

# Imported *Hyalomma* Ticks in The Netherlands 2018-2020

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## Research

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## Abstract

**Background:** Ticks of the genus *Hyalomma*, which are vectors for several tick-borne diseases, are occasionally found in areas outside their endemic range including northern parts of Europe. The objective of this study was to analyse adult *Hyalomma* ticks that were recently found in The Netherlands.

**Methods:** *Hyalomma* ticks were morphologically identified. Cluster analysis based upon sequence data (*cox1* barcoding) for molecular identification and pathogen detection was performed. Additionally, a cross-sectional survey among horses was conducted to actively search for *Hyalomma* ticks in summer 2019. Analysis of temperature was done to assess the possibility of i) introduced engorged nymphs moulting to adults and ii) establishment of populations in The Netherlands.

**Results:** Seventeen adult *Hyalomma* ticks (one in 2018, eleven in 2019, five in 2020) were found by citizens and reported. Fifteen ticks were detected on horses and two on humans. Twelve were identified as *H. marginatum*, one as *H. rufipes* and four, of which only photographic images were available, as *Hyalomma* sp. No Crimean-Congo Hemorrhagic Fever virus or *Babesia/Theileria* parasites were detected. One adult tick tested positive for *Rickettsia aeschlimannii*. In the cross-sectional horse survey, no *Hyalomma* ticks were found. Analysis of temperatures showed that engorged nymphs arriving on migratory birds in spring were able to moult to adults in 2019 and 2020, and that cumulative daily temperatures in The Netherlands were lower than in areas with established *H. marginatum* populations.

**Conclusions:** Our results show that *Hyalomma* ticks are regularly introduced in The Netherlands as nymphs and these are able to develop to the adult stage under the Dutch weather conditions. Vigilant citizens can notify *Hyalomma* adult ticks, especially when attached to horses. Only one human pathogen, *Rickettsia aeschlimannii*, was found in one of the ticks. The risk of introduction of tick-borne diseases via *Hyalomma* ticks on migratory birds is considered to be low.

## Background

Ticks of the genus *Hyalomma* (Acari: Ixodidae) are endemic in semi-arid regions across Asia, Africa and Europe [1, 2, 3]. Incidentally, specimens of *Hyalomma marginatum* sensu lato (Koch, 1844) have since long been reported far from the boundaries of their endemic range [4]. Migrating birds play an important role in long-distance dispersal of immature stages of *H. marginatum* s.l. into new areas. In studies performed in southern and eastern parts of Europe for which thousands of migration birds were checked for ticks, several hundreds of larvae and nymphs of *Hyalomma* spp. were collected [5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. In northern and western parts of Europe, such as in the United Kingdom [11], Sweden [12], Norway [13] and The Netherlands [15], immature stages of *Hyalomma* spp. were also found on migrating birds, but to a lesser extent. Occasionally, adults of *H. marginatum* are found in northern and western European countries [16, 17, 18, 19, 20, 21, 22, 23]. In The Netherlands, three adult *Hyalomma* sp. ticks were reported on horses between 2005 and 2009 [24, 25] and one adult *Hyalomma* sp. tick was found attached to a person in 2012 [26].

*Hyalomma marginatum* is a species complex including *H. marginatum*, *H. turanicum* and *H. rufipes* [27]. *Hyalomma marginatum* are two-host ticks. The larvae and nymphs feed on small mammals, ground-dwelling birds and reptiles [28]. Larval feeding, moulting to nymphal stage and nymphal feeding will in most cases occur on the same host, on which the immature stages can be present for up to 26 days [29, 30]. Adult *Hyalomma* ticks feed on ungulates, such as Bovidae, Equidae, Cervidae and Suidae, but also (smaller) mammals such as Canidae, Lagomorpha and occasionally humans [28, 31].

*H. marginatum* ticks can transmit tick-borne pathogens such as Crimean Congo Haemorrhagic Fever-virus (CCHFv) and Spotted Fever Rickettsia's to humans, *Babesia caballi* and *Theileria equi* (piroplasmiosis) to horses and *T. annulata* (tropical theileriosis) to bovines. Therefore, this tick species is of public and veterinary health concern [28, 32, 33, 34].

In this study we describe reports of adult *Hyalomma* ticks in The Netherlands by citizens, and the results of a survey that was undertaken to actively search for *Hyalomma* ticks after the first findings in 2019. The results of the molecular identification of the specimens and of the pathogen analysis of the specimens will be discussed. Furthermore, we evaluated for the Netherlands the possibility of nymph moulting, wintering and establishment of *Hyalomma* ticks after introduction, based upon climatic data.

## Methods

### Tick collection

### Citizens' notification

From the beginning of summer 2019, and after receiving the first notification of *Hyalomma*, citizens were engaged to report *Hyalomma* ticks, by posting information on the website of the Netherlands Food and Consumer Product Safety Authority (NVWA) ([www.nvwa.nl/reuzenteek](http://www.nvwa.nl/reuzenteek)) and the National Institute for Public Health and the Environment (RIVM) ([www.rivm.nl/tekenbeten-en-lyme/hyalomma-teken](http://www.rivm.nl/tekenbeten-en-lyme/hyalomma-teken)). Citizens could send photographic images of *Hyalomma* ticks to the Centre for Monitoring of Vectors (CMV, NVWA, National Reference Centre, the Netherlands) via email or using the specific *Hyalomma* tick report system included on the NVWA website. After evaluation of the photographic images by entomologists, suspected *Hyalomma* specimens were sent to the laboratory and the contributors were questioned for additional information by telephone or on-site. When on-site, relevant animals present and vegetation nearby were carefully inspected for other possible *Hyalomma* ticks.

### Cross-sectional horse study

After receiving the first notification of *Hyalomma*, a cross-sectional study was performed to investigate the presence of *Hyalomma* ticks on horses. For this, 40 horse farms and equestrian centres, located at rural wooded areas in the northern and eastern part of The Netherlands, were visited between August and October 2019 (Fig. 1). Locations were visited one time and at each location, five horses were carefully examined for ticks, in particular the nose, neck, chest, under the manes and tail, in between the legs and the legs themselves. Horses were selected based upon having outdoor activities on a regular base and/or being grazed and/or frequently being infested with ticks in the past.

## Tick identification

### Morphological identification

In the laboratory, arrived ticks (Table 1) were morphologically identified by tick specialists according to the keys of Estrada-Pena et al. (2017) and Walker et al. (2014) [35, 36].

### Molecular identification & cluster analysis

Molecular identification was performed by Sanger sequencing of the partial mitochondrial *cox1* gene using primers LCO1490 and HCO2198 [37] according to the EPPO DNA Barcoding standard PM7/129(1) [38]. In short, a few legs of the specimen were used as input for the DNA extraction. Tick tissue was ground in a lysis buffer with a micro-pestle prior to DNA extraction with the High Pure PCR template preparation kit (Roche, Basel, Switzerland). Positive and negative controls were always included to detect possible contamination and to monitor the efficiency of Sanger sequencing and assembly. Amplicons were purified with the QIAquick PCR Purification Kit (Qiagen, Venlo, The Netherlands) prior to cycle sequencing with both amplification primers in individual runs using the Big-Dye Terminator v1.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA). Cycle sequence products were purified with DyeEx 2.0 Spin Kit columns (Qiagen, Venlo, The Netherlands) prior to sequencing on a 3500 Genetic Analyzer (Life Technologies, Carlsbad, CA).

Electropherograms were assembled in Geneious Prime 2019.2.3 (Biomatters, Auckland, New Zealand), and consensus sequences were aligned with selected NCBI accessions with MAFFT [39]. After masking 5' and 3' overhang sequences in the 896 bp alignment, 542 bp were represented by all specimens in the alignment which were included in the clustering analysis. The alignment was subjected to an UPGMA clustering analysis using the tree builder tool incorporated in Geneious Prime (HKY85 substitution model; 100 bootstraps).

*Hyalomma* sequences generated in this study were deposited in the NCBI GenBank database under the following accession numbers: MT757612 to MT757622, and MW495246 to MW495248.

## Pathogen detection

### DNA and RNA extraction

For pathogen detection, remaining tick bodies were lysed and homogenized in RNAlater (Thermo Fisher Scientific, Leiden, Netherlands), and subsequently processed according to the manufacturer's instructions in a diagnostic laboratory setting (Nucleic Acid Isolation Kit I; Roche). Extraction of whole nucleic acids from the ticks was performed using robot-extraction (MagNA Pure Compact Extraction Robot; Roche, Basel, Switzerland). To detect potential cross-contamination, negative controls were included in each batch of extraction. Samples were analysed with different (real-time) PCRs based on various genes specific for the microorganism of interest and carried out on a LightCycler 480 (Roche Diagnostics Nederland B.V, Almere, The Netherlands). Positive (plasmid) controls and negative water controls were used on every plate tested. To minimize contamination leading to false-positive samples, the DNA/RNA extraction, PCR mix preparation, sample addition, and (q)PCR analyses were performed in separated air locked dedicated labs.

### Crimean Congo Hemorrhagic Fever Virus

For detection of CCHFV, a reverse transcription real-time PCR was performed [40].

### Babesia and Theileria

Primers recognizing a 400–440 bp fragment of the 18S rRNA gene of both *Babesia* spp. and the closely related *Theileria* spp. were used to amplify with PCR [41].

### Rickettsiae

Lastly, for the detection and identification of spotted fever rickettsiae, a real-time PCR on citrate synthase (*gltA*) gene was performed [42]. On positive samples, partial outer membrane protein *ompB* gene was amplified with conventional PCR and used for sequencing [43]. *Rickettsia* sequence generated in this study was deposited in the NCBI GenBank database under the following accession number: MW498244.

### Evaluation of *Hyalomma* tick moulting and wintering after introduction

We assume that the *Hyalomma* ticks found in The Netherlands were introduced on migratory birds in early spring. These ticks were introduced as feeding nymphs, and after processing the blood meal they completed the moult to the adult stage in the environment. This last step is a temperature dependent process. We evaluated the possibility of engorged nymphs to successfully moult to adults using two different criteria: (i) about 300°C cumulative degrees above the developmental zero (14–16°C) are necessary to complete the moult [44] and (ii) temperatures of >8°C for 15 continuous days assure the moulting of nymphs [45].

We also evaluated the possibility of *Hyalomma* wintering and establishment using the temperature-related limiting factor of Gray et al. (2009), where temperatures between September and December are critical for wintering of *H. marginatum* ticks. As suggested by these authors and using a threshold of 10°C, we considered that areas with cumulative temperatures between September and December with an average of 800°C would be suitable and areas below 400°C would be unsuitable for the species [46].

Daily average temperature values were obtained from available dataset of the Royal Netherlands Meteorological Institute (KNMI). For the calculations we downloaded the data of the weather stations “De Bilt” (situated central in The Netherlands), and “Heino” and “Hoogeveen” situated in the vicinity of most of the *Hyalomma* finding locations (Fig. 1).

## Results

### Tick specimens

#### Citizens’ notifications

From July 2019 to December 2020, 118 findings of ‘*Hyalomma* ticks’ were reported (Additional file 1: Table S1). Also, one adult *Hyalomma* sp. tick was already found in 2018 but reported in 2020. Of these, 17 specimens (1 in 2018, 11 in 2019, 5 in 2020, see Table 1) could be classified as adult *Hyalomma* sp. ticks based upon photographic images made by the citizens. Figure 1 shows the locations where the ticks were found.

The 102 reports which were not *Hyalomma* ticks, could be identified as other tick species (n = 51), mostly of genera *Ixodes* sp. and *Dermacentor* sp., but also hemipterans (n = 15), spiders (n = 10), louse flies (n = 6), other arthropods or unidentifiable.

Of all reported adult *Hyalomma* ticks, nearly all (15/17) were discovered on horses. Two ticks were discovered on humans, both not attached. The horses on which the ticks were found were all kept (partly) on pasture and most horses were taken for outdoor rides on a regular basis. None of the horses or persons on which the adult ticks were found had (recently) been abroad. We inquired where the ticks were most likely acquired, and whether similar ticks were noticed recently or before. In about half of the reports, we checked on-site whether other specimens were present on animals or in the vegetation. As a result, no other *Hyalomma* ticks were reported or detected.

### Morphological and molecular species identification, and pathogen detection

Thirteen of the 17 reported adult *Hyalomma* ticks, and one nymph that was found on a migratory bird in 2012, were available for further investigations (Table 1).

**Table 1.** Information about *Hyalomma* ticks and results of laboratory analyses (morphological and molecular identification, and pathogen detection). *Notes:* +, present; -, not detected; \*, identification based on photographic images; x, specimen not available for laboratory analyses. *Abbreviations:* M, male; F, female; CCHFv, Crimean Congo Hemorrhagic Fever virus; n.a., not applicable; n.t., not tested

Date found	Municipality name	Province	Found on	Stage	M/F	Morphological identification	Cluster analysis	Genbank acc. no.	CCHFv	<i>Rickettsia</i>	<i>The</i>
5 June 2012	Almere	Flevoland	bird	nymph	n.a.	<i>Hyalomma</i> sp.	<i>H. rufipes</i>	MT757612	-	-	-
18 September 2018	Yerseke	Zeeland	horse	adult	M	<i>Hyalomma</i> sp.*	x	x	x	x	x
4 July 2019	Zelhem	Gelderland	horse	adult	F	<i>Hyalomma</i> sp.*	x	x	x	x	x
14 July 2019	Odoorn	Drenthe	horse	adult	F	<i>H. marginatum</i>	<i>H. marginatum</i>	MT757622	-	+	-
30 July 2019	Dronten	Flevoland	horse	adult	F	<i>H. marginatum</i>	<i>H. marginatum</i>	MT757619	-	-	-
6 August 2019	Oldeouwer	Friesland	human	adult	M	<i>Hyalomma</i> sp.*	x	x	x	x	x
7 August 2019	Emmer-Compasuum	Drenthe	horse	adult	M	<i>Hyalomma</i> sp.	<i>H. marginatum</i>	MT757618	-	-	-
14 August 2019	Linde	Drenthe	horse	adult	F	<i>H. marginatum</i>	<i>H. marginatum</i>	MT757613	-	-	-
14 August 2019	Eén	Drenthe	horse	adult	F	<i>H. marginatum</i>	<i>H. marginatum</i>	MT757621	-	-	-
20 August 2019	Tiendeveen	Drenthe	horse	adult	M	<i>H. marginatum</i>	<i>H. marginatum</i>	MT757616	-	-	-
23 August 2019	Noordwolde	Friesland	horse	adult	F	<i>H. marginatum</i>	<i>H. marginatum</i>	MT757615	-	-	-
27 August 2019	Rucphen	Noord-Brabant	human	adult	M	<i>H. marginatum</i>	<i>H. marginatum</i>	MT757614	-	-	-
20 September 2019	Wageningen	Gelderland	horse	adult	F	<i>H. marginatum</i>	<i>H. marginatum</i>	MT757617	-	-	-
4 May 2020	Amen	Drenthe	horse	adult	F	<i>Hyalomma</i> sp.	<i>H. marginatum</i>	MT757620	-	-	-
4 August 2020	Oosterhout	Gelderland	horse	adult	F	<i>Hyalomma</i> sp.	<i>H. marginatum</i>	MW495246	-	-	-
11 August 2020	Rockanje	Zuid-Holland	horse	adult	F	<i>H. rufipes</i>	<i>H. rufipes</i>	MW495248	-	-	-
15 August 2020	Oostvoorne	Zuid-Holland	horse	adult	F	<i>Hyalomma</i> sp.	<i>H. marginatum</i>	MW495247	-	-	n.t.
4 September 2020	Harskamp	Gelderland	horse	adult	unknown	<i>Hyalomma</i> sp.*	x	x	x	x	x

#### Morphological species identification

Morphological identification in the laboratory identified 8 of the adult *Hyalomma* ticks as *H. marginatum* and 1 as *H. rufipes* (Table 1). Four adult ticks and the nymph could not be morphologically identified to species level in the laboratory with certainty (Table 1).

#### Molecular species identification and cluster analysis

A total of 91 partial *cox1* gene sequences were included in the alignment, representing 77 selected sequences deposited in NCBI (see Additional file 1: Table S2) and 14 specimens (13 adults and 1 nymph) from The Netherlands generated in this study. Results of the *cox1* sequence molecular analysis (UPMA; HKY85, 100 bootstraps) showed that the specimens found in our study cluster in two major clades (Fig. 2). The first clade, with 98% bootstrap support, clusters 12 adult specimens with *H. marginatum* (24), *H. turanicum* (5) and *H. rufipes* (1) NCBI sequences. The second clade, with 100% bootstrap support, clusters one adult specimen and the nymph with *H. rufipes* (9), *H. dromedarii* (1) and *H. truncatum* (1) NCBI sequences. As shown in Fig. 2, *H. dromedarii* and *H. truncatum* sequences are also represented in other clades. According to combined morphological and molecular identification (Table 1), twelve adult ticks can be classified as *H. marginatum* and one as *H. rufipes*. The nymph can be classified as *H. rufipes*.

#### Pathogen detection

Results of the pathogen detection analysis (Table 1) show that one adult tick reported in 2019 tested positive for *Rickettsia* sp., more specifically *R. aeschlimannii*. In the other 13 *Hyalomma* ticks that were tested, no pathogens were detected.

## Cross-sectional horse study

A total of 202 horses from 40 equestrian centres (Fig. 1) were examined for the presence of ticks in 2019. In this survey, no *Hyalomma* ticks were found during the inspections or reported by the caretakers. Six *Ixodes* ticks were found on five horses.

## Accumulated daily temperature analysis

When using the parameters obtained in the laboratory by Emelyanova et al. (2005), and using the lowest developmental zero temperature suggested ( $T_0 = 14^\circ\text{C}$ ), if *Hyalomma* nymphs were introduced early in spring in 2019 (1st of April), results show that the first adult would be expected to appear approximately the first days of August in 2019. This result does not match with the first *Hyalomma* adult finding being in 2019 notified one month before (on 4th of July).

However, results applying the temperature parameters used in the study of Gale et al. (2012) show that in the spring of 2019 (from 15-04-2019 until 02-05-2019) and 2020 (from 15-04-2020 until 02-05-2020) temperatures of  $> 8^\circ\text{C}$  for 15 or more continuous days were accounted previously to the citizens' notifications of *Hyalomma* adults, allowing the engorged nymphs to moult to adults.

In 2018 and 2019, cumulative temperatures above  $10^\circ\text{C}$  between September and December (weather station "Heino") accounted  $216^\circ\text{C}$  and  $192^\circ\text{C}$  respectively, indicating that this area is not considered suitable for the establishment of populations of *H. marginatum* (below  $400^\circ\text{C}$ ), according to Gray et al. (2009).

## Discussion

Ticks of the *H. marginatum* complex are known vectors of diseases of veterinary and public health importance, and able to survive in a wide range of climatic conditions and a variety of habitats [31]. In this study, we report the first citizens' notifications of *Hyalomma* adults in the Netherlands. Results of this study raise questions about the frequency of introductions and the ability to establish endemic *Hyalomma* populations in The Netherlands and warrants for vigilance because of the risks for human and animal health.

Even though *Hyalomma* ticks have been found and reported before in The Netherlands [15, 24, 25, 26], the numbers of adult *Hyalomma* ticks reported in the last few years seem unprecedented. In the weeks preceding the findings in 2019, the media reported about adult *Hyalomma* ticks in horse farms in Germany [47] in a sensationalistic way. The finding of the first *Hyalomma* ticks in The Netherlands that year boosted the media attention. There, the *Hyalomma* ticks were classified as 'giant', 'monster' or 'horror' ticks and were connected to 'a deadly Ebola-like virus' (meaning CCHFv), which probably led to an increased awareness among the public. Fifteen out of seventeen adult *Hyalomma* ticks were discovered on horses. This is most likely due to the fact that horses, in contrast to other hosts for *Hyalomma* adults, are on a regular base closely inspected and handled by their carers during grooming and saddling.

Of the thirteen available adult *Hyalomma* ticks (Table 2), eight could be morphologically identified as *H. marginatum* and one as *H. rufipes*. Four could not be morphologically identified to species level with certainty, due to the poor state (dried out and/or moldy) of the received specimens. Also, the engorged nymph that was found on a migratory bird in 2012 [15] could not morphologically be identified to species level, because of the difficulties arising morphological identification of *Hyalomma* nymphs to species level [27, 35].

Molecularly, twelve adults, including the eight ticks that were morphologically identified as *H. marginatum*, clustered mainly with *H. marginatum* (24). Also in this cluster were two other members of the *H. marginatum* species complex, *H. turanicum* (5) and *H. rufipes* (1) (Fig. 2). The one adult that was morphologically identified as *H. rufipes*, and the nymph clustered mainly with *H. rufipes* (9). One *H. dromedarii* and one *H. truncatum* were also in this cluster, which also clustered together in their own cluster (Fig. 2). Ticks from the *H. marginatum* species complex are known to be taxonomically challenging to identify [27]. Also, cryptic hybridization in *Hyalomma* ticks might, at least partly, account for the apparent incongruence between morphology and molecular clustering [48].

*H. rufipes* has also been found in western and northern European countries [13, 18]. To our knowledge, *H. dromedarii* or *H. truncatum* specimens have not been found in western and northern European countries. In our consideration, we also took into account the fact that *H. rufipes* is a two host tick of which the immatures are most likely to seek (migratory) birds as hosts, in contrast to *H. dromedarii* and *H. truncatum* [35, 36]. Combining morphological and molecular identification, we interpret that the twelve adults are *H. marginatum*, and both the one adult and nymph are *H. rufipes*.

The ticks were most likely introduced via migratory birds. In general, migrating birds that are breeding in The Netherlands and wintering in warmer climates can be divided into two groups. Birds belonging to the first group migrate over long distances, and winter in Africa (Sahel to more southern regions). The mass arrival in The Netherlands of these birds, such as Garden warbler (*Sylvia borin*), Common whitethroat (*S. communis*), Willow warbler (*Phylloscopus trochilus*) and Common redstart (*Phoenicurus phoenicurus*) is from half of April to May. The second group consists of birds that winter much closer by, in southern Europe and northern Africa. These birds, such as European robin (*Erithacus rubecula*), Song thrush (*Turdus philomelos*), Common chiffchaff (*Phylloscopus collybita*) and Eurasian blackcap (*Sylvia atricapilla*) arrive from March onwards, leaving the African and Mediterranean European countries already in February. In the wintering and stop-over areas of both groups, *Hyalomma* ticks are endemic and can be dispersed via returning migrating birds [49]. Indeed, *Hyalomma* immatures have been found on bird species of both groups returning to or present in their breeding area [5, 6, 7, 8, 9, 10, 11, 12, 13, 50, 51, 52], also in The Netherlands [15]. The *Hyalomma* ticks reported in this study were most likely introduced at the locations in early spring by migratory birds as engorged nymphs, and moulted to adult stage. The horses and persons on which the adult ticks were found had not (recently) been abroad. Also, in case of introduction of adult ticks with animal hosts (e.g. introduced via imported horses), more specimens found at one location would be expected [31, 33, 46].

As shown by the results of the cumulative daily temperature analyses, the accounted temperatures between September and December of 2018 and 2019 do not correspond with the areas where *H. marginatum* has permanent populations. As suggested by Gray et al. (2009), these parameters (average of  $800^\circ\text{C}$  in places where the tick has permanent populations, and below  $400^\circ\text{C}$  in sites not colonized) are related to the local factors that affect the moulting of nymphs

to adult stage before the onset of winter. These parameters are not connected to (extreme) cold winter temperatures that prevent wintering adults surviving into the next year, because unfed adults of *Hyalomma* are very capable of surviving even harsh winter circumstances in continental climate, probably hidden deeply in the litter layer [32, 46]. So, temperatures in late summer and autumn are more critical for potential *Hyalomma* survival and establishment of permanent populations than temperatures in winter.

Results according to the criteria of Emylyanova et al. (2005) [44] show that the early findings (before August) of adult *Hyalomma* ticks in The Netherlands do not match with the suggested temperature accumulation. According to these criteria, introduced engorged nymphs in 2019 and 2020 could not moult to adults within this time period, suggesting that adult *Hyalomma* ticks that were notified before August wintered at these locations. A period of at least four months would have been necessary in 2019 for engorged nymphs to moult into adults. Taking into consideration the probability of survival of the moulting ticks during such a four months period exposed to (environmental) factors such as predation, parasitism, fungal infections, drowning or desiccation [31, 53, 54], this period can be considered too long and the proposed parameters less appropriate. Applying the criterion proposed by Gale et al. (2012) [45], engorged nymphs that arrived at the end of March or in April, could moult to adults in the first days of May in 2019 and 2020, matching with the findings of the first adults. The criterion used by Gale et al. (2012) [45] and accepted for the *H. marginatum* populations maintained in Spain (Estrada-Peña personal communication) is based on temperature as the main factor affecting the seasonal pattern of the *H. marginatum* tick (Estrada-Peña et al. 2011) and is therefore used as the sole parameter for moulting. Other critical parts of the life cycle necessary for establishment of permanent populations besides nymphal moulting and wintering of adults [46, 49, 53], such as oviposition by the adult females and questing and moulting activity of all stages, are depending heavily on (micro)climatic circumstances as well [53]. This explains why in areas where climatic circumstances are in favour of moulting from nymph to adult, this does not automatically lead to permanent populations of *Hyalomma* ticks.

Besides climatic factors, tick densities and dispersal are also important factors for possible establishment of *Hyalomma* [28, 53]. All findings of *Hyalomma* ticks so far were singular, and no other *Hyalomma* ticks were reported by the citizens or detected by us in the vicinity of the reported adult *Hyalomma* tick. Also, in the horse screening part of this study no *Hyalomma* ticks were found. Although it is likely that more *Hyalomma* ticks are present than noticed, we hypothesize that these ticks are still too widespread to find a mating partner (Allee effect [55, 56]). With the current seemingly occasional introduction on migration birds, the chance for adult females to encounter adult males for mating on the same mammalian host can be considered very low. However, we cannot conclude that this event is impossible or could not already have happened. To detect attached larvae or nymphs, active (more site-directed) monitoring on small mammals and resident birds would need to be implemented in early spring, at least in areas where *Hyalomma* has been notified. Besides the previous mentioned factors, the availability and densities of suitable hosts are also of importance. Since in The Netherlands birds, rodents, lagomorphs and wild and domestic ungulates are abundant, this will probably not be the most limiting factor.

Taking the abovementioned into account, we hypothesize that engorged nymphs arriving on migratory birds in spring are able to moult to adults under the current Dutch climatic circumstances of spring and summer. *H. marginatum* is currently not able to establish permanent populations due to the climate. For *H. rufipes*, which is distributed in drier areas of Africa and prefers arid conditions [35], it seems even more unlikely that permanent populations will be established in The Netherlands, which classifies as Cfb (temperate oceanic), according to the Köppen–Geiger climate classification [57].

In none of the tested ticks (13 adults and 1 nymph) CCHFv was detected. This is in accordance with other studies performed in western and northern countries which tested for CCHFv in *Hyalomma* ticks [11, 16, 17, 18, 20, 22]. CCHFv positive *Hyalomma* ticks can be found in ticks collected in regions in and around Europe that lay closer to CCHFv endemic regions [8, 9, 58]. CCHFv seems to have a focal geographic distribution range, also in the regions where competent vector tick species are established [33]. Complex interactions between tick vector species, reservoir hosts, climate (change) and social changes leading to alterations in vegetation and use of landscape determines the enzootic cycle of CCHFv and human outbreaks [59]. Widespread occurrence of vectors and reservoirs do not necessarily lead to outbreaks of CCHFv in humans and even though climate may favour competent tick populations, it is not directly linked to presence of CCHFv [60]. This makes it difficult to predict the dynamics of CCHFv in areas where potential reservoirs are present, the competent vectors are occasionally introduced and climate is changing, as is the current situation in The Netherlands. Recently, CCHFv was found in *Hyalomma* ticks in Spain [61] and at a later stage evidence of circulation of the virus in wildlife in parts of Spain was found, including human cases [62, 63]. The virus strain found in Spain belongs to a cluster of strains isolated in western Africa and it was calculated that it was introduced in Spain around 50 years prior to the first human cases [64]. This finding shows that enzootic cycles can be present unnoticed for quite some time. Although other tick species are also involved in epidemiology of CCHFv, *H. marginatum* is considered the principal vector in Europe [33, 65]. Because the virus can be transmitted in ticks both transstadial and transovarial, *Hyalomma* ticks can also form a reservoir of CCHFv [53]. To estimate the risks of introduction of CCHFv via *Hyalomma* ticks imported by migratory birds, the dynamics between the virus, the tick and feeding on a non-viremic host need to be clarified. In summary, taking into account that i) only a small percentage of larvae in endemic areas are infected via transovarial transmission [45, 60, 66], ii) viremic transmission of ticks via migratory birds [60, 67] and transmission of CCHFv via co-feeding on migratory birds seems unlikely [33], iii) transstadial transmission is only successful in over a third of the ticks and perhaps lower in ticks feeding on birds [66, 68], iv) most probably only occasionally *H. marginatum* immatures are dropped off in northern and western European regions, and v) no CCHFv has been found so far in *Hyalomma* ticks in northern and western European countries, the chance of human exposure to CCHFv via *Hyalomma* ticks in The Netherlands is currently considered very low.

Presence of the strictly intracellular bacterium *Rickettsia*, of which the species *aeschlimannii* was found in our study in one tick, is much more often reported in *Hyalomma* ticks [14, 17, 18, 19, 23, 51, 58, 69, 70]. The reason for this might be that *Rickettsia* spp. are considered heritable symbionts from invertebrates [71, 72].

The ticks tested negative for the parasites *Babesia* and *Theileria*. This is in accordance with other studies that tested *Hyalomma* ticks for *Babesia* and *Theileria* species in northern and western European countries [17, 18, 20]. Bovine tropical theileriosis (*T. annulata*) is only present in southern parts of Europe and has to our knowledge not been detected in north western European countries, but equine piroplasmiasis (*B. caballi*/*T. equi*) is occasionally diagnosed outside its endemic range [73]. In fact, a study revealed piroplasmiasis in horses in the south western part of The Netherlands in 2010. Not all horses had been

abroad, suggesting autochthonous infections, most likely caused by *Dermacentor reticulatus* ticks [74, 75]. Because *Theileria* parasites are not transmitted transovarially, the risk of introduction of *Theileria* with *Hyalomma* ticks seem negligibly low [32]. We estimate the risk of introduction of *Babesia* through *Hyalomma* ticks also very low, although it must be taken into account that this protozoan can be transmitted transovarially [76].

## Conclusion

*Hyalomma* ticks are on a regular basis introduced into areas outside their current living range, and our results show that alert citizens can notice the presence of a *Hyalomma* tick and report the finding to the authorities. We hypothesize that as of summer 2019, citizens were more alert to *Hyalomma* ticks than in the previous years. The awareness, caused by media reports of findings, in combination with the intensive care (e.g. brushing) of horses could be the reasons that relatively many ticks, compared to previous years, were reported in 2019 and 2020 and that most *Hyalomma* ticks have been found on horses. Under our current climatic conditions, introduced engorged nymphs are able to moult to adults, but establishment of permanent *Hyalomma* populations is considered not likely. We estimate the current probability of introduction of *Hyalomma* tick-borne pathogens in The Netherlands as very low, with the exception of *R. aeschlimannii*, of which there is existing risk as demonstrated by our results, and to a lesser extent *B. caballi*.

## Abbreviations

CCHFv: Crimean Congo Haemorrhagic Fever-virus, CMV: Centre for Monitoring of Vectors, *gltA*: citrate synthase gene, NVWA: Netherlands Food and Consumer Product Safety Authority, *ompB*: outer membrane protein B gene, RIVM: National Institute for Public Health and the Environment, s.l.: sensu lato, UPGMA: Unweighted Pair Group Method with Arithmetic Mean

## Declarations

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### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

*Hyalomma* sequences generated in this study were submitted to NCBI GenBank under accession numbers MT757612 to MT757622, and MW495246 to MW495248. *Rickettsia aeschlimannii* sequence generated in this study was submitted to the NCBI GenBank under accession number MW498244. Data supporting the conclusions of this article are included within the article and its additional file. Limited amount of DNA/RNA from samples are available upon reasonable request.

### Competing interests

The authors declare that they have no competing interests

### Funding

Not applicable

### Authors' contributions

MU, AIJ, AS and FJ were involved in collecting and processing of the samples reported by citizens. FJ, AIJ and AS performed the morphological identification of the reports made by citizens. The cross-sectional horse study was designed by HS, RN, PO and CD and performed by CD. HS, RN and PO supervised CD (veterinary student). BvdV performed the cluster analysis, in consultation with MU, AIJ and HS. AIJ performed the climate analyses, in consultation with MU. HS supervised the pathogen detection work. MU and AIJ wrote the manuscript. All authors provided relevant input at different stages of manuscript preparation. All authors read and approved the final manuscript.

## References

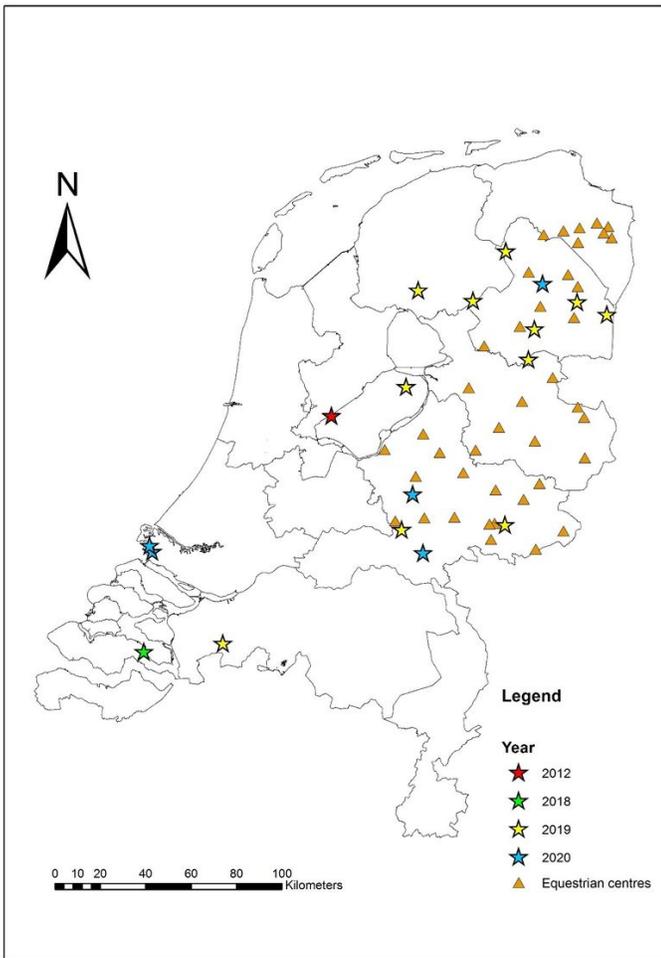
1. Gargili A, Thangamani S, Bente D. Influence of laboratory animal hosts on the life cycle of *Hyalomma marginatum* and implications for an in vivo transmission model for Crimean-Congo hemorrhagic fever virus. *Front Cell Infect Microbiol*. 2013;3:39.
2. Vial L, Stachurski F, Leblond A, Huber K, Vourc'h G, René-Martellet M, et al. Strong evidence for the presence of the tick *Hyalomma marginatum* Koch, 1844 in southern continental France. *Ticks Tick Borne Dis*. 2016;7:1162–7.
3. ECDC. *Hyalomma marginatum* - current known distribution: May 2020 ECDC. <https://>.

4. Hoogstraal H, Kaiser MN, Traylor MA, Gaber S, Guindy E. Ticks (Ixodoidea) on birds migrating from Africa to Europe and Asia. *Bull World Health Organ.* 1961;24:197–212.
5. Capek M, Literak I, Kocianova E, Sychra O, Najer T, Trnka A, et al. Ticks of the *Hyalomma marginatum* complex transported by migratory birds into Central Europe. *Ticks Tick Borne Dis.* 2014;5:489–93.
6. Poupon M, Lommano E, Humair PF, Douet V, Rais O, Schaad M, et al. Prevalence of *Borrelia burgdorferi* sensu lato in ticks collected from migratory birds in Switzerland. *Appl Environ Microbiol.* 2006;72:976–9.
7. Toma L, Mancini F, Di Luca M, Cecere JG, Bianchi R, Khoury C, et al. Detection of microbial agents in ticks collected from migratory birds in central Italy. *Vector Borne Zoonot Dis.* 2014;14:199–205.
8. Mancuso E, Toma L, Polci A, d'Alessio SG, Di Luca M, Orsini M, et al. Crimean-Congo Hemorrhagic Fever Virus Genome in Tick from Migratory Bird, Italy. *Emerg Infect Dis.* 2019;25:1418–20.
9. England ME, Phipps P, Medlock JM, Atkinson PM, Atkinson B, Hewson R, et al. *Hyalomma* ticks on northward migrating birds in southern Spain: Implications for the risk of entry of Crimean-Congo haemorrhagic fever virus to Great Britain. *J Vector Ecol.* 2016;41:128–34.
10. Sparagano O, George D, Giangaspero A, Špitalská E. Arthropods and associated arthropod-borne diseases transmitted by migrating birds. The case of ticks and tick-borne pathogens. *Vet Par.* 2015;213:61–6.
11. Jameson LJ, Morgan PJ, Medlock JM, Watola G, Vaux AG. Importation of *Hyalomma marginatum*, vector of Crimean-Congo haemorrhagic fever virus, into the United Kingdom by migratory birds. *Ticks Tick Borne Dis.* 2012;3:95–9.
12. Labbe Sandelin L, Tolf C, Larsson S, Wilhelmsson P, Salaneck E, Jaenson TG, et al. *Candidatus Neorhlichia mikurensis* in Ticks from Migrating Birds in Sweden. *PLoS One.* 2015;107:e0133250.
13. Hasle G, Bjune G, Edvardsen E, Jakobsen C, Linnehol B, Roer JE, et al. Transport of ticks by migratory passerine birds to Norway. *J Parasitol.* 2009;95:1342–51.
14. Battisti E, Urach K, Hodžić A, Fusani L, Hufnagl P, Felsberger G, et al. Zoonotic Pathogens in Ticks from Migratory Birds, Italy. *Emerg Infect Dis.* 2020;26:2986–8.
15. Heylen D, Fonville M, Docters van Leeuwen A, Stroo A, Duisterwinkel M, van Wieren S, et al. Pathogen communities of songbird-derived ticks in Europe's low countries. *Parasites Vectors.* 2017;10.
16. Chitimia-Dobler L, Nava S, Bestehorn M, Dobler G, Wolfel S. First detection of *Hyalomma rufipes* in Germany. *Ticks Tick Borne Dis.* 2016;7:1135–8.
17. Chitimia-Dobler L, Schaper S, Riess R, Bitterwolf K, Frangoulidis D, Bestehorn M, et al. Imported *Hyalomma* ticks in Germany in 2018. *Parasites Vectors.* 2019;12:134.
18. Hansford KM, Carter D, Gillingham EL, Hernandez-Triana LM, Chamberlain J, Cull B, et al. *Hyalomma rufipes* on an untraveled horse: Is this the first evidence of *Hyalomma* nymphs successfully moulting in the United Kingdom? *Ticks Tick Borne Dis.* 2019;10 3:704–8.
19. Rumer L, Graser E, Hillebrand T, Talaska T, Dautel H, Mediannikov O, et al. *Rickettsia aeschlimannii* in *Hyalomma marginatum* ticks, Germany. *Emerg Infect Dis.* 2011;17:325–6.
20. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, et al. First records of adult *Hyalomma marginatum* and *H. rufipes* ticks (Acari: Ixodidae) in Sweden. *Ticks Tick Borne Dis.* 2020:101403.
21. McGinley L, Hansford KM, Cull B, Gillingham EL, Carter DP, Chamberlain JF, et al. First report of human exposure to *Hyalomma marginatum* in England: further evidence of a *Hyalomma* moulting event in north-western Europe? *Ticks Tick Borne Dis.* 2020:101541.
22. Oehme R, Bestehorn M, Wölfel S, Chitimia-Dobler L. *Hyalomma marginatum* in Tübingen, Germany. *SystAppl Acarol.* 2017;22:1–6.
23. Duscher GG, Hodžić A, Hufnagl P, Wille-Piazzai W, Schötta AM, Markowicz MA, et al. Adult *Hyalomma marginatum* tick positive for *Rickettsia aeschlimannii* in Austria, October 2018. *Euro Surveill.* 2018;23.
24. Nijhof AM, Bodaan C, Postigo M, Nieuwenhuijs H, Opsteegh M, Franssen L, et al. Ticks and associated pathogens collected from domestic animals in the Netherlands. *Vector Borne Zoonotic Dis.* 2007;7:585–95.
25. Butler CM, Sloet van Oldruitenborgh-Oosterbaan M, Werners AH, Stout TAE, Jongejan F, Houwers D. Classification of ticks collected from horses in the Netherlands in 2008–2009 and identification of the (zoonotic) agents they contain. *Pferdeheilkunde.* 2016.
26. Graveland H, Roest H, Stenvers O, Valkenburgh S, Friesema I, van der Giessen J, et al: Staat van Zoönosen 2012. RIVM The Netherlands 2013: 44.
27. Estrada-Pena A, Pfäffle M, Baneth G, Kleinerman G, Petney TN. Ixodoidea of the Western Palaearctic: A review of available literature for identification of species. *Ticks Tick Borne Dis.* 2017;8:512–25.
28. Spengler JR, Estrada-Pena A. Host preferences support the prominent role of *Hyalomma* ticks in the ecology of Crimean-Congo hemorrhagic fever. *PLOS Negl Trop Dis.* 2018;12:e0006248.
29. Estrada-Pena A, de la Fuente J. The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral Res.* 2014;108:104–28.
30. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J Med Entomol.* 1979;15:307–417.
31. Valcárcel F, González J, González MG, Sánchez M, Tercero JM, Elhachimi L, et al. Comparative Ecology of *Hyalomma lusitanicum* and *Hyalomma marginatum* Koch, 1844 (Acarina: Ixodidae). *Insects.* 2020;11.
32. Bakheit MA, Latif AA, Vatanserver Z, Seitzer U, Ahmed J. The Huge Risks Due to *Hyalomma* Ticks. In: Mehlhorn H, editor. *Arthropods as Vectors of Emerging Diseases Parasitol Res Monographs.* Vol. 3. Berlin: Springer; 2012.
33. EFSA. Scientific Opinion on the Role of Tick Vectors in the Epidemiology of Crimean-Congo Hemorrhagic Fever and African Swine Fever in Eurasia, EFSA Panel on Animal Health and Welfare (AHAW). *EFSA Journal.* 2010;8:156.

34. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: A geographic approach. *Clin Microbiol Rev.* 2013;26:657–702.
35. Estrada-Pena A, Mihalca AD, Petney TN. *Ticks of Europe and North America.* Springer International Publishing; 2017.
36. Walker AR, Bouattour A, Camicas JL, Estrada-Pena A, Horak IG, Latif AA, et al. *Ticks of Domestic Animals in Africa: a Guide to Identification of Species.* Edinburgh Scotland: Bioscience Reports; 2014.
37. Hebert PD, Cywinska A, Ball SL, deWaard JR. Biological identifications through DNA barcodes. *Proc. Biol. Sci.* 2003;270:313 – 21.
38. EPPO/OEPP. PM 7/129 (1) DNA barcoding as an identification tool for a number of regulated pests. *EPPO Bull.* 2016;46:501–37.
39. Katoh K, Misawa K, Kuma K, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 2002;30:3059–66.
40. Wolfel R, Paweska JT, Petersen N, Grobbelaar AA, Leman PA, Hewson R, et al. Virus detection and monitoring of viral load in Crimean-Congo hemorrhagic fever virus patients. *Emerg Infect Dis.* 2007;13:1097–100.
41. Wielinga PR, Fonville M, Sprong H, Gaasenbeek C, Borgsteede F, van der Giessen JW. Persistent detection of *Babesia* EU1 and *Babesia microti* in *Ixodes ricinus* in the Netherlands during a 5-year surveillance: 2003–2007. *Vector Borne Zoonotic Dis.* 2009;9:119–22.
42. Stenos J, Graves SR, Unsworth NB. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group Rickettsiae. *Am J Trop Med Hyg.* 2005;73:1083–5.
43. Roux V, Raoult D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (*ompB*). *Int J Syst Evol.* 2000;50:1449–55.
44. Emelyanova N. Seasonal changes and host adaptability of ticks of the species *Hyalomma marginatum* in the Stavropol territory. *Zurnal Mikrobiologii epidemiologii i immunobiologii.* 2005;4:115–8.
45. Gale P, Stephenson B, Brouwer A, Martinez M, de la Torre A, Bosch J, et al. Impact of climate change on risk of incursion of Crimean-Congo haemorrhagic fever virus in livestock in Europe through migratory birds. *J Appl Microbiol.* 2012;112:246–57.
46. Gray JS, Dautel H, Estrada-Pena A, Kahl O, Lindgren E. Effects of climate change on ticks and tick-borne diseases in Europe. *Interdiscip Perspect Infect Dis.* 2009:593232.
47. Höhenheim U: Tropische Zecken: Neu eingewanderte Art überwintert erstmals in Deutschland. 2019. [https://www.uni-hohenheim.de/pressemitteilung?tx\\_ttnews%5Btt\\_news%5D=43734&cHash=5fd3c0e9da33d842f4ee127beaf7b078](https://www.uni-hohenheim.de/pressemitteilung?tx_ttnews%5Btt_news%5D=43734&cHash=5fd3c0e9da33d842f4ee127beaf7b078).
48. Rees DJ, Dioli M, Kirkendall LR. Molecules and morphology: evidence for cryptic hybridization in African *Hyalomma* (Acari: Ixodidae). *Mol Phylogenet Evol.* 2003;27:131–42.
49. Bosch J, Muñoz MJ, Martínez M, de la Torre A, Estrada-Peña A. Vector-borne pathogen spread through ticks on migratory birds: a probabilistic spatial risk model for south-western Europe. *Transbound Emerg Dis.* 2013;60:403–15.
50. Wallmenius K, Barboutis C, Fransson T, Jaenson TG, Lindgren PE, Nystrom F, et al. Spotted fever *Rickettsia* species in *Hyalomma* and *Ixodes* ticks infesting migratory birds in the European Mediterranean area. *Parasites Vectors.* 2014;7:318.
51. Socolovschi C, Reynaud P, Kernif T, Raoult D, Parola P. Rickettsiae of spotted fever group, *Borrelia valaisiana*, and *Coxiella burnetii* in ticks on passerine birds and mammals from the Camargue in the south of France. *Ticks Tick Borne Dis.* 2012;5–6:355 – 60.
52. Hubálek Z, Sedláček P, Estrada-Peña A, Vojtíšek J, Rudolf I. First record of *Hyalomma rufipes* in the Czech Republic, with a review of relevant cases in other parts of Europe. *Ticks Tick Borne Dis.* 2020;11:101421.
53. Estrada-Pena A, Martínez Aviles M, Muñoz Reoyo MJ. A population model to describe the distribution and seasonal dynamics of the tick *Hyalomma marginatum* in the Mediterranean Basin. *Transbound Emerg Dis.* 2011;58:213–23.
54. Ogden NH, Mechai S, Margos G. Changing geographic ranges of ticks and tick-borne pathogens: drivers, mechanisms and consequences for pathogen diversity. *Front Cell Infect Microbiol.* 2013;3:46.
55. Lampert A, Liebhold AM. Combining multiple tactics over time for cost-effective eradication of invading insect populations. *Ecol Lett.* 2021;24:279–87.
56. Tobin PC, Berc L, Liebhold AM. Exploiting Allee effects for managing biological invasions. *Ecol Lett.* 2011;14 6:615–24.
57. MacLeod A, Korycinska A. Detailing Köppen–Geiger climate zones at sub-national to continental scale: a resource for pest risk analysis. *EPPO Bulletin.* 2019;49:73–82.
58. Azagi T, Klement E, Perlman G, Lustig Y, Mumcuoglu KY, Apanaskevich DA, et al. *Francisella*-Like Endosymbionts and *Rickettsia* Species in Local and Imported *Hyalomma* Ticks. *Appl. Environ. Microbiol.* 2017;83.
59. Vescio FM, Busani L, Mughini-Gras L, Khoury C, Avellis L, Taseva E, et al. Environmental correlates of Crimean-Congo haemorrhagic fever incidence in Bulgaria. *BMC Public Health.* 2012;12:1116.
60. Estrada-Pena A, Jameson L, Medlock J, Vatansever Z, Tishkova F. Unraveling the ecological complexities of tick-associated Crimean-Congo hemorrhagic fever virus transmission: a gap analysis for the western Palearctic. *Vector Borne Zoonotic Dis.* 2012;12:743–52.
61. Estrada-Pena A, Palomar AM, Santibanez P, Sanchez N, Habela MA, Portillo A, et al. Crimean-Congo hemorrhagic fever virus in ticks, Southwestern Europe, 2010. *Emerg Infect Dis.* 2012;18:179–80.
62. Negrodo A, de la Calle-Prieto F, Palencia-Herrejon E, Mora-Rillo M, Astray-Mochales J, Sanchez-Seco MP, et al. Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *N Engl J Med.* 2017;377:154–61.
63. Monsalve Arteaga L, Muñoz Bellido JL, Vieira Lista MC, Vicente Santiago MB, Fernández Soto P, Bas I, et al. Crimean-Congo haemorrhagic fever (CCHF) virus-specific antibody detection in blood donors, Castile-León, Spain, summer 2017 and 2018. *Euro Surveill.* 2020;25.

64. Cajimat MNB, Rodriguez SE, Schuster IUE, Swetnam DM, Ksiazek TG, Habela MA, et al. Genomic Characterization of Crimean-Congo Hemorrhagic Fever Virus in *Hyalomma* Tick from Spain, 2014. *Vector borne and zoonotic diseases* (Larchmont, NY). 2017;17 10:714–9; doi: 10.1089/vbz.2017.2190.
65. Monsalve-Arteaga L, Alonso-Sardón M, Muñoz Bellido JL, Vicente Santiago MB, Vieira Lista MC, López Abán J, et al. Seroprevalence of Crimean-Congo hemorrhagic fever in humans in the World Health Organization European region: A systematic review. *PLoS Negl Trop Dis*. 2020;14(3):e0008094. doi:10.1371/journal.pntd.0008094.
66. Gargili A, Estrada-Peña A, Spengler JR, Lukashev A, Nuttall PA, Bente DA. The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: A review of published field and laboratory studies. *Antiviral Res*. 2017;144:93–119.
67. Hawman DW, Feldmann H. Recent advances in understanding Crimean-Congo hemorrhagic fever virus. *F1000Research*. 2018;7.
68. Zeller HG, Cornet JP, Camicas JL. Experimental transmission of Crimean-Congo hemorrhagic fever virus by west African wild ground-feeding birds to *Hyalomma marginatum rufipes* ticks. *Am J Trop Med Hyg*. 1994;50:676–81.
69. Palomar AM, Portillo A, