


# First occurrence of Rift Valley fever outbreak in Niger, 2016

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## Abstract

Rift Valley fever (RVF) is a mosquito-borne viral zoonosis causing abortions and high mortality among animals, whereas in humans, the disease is usually mild or asymptomatic. In September 2016, the Republic of Niger declared the first RVF outbreak in the northern region of Tahoua near the Malian border. This study describes the outbreak and reports the results of serological and molecular investigations of the human and animal samples collected. Serum samples from both human and animal suspected cases have been confirmed at the Centre de Recherche Médicale et Sanitaire (CERMES) and the Laboratoire Centrale d'Élevage (LABOCEL) public health and animal reference laboratories, respectively. Techniques for biological confirmation were real time reverse transcription polymerase chain reaction (RT-PCR) and enzyme linked immunosorbent assay (ELISA). Phylogenetic trees were established after genetic sequencing of the small and medium segments of the RVF virus (RVFV) genome. Out of the 399 human samples collected, 17 (4.3%) were confirmed positive for RVFV. Overall, 33 (8.3%) deaths occurred out of which five (29%) were among the 17 confirmed cases. Regarding animals, 45 samples were tested, three of which were RT-PCR positive and 24 were IgG positive. The phylogenetic analyses showed that the Niger strains clustered with Senegal 2013 and Mauritania 2015 RVFV strains. This first outbreak of RVF was very challenging for public and animal health laboratories in Niger. Besides resulting in human deaths, important loss of cattle has been reported. Therefore, vigilance has to be strengthened emphasising vector control strategies and active surveillance among animals.

**Keywords:** Rift Valley fever, outbreak, Niger, ELISA, RT-PCR, sequencing.

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## Introduction

Rift Valley fever (RVF) is an emerging zoonotic vector-borne disease that represents a threat to both animal and human health (Metras *et al.* 2016). The causative agent of RVF is an arbovirus, which belongs to the Phlebovirus genus in the *Bunyaviridae* family and was first identified in 1930 in the Rift Valley of Kenya (Daubney & Garnham 1931; Nanyingi *et al.* 2015). The Rift Valley fever virus (RVFV) genome is about 11.9 kb and organised in three single-

stranded RNA segments termed as large (L), medium (M) with negative-sense polarity and small (S) which is ambisense (Samy *et al.* 2017). Many outbreaks of RVF have been reported in Africa and in the Arabian Peninsula (Ikegami & Makino 2011; Himeidan *et al.* 2014; Nanyingi *et al.* 2015). The largest epidemic occurred in Egypt in 1977–1978, when there were an estimated 200 000 human suspected cases, with some 18 000 confirmed cases and 600 deaths (Johnson *et al.* 1978). Since 1987, countries in West Africa have experienced RVF outbreaks

(Boushab *et al.* 2016; Sow *et al.* 2016). However, few data are available on any previous occurrence of RVF outbreak in Niger (FAO, 2017).

The virus is primarily transmitted to animals through infected mosquito bites. Spatiotemporal rainfall variability is the key parameter controlling the dynamics of mosquito vector-borne diseases and thereby influencing the transmission of RVFV (Guilloteau *et al.* 2014). The disease occurrences follow the unusual trend of heavy rainfall leading to flooding. This provides a conducive environment for dormant mosquito eggs infected by RVFV to hatch and become predominant mosquito populations that transmit virus to animals and humans (Boushab *et al.* 2016). Mosquitoes belonging to the genera *Aedes*, and *Culex* are mostly involved in the transmission (Himeidan *et al.* 2014).

The virus can be transmitted from domestic animals to humans mainly through direct contact with blood, excreta, meat or secretions of infected animals, consumption of raw milk (Lancelot *et al.* 2017). However, there is no current evidence of person-to-person transmission of RVFV (Metras *et al.* 2011; Boushab *et al.* 2016) although vertical transmission in humans from mother to baby has been reported (Samy *et al.* 2017).

The clinical manifestations of the disease among animals are abortion and death of newborns (Daubney & Garnham 1931; Nanyingi *et al.* 2015) while in humans, although the disease is generally mild or asymptomatic, there are several reports of high fatality rates (Madani *et al.* 2003; CDC, 2007). However, 2–5% of infected individuals may develop severe disease such as ocular disease, haemorrhagic fever syndrome or encephalitis (Boushab *et al.* 2016; Mroz *et al.* 2017).

