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Short communication

Serosurvey for Crimean-Congo hemorrhagic fever virus infections in ruminants in Katanga province, Democratic Republic of the Congo

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ABSTRACT

Crimean-Congo hemorrhagic fever virus (CCHFV) has been detected in many African countries. Unfortunately, little is known about the current CCHFV situation in most of those countries including the Democratic Republic of the Congo (DRC). In over 50 years, three human CCHF cases have been detected in DRC but no seroepidemiological investigation was performed so far. To determine the prevalence of CCHFV-specific antibodies we tested 838 serum samples of cattle, goat and sheep from the southern province Katanga, DRC.

The detected seroprevalence in ruminants was 1.6% ranging from 0.4% to 3.4% between the two sampling sites, Kamina and Lubumbashi. The low prevalence indicates only sporadic introduction of CCHFV into this part of the country. DRC is a very large country and the study was performed only at two locations in one province; therefore, the investigations can be only a starting point for further epidemiological activities.

1. Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV) is a *Nairovirus* belonging to the family *Bunyaviridae*. CCHFV is predominantly transmitted by ticks of the genus *Hyalomma*. Other transmission pathways are unprotected contact with blood, other body fluids and tissues of viremic animals or human patients (Hoogstraal, 1979). In humans, CCHFV infections can cause a severe hemorrhagic disease (CCHF) with case fatality rates ranging from 5% in Turkey to 80% in China (Yilmaz et al., 2009; Yen et al., 1985). This variability probably depends on the circulating virus strain, awareness of the population and effectiveness of the public health system (Mertens et al., 2013). Unlike humans, infected animals do not show clinical signs but a one to two weeks' viremia and a seroconversion can be detected (Spengler et al., 2016b, Gonzalez et al., 1998). The detection of antibody positive livestock correlates with the occurrence of human cases and hence can be used to identify CCHFV risk areas (Bente et al., 2013). Ruminants, especially cattle, are used for seroprevalence studies and CCHFV risk assessment worldwide (Spengler et al., 2016b).

CCHFV has been reported in around 50 countries of Africa, Europe and Asia (Hoogstraal, 1979; Whitehouse, 2004; Bente et al., 2013). The first detection in Africa was in a 13-year-old boy from Kisangani with a poorly described clinical history in the Democratic Republic of the Congo (DRC; formerly Belgian Congo) in 1956; followed directly by a laboratory infection caused by the isolated strain of this boy (Simpson et al., 1967; Woodall et al., 1967). The next human CCHF case in DRC was a 26-year-old man from Beruwe in 2008 (Grard et al., 2011). CCHFV and CCHFV-specific antibodies have also been detected in the neighboring countries Uganda, the Central African Republic and Tanzania (Hoogstraal, 1979).

Even though DRC was partly eponymous, few human cases have been reported decades ago and CCHFV was detected in neighboring countries, there have been no seroepidemiological studies carried out in DRC to date using ruminants as indicator species for CCHFV infections in the environment.

2. Material and methods

2.1. Serum samples

Serum samples were collected under the direction of the University of Lubumbashi and the Laboratoire Vétérinaire de Lubumbashi from

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Table 1
Results of the seroepidemiological study in ruminants in DRC

Location	Species	Age (years)	Farm type	n	Positives	Prev. in% (species)	Prev. in% (location)
Kamina (farm)	Cattle	2–4	commercial	514	2	0.4 (0–1.4)	0.4 (0–1.4)
Demers farm (LA)	Goat	0.5–2	small personal	25	1	4.0 (0.1–20.4)	3.4 (0.1–17.8)
	Sheep			4	0	0 (0–60.2)	
Kamalondo market (L)	Goat	1–3	small personal	62	3	4.8 (1.0–13.5)	4.8 (1.0–13.5)
Kamwanya farm (LA)	Goat	0.5–2	commercial	97	7	7.2 (3.0–14.3)	7.1 (2.9–14.2)
	Sheep			1	0	0 (0–97.5)	
Kafubu river farm (LA)	Goat	0.5–2	commercial	2	0	0 (0–84.2)	0 (0–2.7)
	Sheep			133	0	0 (0–2.7)	
Total				**838**	**13**		**1.6 (0.8–2.6)**

LA: Lubumbashi area, L: Lubumbashi city, n: number of samples; Prev.: prevalence; the confidence interval (95%) is shown in brackets

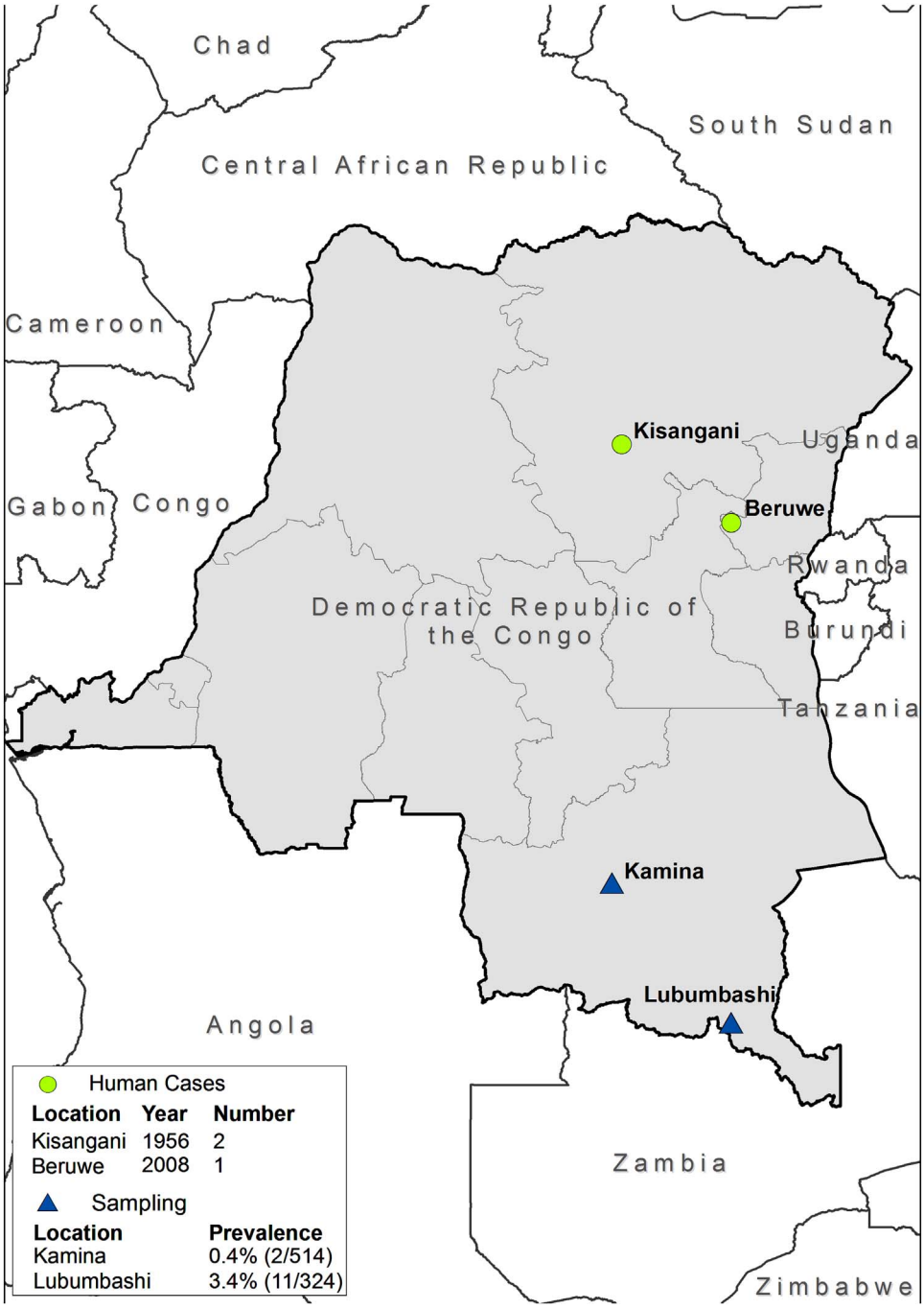

Fig. 1. Map of DRC with human cases and sampling sites.
All sera from Kamina were collected at one farm. The sera from Lubumbashi were collected at different sampling sites in Lubumbashi area (Kafubu road, Kamalondo, Kasumbalesa road and Kasenga road). This Map was designed at FLI using ArcGIS 10.3.1.

July to September 2013 (dry season). All cattle sera (n = 514) originated from a farm in Kamina, Katanga province. The goat (n = 186) and the sheep (n = 138) sera were collected at different farms and a market in and around Lubumbashi. The animals sampled were six months to four years old and originated from commercial and small personal farms. Most of the animals were bred on the farm. However, also animals from Zambia and other Congolese areas were sampled on the Kamalando market (n = 62). All samples were gamma-irradiated before serological investigation at FLI, Germany.

2.2. Serological analysis

All sera were analyzed by a species specific in-house CCHFV-IgG-ELISA and an adapted species specific commercial CCHFV-IgG-ELISA (Vector Best, Novosibirsk, Russia). In case of divergent results, samples were run in a species adapted commercial CCHFV-IgG-IFA (Euroimmun, Lübeck, Germany) to get to a final result.

The serological assays for cattle (Mertens et al., 2015) and for goat and sheep (Schuster et al., 2016) were previously described. The cattle specific IFA was adapted for use in irradiated sera (Sas et al., In preparation).

3. Results

19 cattle, 11 goats and 18 sheep showed divergent results in the in-house and the commercial ELISAs. The commercial IFA clarified the reactivity of 37 of all 38 inconclusive samples. Only one sample remained inconclusive. Two of the 514 bovines tested CCHFV antibody positive (0.4% prevalence; 95% CI: 0%–1.4%), whereas in eleven of 186 tested goats (5.9% prevalence; 95% CI: 3.0%–10.3%) CCHFV-specific antibodies were detected. No sheep was positive for CCHFV-specific antibodies at any sampling site. The overall prevalence for small ruminants was 3.4% (95% CI: 1.7%–6.0%) in Lubumbashi city and in the Lubumbashi area (Table 1 and Fig. 1).

4. Discussion and conclusion

DRC is eponymous in part for CCHFV as the first African cases were detected in Kisangani, Orientale province in 1956. However, the diagnosis of just three human cases in over 50 years raises the question, whether CCHF cases are just underreported or indeed occur only quite rarely.

Kisangani is located in the equatorial rain forest area as is Beruwe, North Kivu, where the CCHF case occurred in 2008 (Fig. 1). The tropical rain forest is not the preferred habitat for *Hyalomma* ticks which are considered to function as the main vector for CCHFV. *Hyalomma* ticks prefer rather grass and tree savanna and semiarid desert habitats and CCHF cases are usually linked to this type of climate and vegetation (Estrada-Pena et al., 2007). The most broadly distributed *Hyalomma* species on the African continent are *Hyalomma marginatum rufipes* and *Hyalomma truncatum* (Walker et al., 2003); both species are also found in DRC (Walker et al., 2003). Therefore, the tree and grassland savanna regions of the Katanga province were chosen for this CCHF seroprevalence study in ruminants, in order to increase the chances for finding antibodies to CCHFV. Surprisingly, only low prevalence rates were found in cattle (0.4%), goat (5.9%) and sheep (0%) in the Katanga province. This probably shows that CCHFV is circulating albeit at a very low level so that the human CCHFV exposure risk might be fairly low. However, risk groups, i.e. health care personnel, slaughterhouse workers, veterinarians and farmers should still not neglect this diagnosis and risk. No difference was evident for animals bred on a farm in Katanga province and for animals imported from Zambia and from other Congolese areas. However, the number of samples (n = 62) collected at Kamalando market was too low to be representative, hence this observation should not be over interpreted. Likewise was it not possible to determine age specific effects as data for individual animals were lacking.

Even though CCHFV is most frequently connected to *Hyalomma* spp. in most endemic areas, it was also detected in neighboring countries of DRC in *Amblyomma* and *Rhipicephalus* ticks in the past (Hoogstraal, 1979). Hypothetically, higher CCHFV prevalences for the northern DRC provinces could apply, if these tick species, which are found particularly frequently in Northern provinces, would locally function as the primary vectors. However, such a particular local vector preference for CCHFV is rather unlikely. Since only animals from two different locations in one province were tested, it would be speculative to believe that the results reflect the situation for the whole country. A seroepidemiological study in cattle in neighboring Tanzania revealed similarly low (0.6%–7.4%) prevalence rates (Hoogstraal, 1979), while a much higher seroprevalence (36.5%) was detected in cattle from Uganda (Spengler et al., 2016a). However, those studies were performed more than 40 years ago by the less sensitive agar gel precipitation test.

To corroborate our results now, vector studies should be carried out by collecting sucking ticks from mammals and assay their CCHFV infection status.

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