for the development of prevention strategies.

Chapter 7 Zusammenfassung

Untersuchung des Infektionszyklus von Rift Valley fever virus in Kamerun und Mauretanien und die Anwendbarkeit deMP-12 Vakzine für Kameliden

Melanie Rissmann

Rift Valley fever virus (RVFV) gehört zur Familie der *Bunyaviridae* und kann zu schwerwiegenden Erkrankungen bei Nutztieren und Menschen führen. Zwar verlaufen Humaninfektionen meist als milde grippeartige Erkrankungen, jedoch kann es in 1-2% der Fälle auch zu schwerwiegender Symptomatik wie Retinitis, Meningoencephalitis oder gar einem hämorrhagischen Fiebersyndrom auf Grund einer fulminanten Hepatitis kommen. Eine Infektion adulter Wiederkäuer und Kamele führt neben einer fieberhaften Grunderkrankung nur selten zu manifesten und letalen Hepatitiden. Ein besonderes Charakteristikum stellt jedoch das massenhafte Verlammen (sog. „abortion storms") infizierter Muttertiere dar. Hierbei wird eine bis zu 100 prozentige Mortalität der Jungtiere beobachtet. Während Humaninfektionen meist durch Kontakt zu virämischen Tieren verursacht werden, erfolgt die Infektion von Nutz- und Wildtieren vorrangig durch RVFV-infizierte Mücken. Derzeit wurde das Virus in über 30 Mückenspezies nachgewiesen. Gegenwärtig ist RVFV in Afrika endemisch und führt dort zu bedeutenden Krankheitsausbrüchen, die neben hohen Verlusten bei Mensch und Tier auch schwere ökonomische Schäden verursachen. Zur Eindämmung und Überwachung dieser Auswirkungen sind Kenntnisse der spezifischen Infektionsbiologie von großer Wichtigkeit.

Dazu wurde ein regionsübergreifendes serologisches und molekulares Screening von Nutztierproben aus Mauretanien und Kamerun implementiert. In Kamerun wurden erhebliche speziesspezifische Differenzen festgestellt, welche sich durch eine signifikant höhere Seroprävalenz von Rindern im Vergleich zu der von kleinen Wiederkäuern äußerte. Zudem wurde ein deutliches Nord-Süd Gefälle in der Gesamtprävalenz nachgewiesen. Neben dem Nachweis einer allgemeinen Serokonversion konnten erstmals für dieses Land akute Infektionen, anhand des Nachweises von IgM Antikörpern in drei Rindern und einem kleinen Wiederkäuer gezeigt werden. Für eines der IgM positiven Rinder wurde außerdem das Vorhandensein viraler RNA gezeigt, womit das Vorliegen einer geringgradigen RVFV-Zirkulation bewiesen wurde. Der erstmalige Nachweis akuter Infektionen während enzootischer Infektionszyklen zeigt, dass auch in Kamerun ein Gefährdungspotential für bedeutende RVF-Ausbrüche vorhanden ist.

Obwohl wiederkehrende RVF-Ausbrüche in Mauretanien in der Vergangenheit zu enormen Verlusten führten, fehlen serologische und molekulare Untersuchungen während inter-epidemischer Perioden derzeit noch gänzlich. Darum wurden Proben kleiner Wiederkäuer, Rinder und Kamele, welche während der inter-epidemischen Periode 2012-2013 gesammelt worden, untersucht. So konnte anhand einer vergleichenden Untersuchung gezeigt werden, dass es in der inter-epidemischen Periode zu einem signifikanten Abfall der Seroprävalenz bei kleinen Wiederkäuern kommt. Hingegen fällt die Seroprävalenz bei Rindern und Kamelen in dieser Phase wesentlich geringer ab. Die erhobenen Daten verdeutlichen somit die maßgebliche Rolle kleiner Wiederkäuer als wichtige Indikatortiere für RVFV, da ein Anstieg der Serokonversionsrate frühzeitig den Beginn eines epizootischen Geschehens anzeigen

kann. Auch in Mauretanien konnte eine akute Infektion eines Rindes nachgewiesen werden, welche die Präsenz eines enzootischen Zyklus verdeutlicht.

Kameliden spielen eine bisher vernachlässigte aber bedeutende Rolle in der Übertragung und Verbreitung von RVFV. Verbindungen zu Humaninfektionen unterstreichen den eminenten Bedarf an effektiven Vakzinen für diese Spezies. Aus diesem Grund wurden Alpakas als Modellorganismus für Kameliden gewählt, um in einer Impfstudie mit der lebend-attenuierten Vakzine MP-12 dessen Sicherheit, Immunogenität und potentielle Pathogenität zu überprüfen. Dabei erwies sich die MP-12 Vakzine als sicherer Impfstoff, der nicht ausgeschieden wurde und zu keinen anhaltenden Veränderungen im Blutbild oder der Blutchemie führte. Die Vakzine war hochgradig immunogen und eine einmalige Immunisierung reichte aus, um stabile Titer neutralisierender Antikörper zu induzieren. Die Untersuchung der Immunantwort zeigte eine deutliche Bildung IgG spezifischer Antikörper gegen die viralen Antigene NSs, NP und Gn. Der Nachweis viraler Replikation in Serum und Leber drei Tage nach Infektion demonstrierte eine geringfügige Residualpathogenität des Impfstoffes. Die pathologische Untersuchung zeigte zusätzlich eine geringgradige multifokale akute nekrotisierende Hepatitis mit Antigennachweis von NP, Gn, Gc und NSm. Hingegen waren entsprechende hepatische Läsionen 31 Tage nach Infektion von lymphohistiozytärem Charakter und ein Antigennachweis blieb aus, welches die effektive immunologische Clearance des Erregers und die Abwesenheit von Spätschäden indizierte. Zudem belegte eine Vollgenomsequenzierung der Vakzine die genetische Stabilität des Impfvirus. Insgesamt konnte gezeigt werden, dass MP-12 eine sichere und hoch immunogene Vakzine mit geringer Restpathogenität für Kameliden ist.

Zusammenfassend konnten in den hier vorgestellten Studien Charakteristika der allgemeinen Infektionsbiologie in Kamerun erhoben, vergleichende Untersuchungen während inter-epidemischer Perioden in Mauretanien durchgeführt und eine Vakzinierungsstudie in Kameliden realisiert werden. Die dabei erhobenen Daten tragen somit sowohl zur Erregercharakterisierung als auch zur Entwicklung effektiver Präventiv- und Bekämpfungsmaßnahmen für RVFV-Infektionen bei.

Chapter 8

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Chapter 9

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