Detection of Crimean-Congo hemorrhagic fever virus (CCHFV) in Hyalomma ticks collected from Mauritanian livestock

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Research

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Abstract

Background: Crimean-Congo hemorrhagic fever virus (CCHFV) belongs to the *Nairovididae* family in the *Orthonairovirus* genus and is an emerging tick-borne virus. It is endemic in most parts of Africa, Asia, as well as southern Europe, and can cause severe hemorrhagic symptoms in humans with high fatality rates (5-30 %).

Methods: *Hyalomma* ticks were collected from four different livestock herds (cattle and camel) from Mauritania in 2018. The tick species was determined morphologically and confirmed on a molecular level by using cytochrome oxidase 1 gene marker (CO1). For the detection of CCHFV, ticks were tested individually with a one-step multiplex real-time RT-qPCR. Subsequently, the S-segment of all positive samples were sequenced to determine the CCHFV genotype.

Results: Overall, 39 of 1,523 ticks (2.56 %) collected from 63 cattle and 28 camels were tested positive for CCHFV. Three *Hyalomma* (*H.*) species were identified. The highest prevalence of CCHFV was found in *Hyalomma rufipes* (5.67 %; 16/282), followed by *H. dromedarii* (1.89 %; 23/1,214) and *H. impeltatum* (0 %; 0/21). Positive ticks were found on only 6 out of 91 host animals. Sequence analysis of the positive samples revealed the presence of two different CCHFV lineages (Africa I and Africa III).

Conclusions: This study reveals a CCHFV prevalence of 2.56 % in *Hyalomma* ticks collected from camels and cattle in Mauritania. The true prevalence of unfed ticks may however be lower since a considerable number of ticks may have been passively infected during the ingestion of the blood meal by co-feeding or viremia of the host. The study shows that tick control measures should be implemented, especially in the examined areas.

Background

Crimean-Congo hemorrhagic fever virus (CCHFV) belongs to the *Nairovididae* family in the *Orthonairovirus* genus and is an emerging zoonotic arthropod-borne virus that causes the Crimean-Congo hemorrhagic fever in humans (CCHF). CCHFV is present in most parts of Africa, southern Asia as well as southern Europe [1, 2]. The virus is characterized by a high genetic diversity [3]. Hard ticks of the genus *Hyalomma* are considered as main vectors and reservoirs of the virus [4]. Most, if not all of the various feeding hosts of *Hyalomma* (ranging from wildlife species to domesticated animals) can be infected, although they seem to develop only a short-term viremia without clinical symptoms [5]. In contrast, CCHFV infections in humans can lead to severe hemorrhagic symptoms with a high lethality rate of up to 30 % [4]. Seroepidemiological studies of different susceptible livestock and wildlife species can provide a first indication whether CCHFV is circulating in a certain region. Hence it became a widely used epidemiological instrument to define potential endemic risk areas [5, 6]. However, serological surveys cannot provide information on the true virus prevalence, CCHFV strain genetics and host-vector dynamics. Such data can only be obtained by complex experimental studies of tick-host transmission, which have to be conducted according to stringent guidelines as described by Gargili et al. [7]. To

circumvent these highly elaborate experiments, ticks from less complicated field studies are often screened for the presence of the virus, although these findings must be interpreted cautiously. The detection of CCHFV in engorged ticks is only an evidence for the virus presence, but it does not imply the vector competence of a tick, since passive contamination from blood of the host cannot be excluded [7]. According to World Health Organization (WHO) data, West African countries, including Mauritania, are considered highly endemic with a large annual incidence of human CCHF cases. Traditional husbandry is common in Mauritania, meaning close contact between farmers and their livestock. The first evidence of a human CCHFV infection in Mauritania was reported in 1983 [8]. Moreover, several serological studies on livestock revealed a high seroprevalence of 15 % in small ruminants [9, 10] and up to 67 % in cattle [11], underlining the high endemic status of the country. Despite those high seroprevalences, CCHFV prevalences in ticks have not been systematically studied in Mauritania. The first comprehensive survey was conducted in 1985 and included 2,539 ticks collected from cattle, sheep, goats, camels and horses [12]. The samples were pooled and analyzed using complement-fixation test, resulting in 12/172 (6-9 %) CCHFV-positive tick pools. Of the four analyzed tick species (*Hyalomma rufipes*, *Hyalomma marginatum*, *Hyalomma impeltatum* and *Hyalomma dromedarii*), only *H. rufipes* pools were positive. In a second study, 378 engorged and non-engorged ticks of two different genera (*Hyalomma* and *Rhipicephalus*) were tested for the presence of CCHFV. Only in four *Rhipicephalus evertsi evertsi* collected from sheep CCHFV was detected, whereas all of the *Hyalomma* ticks showed negative results [10].

The aim of this study was to obtain recent data on the circulation of CCHFV in livestock and *Hyalomma* ticks in Mauritania. Special emphasis was laid on an accurate tick species identification [7, 13] and on recording sample histories to improve our understanding of the host-vector dynamics in the sampled herds. Moreover, CCHFV genotypes were determined. The results contribute to a CCHFV exposure risk analysis for local farmers, butchers and other exposed groups in close contact with livestock.

Materials And Methods

1.1. Collection sites

Mauritania is a large country in West Africa with mostly desert-covered landscape of the Sahara, and a low population density. Samples were collected in the surrounding region of the capital Nouakchott and the town of Rosso. Camels were sampled at a livestock market with an associated slaughterhouse on the outskirts of Nouakchott. In addition, one cattle herd was sampled at a dairy farm 60 km east of Nouakchott near the small village Idini (Trarza). Two other sampling sites for cattle and camels, respectively, were located in the surrounding area of Rosso (Trarza) in southwestern Mauritania. The distance between Nouakchott and Rosso is about 160 km (Fig.).

1.2. Collection of samples

Up to 30 *Hyalomma* ticks were collected per animal from a total of 28 camels and 63 cattle from the four aforementioned herds. Moreover, blood samples were taken from most of these animals (Idini, cattle: n= 49; Nouakchott slaughterhouse, camels: n= 13; Rosso, camels: n=15). No blood samples were available

from the cattle herd in Rosso. In total, 77 blood samples were collected among the different herds. Since *Hyalomma* ticks are considered the main vector and reservoir for CCHFV, ticks from other genera were excluded from this study. The collected ticks were stored at -80°C at the Office National de Recherche et de Développement de l'Elevage (ONARDEL) in Nouakchott. Ethanol (90 %) was added to the samples prior to their shipment to the Friedrich-Loeffler-Institut (FLI), Germany.

1.3. Morphological and molecular tick species identification

All ticks were morphologically identified using the identification keys of Apanaskevich et al. [14-16]. Individual ticks were homogenized in AVL buffer (Qiagen, Hilden, Germany) using a Tissuelyser II (Qiagen, Hilden, Germany) machine. The homogenates were cleared by centrifugation and supernatants were used for nucleic acid extraction. DNA/RNA was extracted using a a KingFisher Flex instrument (ThermoFisher, Waltham, USA) with the NucleoMag® VET kit (Macherey-Nagel, Düren, Germany) according to the manufacturer`s protocol. A selected number of ticks that were hard to determine as well as CCHFV-positive specimens were identified using partial cytochrome oxidase 1 (*CO1*) gene Sanger sequencing and restriction fragment length polymorphism method (RFLP) [17].

1.4. CCHFV genome detection

RNA extracted using KingFisher instrument (ThermoFisher Scientifc, Waltham, USA) alongside the NucleoMag Vet kit (Machery-Nagel, Düren, Germany) from individual ticks and serum samples was used to screen for CCHFV. The screening was performed using a one-step real-time reverse-transcriptase PCR assay (RT-qPCR) as described previously [18]. The assay targets a conserved region within the S-Segment. Samples were considered positive in case of ct-values below 35 and weak-positive if between 35 and 40.

1.5. Molecular and phylogenetic analyses of CCHFV genotypes

In order to get a first insight into the detected CCHFV genotypes, amplicons (127 bp) of the RT-qPCR products of all positively tested samples were sequenced by Sanger sequencing (Eurofins, Luxembourg, Luxembourg) and aligned with GeneBank entries by using the BLAST tool (NCBI, Bethesda, USA). For this purpose, the PCR protocol was performed using only one primer pair specific for the African CCHFV lineage III. Due to the small length of the PCR product, it was necessary to amplify a larger segment from the S-segment, which allows a more meaningful phylogenetic analysis. Therefore, complimentary primers close to the terminal regions were selected based on the most related sequence in the BLAST results. The primers to amplify African linage 1 were: “for 5’- AACACGTGCCGCTTACGC” and “rev 5’ – TATCGTTGCCGCACAGCC”; and for African linage 3, “for 5’ – ATGGAAAACAAAATCGAGGTGAATAACAAAGAT” and “rev 5’ – TTAGATAATGTTAGCACTGGTGGCATT”. Both the reverse transcription using SuperScript IV Reverse Transcriptase (ThermoFisher, Waltham, USA) with the reverse primer as well as the PCR using the KAPA HiFi HotStart ReadyMix PCR Kit (Roche, Basel, Switzerland) were performed according to the manufacturer's instructions. The amplified fragment was

sequenced by Sanger sequencing (Eurofins, Luxembourg, Luxembourg) and used to create a phylogenetic tree.

1.6. Statistical analysis

Statistical analyses included 95 % confidence intervals (CI), Fisher's exact test and Chi-square test. Therefore, R software and R-Studio (an integrated development interface for R) were used for the calculation [19]. Those ticks that could not be species identified were excluded for the Fisher's exact test and Chi-square test.

Results

1.1. Tick species and their distribution on hosts

Overall, 1,523 blood-fed *Hyalomma* ticks were collected from 63 cattle and 28 camels. Morphological identification revealed the presence of three different tick species (*H. dromedarii*, *H. rufipes* and *H. impeltatum*) in the surveyed areas. Due to the large morphological diversity of *Hyalomma* ticks and the similarity of *H. dromedarii* and *H. impeltatum*, 219 specimens were identified genetically by the RFLP approach. Furthermore, the *CO1* gene amplicon used for restriction digest was sequenced from 47 ticks to unambiguously identify their species. Due to the very poor condition of six ticks, neither a morphological nor a molecular identification of these specimens was possible. Therefore, those ticks were only determined up to genus level. Results of species identification and distribution among the different collection sites and hosts are summarized in Table 1. *H. dromedarii* represented the by far largest population both in cattle and camels (79.58 %), followed by *H. rufipes* (18.52 %) and *H. impeltatum* (1.51 %). Despite the predominance of *H. dromedarii*, a great variation was observed regarding the regional distribution of tick species in their hosts. In both camel herds, the percentage of *H. dromedarii* was very high (97.01 – 98.58 %), while ticks of the other species were found only sporadically. In contrast, the distribution of tick species among the two cattle herds was considerably more heterogeneous. A large number of *H. dromedarii* individuals (88.76 %) were identified on the dairy farm in Idini, although *H. rufipes* (6.94 %) and *H. impeltatum* (3.31 %) were also recorded. On the other hand, 85.34 % of the collected ticks from the cattle herd in Rosso were identified as *H. rufipes* and only 14.66 % as *H. dromedarii*. The distribution of sex showed nearly a 70:30 ratio of males to females across all four collection sites. In the cattle herd from Rosso, the proportion of male ticks was slightly higher compared to the other sampling sites.

1.2. CCHFV prevalence in ticks and sera

Of the 1,523 individual *Hyalomma* ticks analyzed, 39 tested positive for CCHFV by RT-qPCR and 23 were considered weak-positive. A comprehensive summary is given in Table 1. The CCHFV prevalence between species and sampling sites showed significant differences. Overall, 23 out of 1,214 *H. dromedarii* (1.89 %) and 16 out of 282 *H. rufipes* (5.67 %) were found to be positive for CCHFV, respectively. All 21 examined *H. impeltatum* ticks were negative. Only three *H. dromedarii* collected from camels at the

Tenweich slaughterhouse tested positive (0.87 %). In contrast, all ticks from the camel herd nearby Rosso (Trarza) tested negative, whereas 7 out of 266 ticks (all *H. rufipes*) originating from cattle of the same region were positive (2.63 %). The highest prevalence (4.79 %) was found in the cattle herd from Idini (Trarza), where 29 positive ticks were identified out of 605 collected ticks. Overall, 20 of 537 *H. dromedarii* (3.72 %) and 9 of 42 *H. rufipes* (21.43 %) were CCHFV-positive, while all *H. impeltatum* ticks (n = 26) were negative. In total, 2.28 % of the female and 2.67 % male sampled ticks were CCHFV-positive. No significant sex differences were observed in terms of CCHFV prevalence. All 77 collected serum samples of camels and cattle were negative for CCHFV.

1.3. Distribution of CCHFV-positive ticks per sampled livestock

The distribution of all CCHFV-positive *Hyalomma* spp. on cattle and camels is summarized in Table 2. CCHFV-positive ticks were collected on 6 out of 91 (6.59 %) sampled animals (five cattle: “no. 1 to no. 5“ and one camel: “no. 1”). In one case (“cattle no. 1”), all 22 collected ticks were CCHFV-positive. In four cattle, negatively tested *Hyalomma* spp. were associated with a variable number of positively and weak-positively tested ticks. In each of these four cattle, one of the collected ticks was highly-positive with much higher levels of S-segment RNA (lower ct-value) compared to the other ticks collected from the same cattle. In three of the four cases, these highly positive ticks were identified as *H. rufipes,* and in one case identified to be *H. dromedarii*. In "cattle no. 5“, only a single *H. rufipes* was CCHFV-positive, while the remaining ticks were negative. There was only one camel ("camel no. 1") found with CCHFV-positive ticks (n = 3) which were identified as *H. dromedarii*. Viral RNA concentrations of the S-segment were almost identical in all three samples, and no “weak-positive” ticks were found.

1.4. Phylogeny of CCHFV genotypes

To determine the CCHFV genotypes, the 127 bp long RT-qPCR amplicons were sequenced. Consistent sequence data were obtained for 28 of the 39 CCHFV-positive ticks and compared by using the BLAST tool (NCBI). Essentially, two different CCHFV genotypes – Africa I and Africa III – were detected (Tables 2 and 3). Ticks originating from the same animal always carried the same CCHFV genotype and haplotype. However, two different lineages (Africa I and III) circulated simultaneously in tick vectors of cattle from the Idini region (Table 2). The CCHFV lineages also differed between the collection sites and/or host species (Table 3). The Africa I lineage detected in ticks from cattle (Idini) and camel (Nouakchott slaughterhouse) showed nucleotide differences of 3.15 %. The Africa III genotypes found in ticks feeding on cattle from Idini and from Rosso varied by 2.17 %. The overall genetic distance between the Africa I and Africa III lineages ranged from 10.04 % between the cattle herds from Idini (I) and Rosso (III) up to a maximum of 13.19 % between camels from Nouakchott slaughterhouse (I) and cattle from Rossi/Idini (III). To confirm that the short amplicon (127 bp) was representative for the whole segment, the complete coding region of the S-segment from two CCHFV-positive ticks was RT-PCR amplified and sequenced. Alignments with GenBank database sequences showed a nucleotide homology of 97.5 % and 99.4 % for the consensus sequences of Africa I and Africa III strains, respectively. Construction of a phylogenetic tree

(Fig. 2) showed that both sequences clustered well with the reference strains of Africa I (Senegal) and III (Mauritania/Mali).

Discussion

Previous serological studies have shown a high CCHFV antibody seroprevalence in the livestock population of Mauritania. Several severe CCHF cases have been reported in humans. Hence, Mauritania is considered as a high-endemic country for CCHFV [8–11, 20]. CCHF cases were also reported in Senegal which borders Mauritana to the south [21, 22]. In Mali, eastwards of Mauritania, no human cases of CCHF have been reported, but serological data [23] and virus detection in ticks [24] have also proven a CCHFV circulation in Mali. However, CCHFV monitoring in ticks (especially of the genus *Hyalomma*) has not yet been conducted systematically in this West African region. Existing datasets are either small-scaled, outdated or generated by the analysis of tick pools with focus on virus detection thus only allowing limited conclusions on vector species distribution and competence [10, 12, 24, 25]. Therefore, this study was carried out to provide a better understanding of host-vector dynamics and the current epidemiological situation in Mauritanian livestock herds.

The presence of the three tick species *H. rufipes*, *H. dromedarii*, and *H. impeltatum* in Mauritania is consistent with previous reports from the region [14–16]. The primary host of *H. dromedarii* are camels [16], which explains the high proportion of specimens (97.01–98.58%) found on camels in Rosso and Nouakchott slaughterhouse (Table 1). Due to the important role camels play as agricultural livestock for milk and meat production in Mauritania, there is a relatively high camel density in the country [26]. Moreover, cattle and other ungulates can also be infested by adult stages of *H. dromedarii* [16], especially if camels and cattle are held in close contact. Thus, the 88.71% of *H. dromedarii* ticks found on cattle from Idini were not an exceptional finding. The higher rate of male ticks across all four sampling sites is probably caused by the absence of female ticks from the host during oviposition, which is taking place in the environment and not on the host itself [27].

In total, 39/1,523 (2.56%) of the blood-fed ticks collected from cattle and camels were CCHFV-positive (Table 1). The highest prevalence was found in both cattle herds from Idini (4.79%) and Rosso (2.63%), followed by the camels from the Nouakchott slaughterhouse (0.85%). No CCHFV-positive ticks were found on camels from Rosso. The reasons for different CCHFV prevalences across collection sites and host species are various and require careful interpretation. One explanation could be a difference in susceptibility of cattle and camels for the virus and/or a better capability of cattle to support a longer lasting viremia. So far, only a small number of experimental CCHFV infection studies have been conducted with different livestock species (cattle, sheep and horses), which showed that all species develop a short-term viremia of similar durations [6]. However, no experimental data of CCHFV infections of camelids are available to date to prove this assumption. There remains a need for further infection experiments comparing the susceptibility for CCHFV in different host species.

The high CCHFV prevalence of ticks from cattle in Idini may be related to the geographically isolated location of the sampled farm. It is assumed that fragmented CCHFV foci consisting of susceptible hosts and competent vector ticks may induce stable virus amplification, leading to a high prevalence in these isolated geographical clusters [28]. This dairy farm is located far away from the next village Idini in a desert-like region. The remote location results in limited contact with new naive hosts (wildlife, livestock), which might negatively affect the CCHFV prevalence [29]. In contrast, the fertile lands around Rosso lead to a higher density of livestock as well as human population and thus to an increased movement and interaction between the animals. A similar situation exists at the livestock market in Nouakchott, where a large number of cattle, sheep, goats and camels from various regions of Mauritania are sold or slaughtered every day (Fig. 3).

The tick species themselves may also have an impact on the CCHFV prevalence. Ticks of the genus *Hyalomma* are considered as main vector and reservoir of CCHFV [7], but it is still unknown whether all of the currently recognized 27 *Hyalomma* spp. [30] can function efficiently as virus reservoir and/or vector. Despite the considerably higher occurrence of *H. dromedarii* (79.71%) ticks in the study region (Table 1), significantly more *H. rufipes* (5.67%) than *H. dromedarii* (1.89%) ticks were CCHFV-positive. This difference was most obvious among the cattle herd from Idini, where 21.43% of *H. rufipes* and only 3.72% of *H. dromedarii* were CCHFV-positive. Nevertheless, since our data were derived from blood-fed ticks, speculations on vector competence have to be interpreted cautiously. Furthermore, feeding on viremic hosts and/or co-feeding transmission [31–34] may also have contributed to the concentrated occurrence of some of the 39 positively tested ticks collected from 6 (of 91) animals (Table 2). Interestingly, one of the CCHFV-positive ticks collected from four bovines in Idini and Rosso (no. 1- no. 4) each had much higher levels of S-segment RNA than the co-infesting ticks of the same animal. It is also noteworthy that three of four highly positive ticks in Idini (total occurrence: 88.76% *H. dromedarii* vs. 6.94% *H. rufipes*) were identified as *H. rufipes* (Table 2), which is suggestive for a better vector competence of *H. rufipes* for CCHFV. The genomic data proving a 100% sequence identity of CCHFV for all infected ticks from a given bovine host supports this assumption (Tables 2 and 3). Therefore, the true CCHFV prevalence in the tick population and thus the absolute risk of exposure for local farmers may actually be considerably lower than the measured 2.56%. Nevertheless, it is recommended to enforce tick control strategies and encourage public awareness of tick bite prevention in the examined areas.

In addition, at least two different CCHFV genotypes (Africa I and III) were found in ticks in Mauritania, either alone or even circulating side by side simultaneously as observed in one cattle herd from Idini (Tables 2 and 3). Significant genetic variability also occurred within the genotypes. The underlying mechanisms of the high genetic diversity are still not fully understood and require further research on the driving factors.

Conclusion

This study reveals a high CCHFV prevalence (2.56%) in *Hyalomma* ticks collected from camels and cattle in Mauritania. *H. rufipes* showed significantly higher CCHFV infection rates compared to *H. dromedarii*

and *H. impeltatum*. However, it must be considered the data was obtained from engorged ticks thus skewing statements on true vector competence. Two different CCHFV genotypes (Africa I and III) were found in the ticks. The absolute risk of exposure for local farmers is probably lower than this determined prevalence would suggest, because a considerable number of ticks may have been passively infected during ingestion of the blood meal by co-feeding with infected ticks or feeding on a viremic host. It is recommended to enforce tick control strategies and encourage public awareness of tick bite prevention in these areas.

Declarations

Ethics approval and consent to participate

The sample collection was carried out by the Mauritanian State Veterinary Laboratory “Office National de Recherches et de Développement de l'Elevage“ (ONARDEL) following all relevant national as well as international regulations and according to fundamental ethical principles for diagnostic purposes in the framework of a governmental program for the animal health surveillance.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

There were no competing interests among the authors.

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Authors contribution

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35. Legends.

Tables

Table 1. CCHFV prevalence rates in ticks at the different sampling sites

Overview showing results of all four sampled herds, including the collected number of different tick species, CCHFV-positive ticks and the analysis of the correlation between the three identified species and CCHFV status by chi-square test ($p < 0.05$).

Location, Number of sampled animals	Tick species	Tick number collected	%	Number of CCHFV positive ticks	Prev./total	Prev./tick species
Idini 43 cattle	*Hyalomma dromedarii*	537	88.76 %	20	3.31% (2.03-5.06 %)	3.72% (2.29-5.69 %)
	Hyalomma impeltatum	20	3.31 %	0	0% (0-0.06 %)	0% (0-16.84 %)
	Hyalomma rufipes	42	6.94 %	9	1.49% (0.06-2.8 %)	21.43% (10.3-36.81%)
	Hyalomma spp.	6	0.99 %	0	0% (0-0.06 %)	0% (0-16.84 %)
	Total	**605**		**29**	**4.79 % (3.23-6.81 %)**	**p-value: <0.001**
Nouakchott slaughterhouse 14 camels	*Hyalomma dromedarii*	346	98.58 %	3	0.85% (0.78-2.48 %)	0.87% (0.18-2.51 %)
	Hyalomma rufipes	5	1.42 %	0	0 % (0-1.05 %)	0% (0-52.18 %)
	Total	**351**		**3**	**0.85 % (0.78-2.48 %)**	**p-value: N.A***
Rosso 20 cattle	*Hyalomma dromedarii*	39	14.66 %	0	0% (0-1.38 %)	0% (0-9.03 %)
	Hyalomma rufipes	227	85.34 %	7	2.63% (1.06-5.35 %)	3.08% (1.25-6.25 %)
	Total	**266**		**7**	**2.63 % (1.06-5.35 %)**	**p-value: N.A***
Rosso 14 camels	*Hyalomma dromedarii*	292	97.01 %	0	0% (0-1.21 %)	0% (0-1.26 %)
	Hyalomma impeltatum	1	0.33 %	0	0% (0-1.21 %)	0% (0-95.7 %)
	Hyalomma rufipes	8	2.66 %	0	0% (0-1.21 %)	0% (0-36.94 %)
	Total	**301**		**0**	**0 % (0-1.21 %)**	**p-value: 0.531**
Total 91 cattle and camels	*Hyalomma dromedarii*	1,214	79.71 %	23	1.51% (1.2-2.83 %)	1.89% (0.96-2.26 %)
	Hyalomma impeltatum	21	1.38 %	0	0% (0-0.24 %)	0% (0-16.11 %)
	Hyalomma rufipes	282	18.52 %	16	1.05% (0.6-1.7 %)	5.67% (3.28-9.05 %)
	Hyalomma spp.	6	0.39 %	0	0% (0-0.24 %)	0% (0-45.93 %)
	Total	**1,523**		**39**	**2.56 % (1.83-3.48 %)**	**p-value: <0.001**

Prev. = CCHFV prevalence; *Hyalomma* spp. = not identified species; 95 % confidence interval (CI %) in brackets; *= no p-value available, because of the absence of *H. impeltatum* at this sampling site

Table 2. Distribution of positive ticks over the sampled animals

All sampled animals on which CCHFV-positive ticks were found (6 out of 91), including weak-positive tested ticks on those individuals and the species of the most positive sample. The CCHFV genotype was determined by comparing the RT-qPCR products (127 bp) using the BLAST Tool (NCBI).

Location	Host	n	p	(+)	Tick sample with the lowest CCHFV ct-value				CCHFV
					Lowest ct-value	Tick species (lowest ct-value)	Second-lowest ct- value	Tick species (sec.- lowest ct-value)	genotype
Idini	Cattle no. 1	22	22	0	18.26	*H. rufipes*	28.62	*H. dromedarii*	Africa I
Idini	Cattle no. 2	30	1	8	19.44	*H. dromedarii*	35.22	*H. dromedarii*	Africa I
Idini	Cattle no. 3	22	5	9	19.68	*H. rufipes*	34.44	*H. dromedarii*	Africa I
Rosso	Cattle no. 4	21	7	3	28.8	*H. rufipes*	31.52	*H. rufipes*	Africa III
Idini	Cattle no. 5	8	1	0	26.66	*H. rufipes*	-	-	Africa III
Nouakchott	Camel no. 1	24	3	0	28.67	*H. dromedarii* (3x)	(29.89; 29.56)	-	Africa I
Total		127	39	20					

n= number of ticks; p= positive ticks; (+) = weak-positive ticks

Table 3. Genetic distances (%) between the CCHFV genotypes

Genetic distances (%) between the CCHFV lineages found in the respective positive ticks on cattle and camels by comparing the RT-qPCR amplicons (127 bp). In all positive ticks originated from the same host animal, both the genotype and the detected gene sequence were identical.

Host/location/genotype			Cattle No.1	Cattle No.2	Cattle No.3	Cattle No.4	Cattle No.5	Camel No.1
			Idini	Idini	Idini	Rosso	Idini	Nouakchott
			Africa I	Africa I	Africa I	Africa III	Africa III	Africa I
Cattle No.1	Idini	Africa I	-	100 %	100 %	89.96 %	89.76 %	96.85 %
Cattle No.2	Idini	Africa I	100 %	-	100 %	89.96 %	89.76 %	96.85 %
Cattle No.3	Idini	Africa I	100 %	100 %	-	89.96 %	89.76 %	96.85 %
Cattle No.4	Rosso	Africa III	89.96 %	89.96 %	89.96 %	-	97.83 %	86.81 %
Cattle No.5	Idini	Africa III	89.76 %	89.76 %	89.76 %	97.83 %	-	86.81 %
Camel No.1	Nouakchott	Africa I	96.85 %	96.85 %	96.85 %	86.81 %	86.81 %	-

Figures

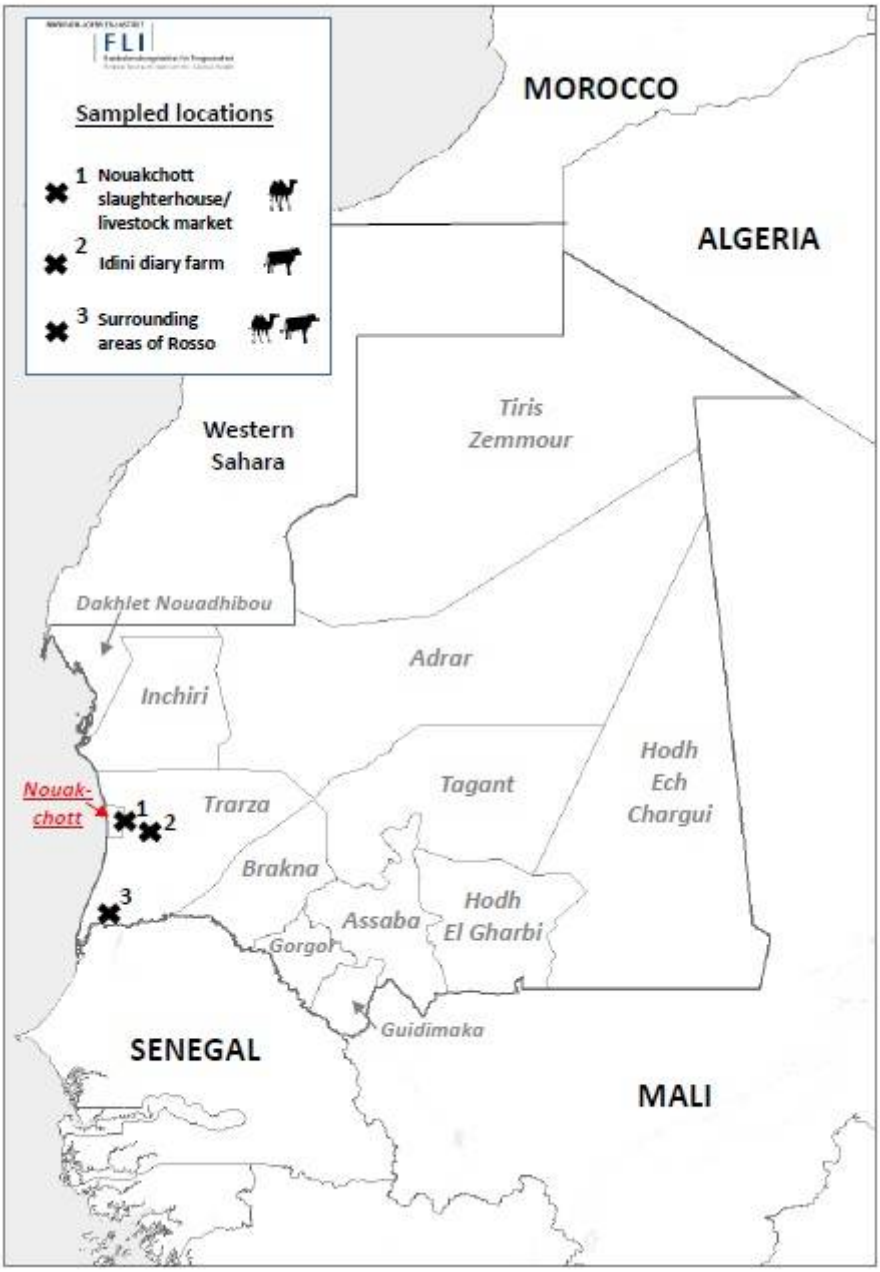

Figure 1

Map of Mauritania and the neighboring countries showing the different sampling sites of cattle and camels.

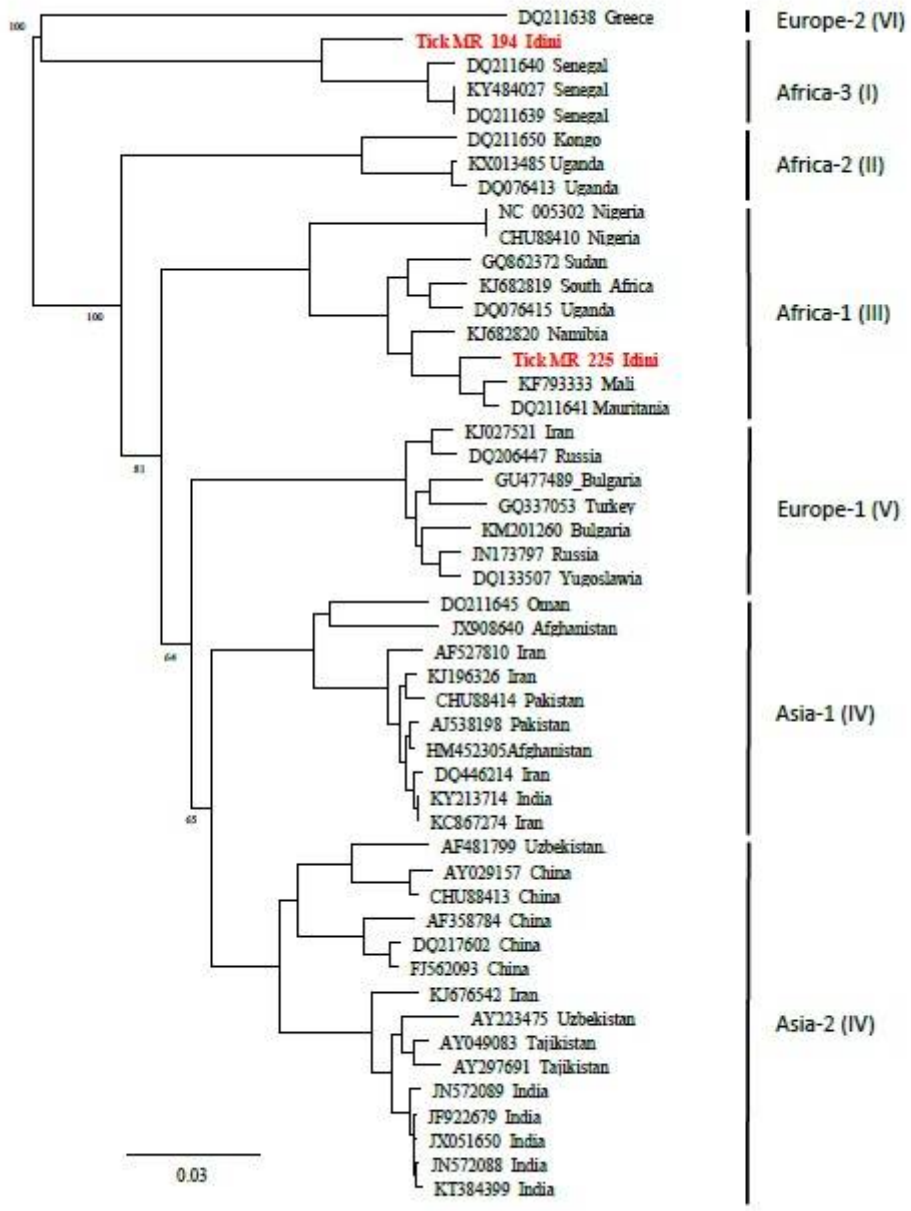

Figure 2

Phylogenetic tree showing the genetic distances of the small (S-) segment of the consensus strains and selected CCHFV-positive samples from Mauritania (1384 bp). The tree was generated using Neighbor-Joining algorithm and Jukes-Cantor distance model in Geneious version 2019.2 (Biomatters, available from https://www.geneious.com). The tree was midpoint-rooted using FigTree v1.4.4 (available from https://github.com/rambaut/figtree/releases). Values at branches represent support in 1,000 bootstrap replicates. Bootstrap values are only shown at major branches.

Figure 3

Different habitats of the livestock herds (cattle and camels) selected for sampling Cattle (a) and camel herds (c) grazing nearby Rosso (Trarza) close to the Senegal River (southern Sahel). The favourable climatic conditions permit the growth of larger trees in the savanna landscape and also the simple cultivating of field crops. In contrast, a modern dairy farm in Idini (b) is fully surrounded by arid desert. Picture d) depicts camels that are gathered for being sold at the highly-frequented Nouakchott livestock market/slaughterhouse.