In September 2016, the Republic of Niger declared the first RVF outbreak with human cases reported in the Northern Region of Tahoua near the Malian border particularly in the departments of Tchintabaraden, Tassara and Abalak (WHO, 2016; Douchi *et al.* 2017).

In this article, we describe the outbreak and report the results of serological and molecular investigations of human and animal samples collected.

## Materials and Methods

### Area of study

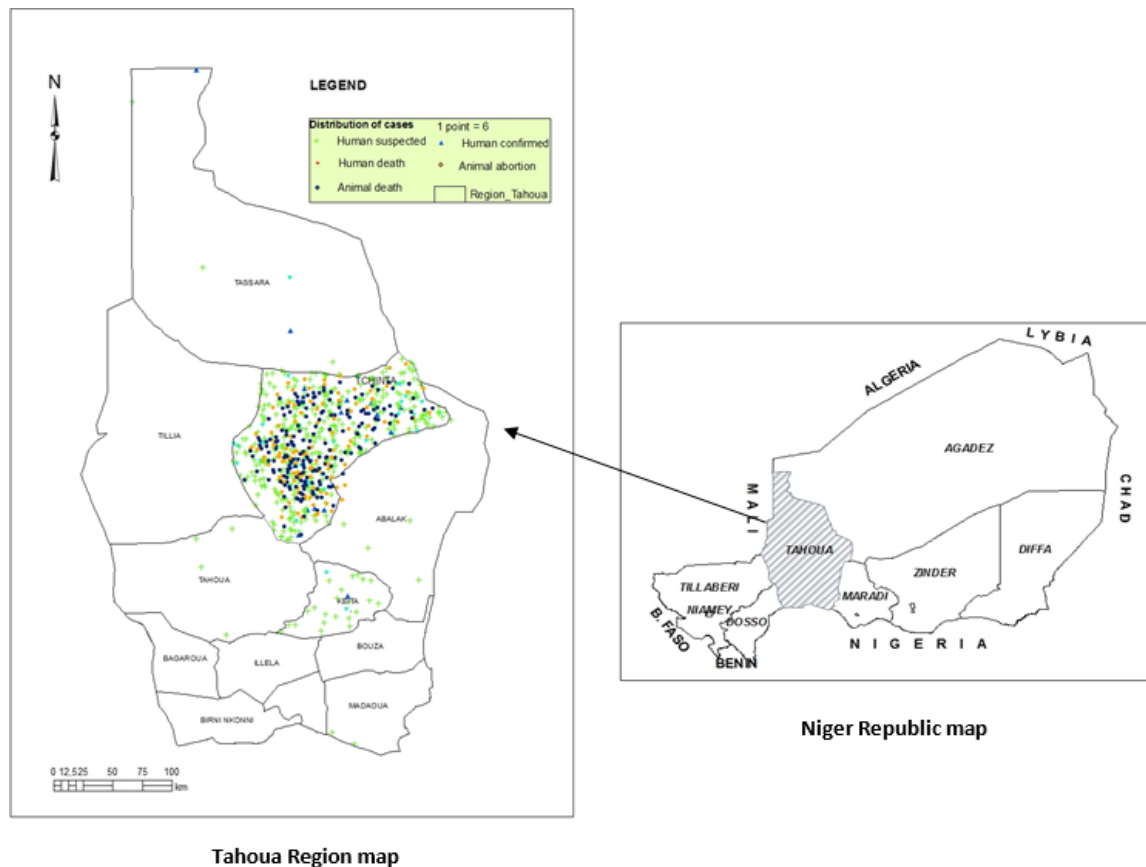
Niger Republic is a landlocked Sub-Saharan country with a land area of 1 270 000 km<sup>2</sup> lying between latitudes 11° and 24°N, and longitudes 0° and 16°E. The country is bordered by Nigeria and Benin to the south, Burkina Faso and Mali to the west, Algeria and Libya to the north and Chad to the east. Administratively, the country is composed of eight regions including the city Niamey (Fig. 1). Tahoua region is a nomadic area situated in the northern part bordering Mali Republic, an area where stockbreeders from Niger and surrounding countries gather with their animals annually (World Health Organization 2016).

In July 2016, reports from the Regional Directorate of Livestock and Agriculture of Tahoua described unusual death and abortion among domestic animals in the department of Tchintabaraden notably in the Maya Valley (Douchi *et al.* 2017). On 30 August, the Ministry of Health in Niger notified the World Health Organization (WHO) of unexplained deaths among humans, along with deaths in livestock in the Tahoua region of Niger (World Health Organization 2016). In September 2016, a joint field investigation mission composed of officers from the Ministry of Health, Ministry of Livestock and the WHO country office collected samples from both human and animal suspected cases in Tchintabaraden and Abalak (Investigation report). All human suspected cases presented icterohaemorrhagic symptoms with fever while sampled animals presented symptoms including fever, abortion and high salivation.

### Sampling and testing

The case definitions used for both RVF human suspected and confirmed cases were those reported in the Technical Guidelines for Integrated Disease Surveillance and Response (IDSR) in the African Region ([http://www.afro.who.int/sites/default/files/2017-06/IDSR-Technical\\_Guidelines\\_Final\\_2010\\_0.pdf](http://www.afro.who.int/sites/default/files/2017-06/IDSR-Technical_Guidelines_Final_2010_0.pdf)).

Blood samples were collected from all suspected human and animal cases in dry tubes and sent to



**Fig. 1** Distribution of RVF human cases and animal cases of abortion and death in the epidemic zone.

Institut Pasteur de Dakar (IPD), CERMES and LABOCEL for biological confirmation. Sera were obtained after centrifugation at 3000 rpm for 3 min and aliquots were made before testing.

For early outbreak confirmation, 13 human and 6 animal (1 camel, 3 goats, 1 sheep and 1 cow) serum samples from suspected cases collected during field investigation were sent to IPD, a WHO collaborating centre for arboviruses and haemorrhagic fever viruses. The samples collected were tested for RVF, dengue, yellow fever, West Nile, Crimean Congo haemorrhagic fever and Chikungunya viruses by RT-PCR and ELISA.

After the official declaration of the outbreak, the IPD team was deployed in Niamey for technology transfer of the molecular and serological diagnosis of arboviruses. Subsequently, CERMES and LABOCEL performed the laboratory diagnosis.

Between September and December 2016, a total of 399 samples from suspected human cases and 45 samples from suspected animal cases were collected for RVF diagnosis.

ELISA test for RVF (human IgM and IgG, and only IgG for animals) and other arboviruses (only human IgM) were performed using IPD in-house methods with antigens and immune ascites produced in mice.

For the RVFV RT-PCR, the primers (forward TGCCACGAGTYAGAGCCA, reverse TTGAA-CAGTGGGTCCGAGA and probe 6FAM-TCCTTCTCCCAgTCAgCCCCA C-BHQ1) were used (Weidmann *et al.* 2008). For other arboviruses, RT-PCRs were done as already described (Bob *et al.* 2017). The RNA was amplified using ABI Prism 7500 SDS Real-Time apparatus (Applied Biosystems) with the QuantiTect Probe kit (Qiagen).

RT-PCR positive samples for RVFV were used for sequencing of partial small (S) and medium (M) segments using specific primers (Bob *et al.* 2017). The PCR products of the expected sizes were purified directly from the agarose gel using a QIAGEN Gel extraction kit and sequenced by Cogenics (Beckman Coulter Genomics, Essex, United Kingdom) using the Sanger method.

An entomological field survey was conducted by the Malaria Programme and IPD teams in the outbreak area and samples of different mosquito populations were tested for RVFV by RT-PCR. Mosquito pools were homogenised as described (Fall *et al.* 2016) and the same RT-PCR method used for blood samples was used for the detection of RVFV.

A selection of 20% of the human serum samples has been sent to National Institute for Communicable Diseases (NICD), Johannesburg, South Africa for quality control of the molecular and serological diagnosis of RVF.

### Data analysis

Patient data including RT-PCR and ELISA results were analysed using Excel spreadsheet software.

The spatial distribution of cases was assessed using ARC GIS 10.3 software.

Sequences were analysed using online tools revseq and merger emboss (<http://www.bioinformatics.nl/cgi-bin/emboss/merger>; <http://emboss.bioinformatics.nl/cgi-bin/emboss/revseq>). Phylogenetic studies were conducted using maximum likelihood method and MEGA software (MEGA 6. 06-mac) (Tamura *et al.* 2013).

### Results

From August to December 2016, a total of 399 human samples were collected, of which 17 (4.3%) cases were confirmed positive for RVFV. RT-PCR positive cases accounted for nine (52.9%) while eight (47.1%) cases were confirmed by ELISA technique. All the differential tests for dengue, yellow fever, West Nile, Crimean Congo haemorrhagic fever, and Chikungunya viruses gave negative results. Overall, 33 (8.3%) deaths were reported among human cases.

Of these, five (29%) were laboratory confirmed during this epidemic episode. More than 90% of RVF cases (suspected, confirmed and deceased) were reported from the district of Tchintabaraden (Fig. 1).

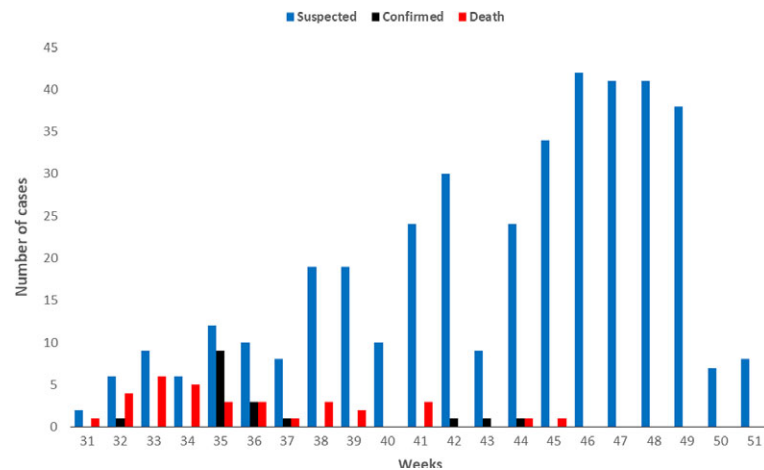
Overall, 15 (88%) of the confirmed cases and 31 (91%) of deaths occurred in those over 15 years of age. There was no significant difference between the number of males and females among the suspected cases ( $P \geq 0.05$ ). However, the sex ratio male/female for confirmed cases was 1.8 and the average age was 23 years (range: 3–70 years). Among the deceased cases, 29 (85%) were breeders and 21 (62%) were among males. A total of 285 (71%) suspected cases reported having fever while bleeding was reported among 223 (56%). Jaundice was reported in 15 (44%) of all deceased cases (Table 1).

The weekly reporting of RVF cases showed that most of the confirmed and deceased cases were recorded during the rainy season (week 31–39); however, notification of suspected cases continued until after the rainy season between week 41 and 51 with peak of notification between week 46 and 49 (Fig. 2).

Regarding animals, 363 cases of abortion and 370 cases of death were reported by the epidemic

**Table 1.** Distribution of RVF human cases by age, gender, symptoms and occupation

	Suspected N = 399 n (%)	Confirmed N = 17 n (%)	Death N = 33 n (%)
<b>Age (years)</b>			
<1	3 (1)	0 (0)	0 (0)
1–4	33 (8)	1 (6)	1 (3)
5–14	108 (27)	1 (6)	2 (6)
>15	255 (64)	15 (88)	31 (91)
<b>Sex</b>			
Male	194 (49)	6 (35)	21 (62)
Female	205 (51)	11 (65)	13 (38)
<b>Clinical symptoms</b>			
Fever	285 (71)	12 (71)	27 (79)
Headache	214 (54)	7 (41)	14 (41)
Bleeding	223 (56)	9 (53)	19 (56)
Jaundice	60 (15)	4 (24)	15 (44)
<b>Occupation</b>			
Farmer	7 (2)	1 (6)	0 (0)
Housewife	124 (31)	1 (6)	2 (6)
Breeder	136 (34)	13 (76)	29 (85)
Student	30 (8)	0 (0)	1 (1)
Others	102 (26)	2 (12)	2 (6)



**Fig. 2** Weekly notification of RVF human cases (suspected, confirmed and death).

surveillance network of the Ministry of livestock at the early stage of the outbreak. However, at the end of the outbreak more than 2000 deaths of domestic animals had been estimated including sheep, goats, camels and cattle. Among the six animal samples sent to IPD for confirmation, three (50%) tested positive for RVFV by RT-PCR (one cow, one sheep and one goat). In addition, out of 39 other animal samples tested by LABOCEL and IPD, none were RT-PCR positive while 24 were positive for IgG.

An entomological field survey of two sites (Tasnala and Egawane villages) in the affected area was conducted using three capture methods: night capture on human bait; spray catch capture and bright traps capture. In addition, inspection of larvae breeding grounds was also conducted. Also, 181 pools of mosquitoes, sandflies and Culicoides were captured. The mosquitoes were mainly *Anopheles* and *Culex* species with *Aedes* species found only rarely. Analysis by RT-PCR did not reveal any positive results for RVFV.

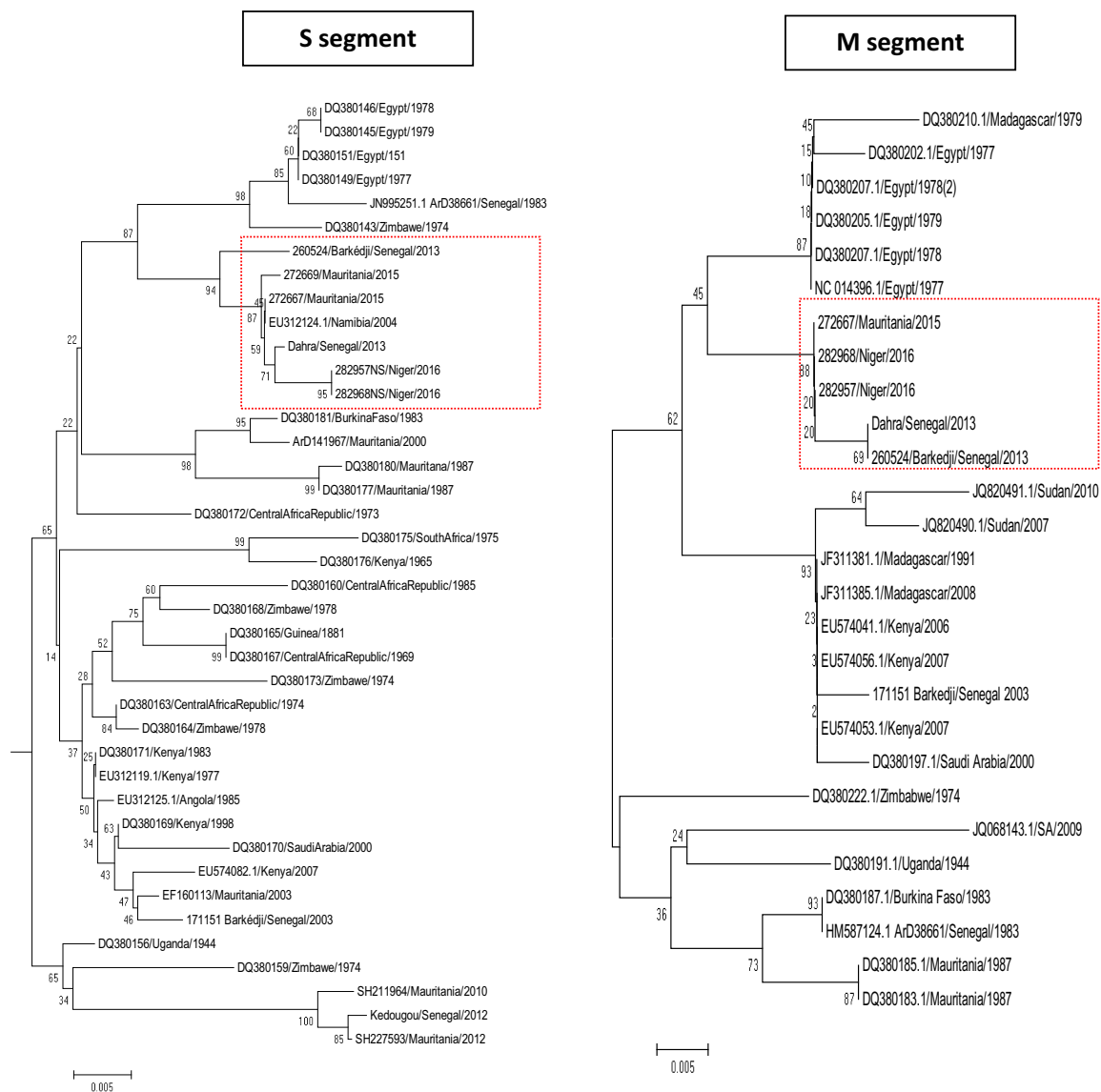
The phylogenetic analysis of the small and medium segments of the RVFV gene demonstrated the circulation of the same strains in both humans and animals. These strains clustered with RVFV strains which circulated in Senegal and Mauritania, respectively in 2013 and 2015 (Fig. 3).

A total of 64 (20%) of the 320 human samples tested by the end of November 2016 were sent to NICD for quality control. All the RT-PCR results

were in accordance with those obtained in CERMES. However, three additional cases found negative at CERMES were confirmed positive by IgM testing at NICD.

## Discussion

This epidemiological surveillance study was conducted during the first official RVF outbreak in Niger that occurred in the second half of 2016. This outbreak occurred in the northern part of the country where few studies since the late 1980s, have reported the presence of RVF antibodies in cattle (Akakpo *et al.* 1991; Mariner *et al.* 1995). These findings show that the Niger outbreak was likely related to the major RVF epidemics reported in West African countries particularly in Senegal and Mauritania during the last three decades (Jouan *et al.* 1988; Akakpo *et al.* 1989; Thiongane *et al.* 1991). Phylogenetic studies showed similarities between strains of RVFV from Niger 2016 and that of Senegal 2013 and Mauritania 2015. Interestingly, it has been shown that Mauritania 2015 strains belong to Northeastern African lineage found in Egypt (Bob *et al.* 2017). This suggests that the outbreak in Niger might be due to an introduction of RVFV from Senegal, Mauritania or Egypt. However, further molecular studies are needed to confirm the precise origin of Niger strains. Trans-border circulation of RVFV has been shown by many studies (Metras *et al.* 2016; Samy *et al.* 2017) and can lead to



**Fig. 3** Phylogenetic trees based on the partial sequencing of the small and medium genes of RVFV from Niger 2016.

the dispersal of the virus as a result of climate variability, animal human association and trade (Nanyingi *et al.* 2015; Sow *et al.* 2016). The occurrence of RVF in Niger has been related to two important annual events held in August. The first event was the islamic Eid-El Kebir feast which increased the importation of ruminant animals for ritual sacrifice, while the second was the annual breeders manifestation called « Cure Salée », an important regional gathering of more than 2 million domestic animals at Ingal district close to the

affected area (Doutchi *et al.* 2017; Food and Agriculture Organization 2017). The Eid-El Kebir hypothesis was supported by a study from Senegal to represent a major risk factor of RVF propagation between animals and humans as well (Sow *et al.* 2016).

Most of the confirmed and death cases were recorded during the rainy season between August and September. Hence, significant rainfall due to climate variability could be an important contributor to the occurrence of RVF infection, because it increases the

population of mosquitoes leading to transmission of the infection (Lancelot *et al.* 2017). The presence of RVFV in the mosquito population indicated their role as a possible source of transmission particularly among animals (Johnson *et al.* 1978; Hassan *et al.* 2011). However, because of the very low number of mosquitoes collected due to late initiation of the entomological field survey, this transmission route could not be well established. The increased number of RVF suspected cases (>30%) recorded after the rainy season between October and December, although without confirmed cases and deaths, was the result of the active surveillance conducted in the area.

The relatively few number of laboratory-confirmed human cases found in this study might be due to the delay in field investigation and the lack of early case confirmation. This low number of confirmed cases among all tested samples could also suggest co-circulation in the area of RVFV with other pathogens, other than the arboviruses tested during the RVF outbreak. Therefore, a larger panel of laboratory tests including other viruses, bacteria and parasites may be needed for differential diagnosis in Africa to better track outbreaks. A syndromic approach with multiplex tests should be used to reinforce laboratory diagnosis.

The difference recorded between IgM test results from NICD and CERMES was due to the high specificity and sensitivity of the method used at NICD, namely the serum bactericidal antibody (SBA) assay. All three IgM positive samples had cut-off values >40, and therefore could not have been detected by the normal ELISA test. In the field of arboviruses, existing diagnostic tools were not numerous. Therefore, the results obtained in this study highlighted the need of more comparative studies between in-house methods and commercial kits, and also development of new, more sensitive tools in order to improve arbovirus diagnosis in Africa.

Historically, the disease has had a case fatality rate in humans of less than 2% (Daubney & Garnham 1931; Johnson *et al.* 1978), but in recent outbreaks, higher mortality rates have been reported. This was in concordance with the fatality rate among confirmed cases we observed in this outbreak (Boushab *et al.* 2016; Sow *et al.* 2016).

The high fatality rate among breeders (85%) suggested that contaminations have occurred mainly through direct contact particularly with animal products like consumption of fresh milk (Boushab *et al.* 2016). However, other related contamination sources including abortion materials and cadaver handling could also favour this route of infection although the unavailability of data. These obvious facts were particularly objective as the infection did not spread beyond the affected districts.

However, there is need to assess the risk of future RVF outbreak in Niger and in neighbouring countries. An FAO study assessed the risk of RVF recurring in Niger and in neighbouring countries as low to medium for public health and high to medium for animal health (Food and Agriculture Organization 2017).

Our study has two important limitations that warrant discussion. First, the very limited data on animal surveillance hindered the ability to assess accurately the geographical source and distribution of the infection. Although sequencing analysis showed close similarities between strains of Niger and those of Senegal 2013 and Mauritania 2015, more remains to be done in order to determine the source of infection. Knowledge of the distribution of RVF cases among animals has been limited by the difficulty to continue case confirmation on animal samples. Indeed, only animal samples collected from the early disease investigation have been confirmed. Second, the late entomological survey did not allow for collection of sufficient information on vector distribution and disease transmission route. All the few mosquito samples collected were tested negative for RVFV by RT-PCR.

## Conclusion

These first epizootic and epidemic episodes of RVF disease have been a challenge for public and animal health in Niger. Besides human deaths, important loss of cattle has been reported. The rapid response and laboratory confirmation capacity through the support of partners allowed the outbreak evolution to be monitored. However, vigilance has to be strengthened using a 'one health approach', particularly in vector-

control strategies and active surveillance among animals. The route of transmission of RVFV needs also to be determined in order to accurately assess the risk of reemergence, particularly in areas where livestock breeding in households is common.

## Acknowledgements

We acknowledge the supports and expertise of World Health Organization and Institut Pasteur of Dakar but also the implication of the RVF National Committee for the control of this outbreak. Besides, we namely thank Idi Issa for mapping and Mariama Sambo, Aichatou Moussa, Bassira Alkassoum and Adama Moumouni for technical assistance. Also, we acknowledge the contribution of the entomology team from IPD and Malaria National Program.

## Source of funding

The authors declare no funding source for this study.

## Conflicts of interest

The authors have no conflicts of interest to declare.

## Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a surveillance study with no original research data.

## Contributions

AL, GF, AI, SO, BS, MA, AA, AEM, BI, FS, MZ, BB, HDM, ABD, GK, JT, HBM and OF have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; AL, GF, HDM, ABD, GK, JT, HBM and OF have been involved in drafting the manuscript or revising it critically for important intellectual content; AL, GF, AI, SO, BS, MA, AA, AEM, BI, FS, MZ, BB, HDM, ABD, GK, JT, HBM and OF have given final approval of the version to be published.

Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; AL, GF, AI, SO, BS, MA, AA, AEM, BI, FS, MZ, BB, HDM, ABD, GK, JT, HBM and OF have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig. S1.** Rift Valley fever virus structure.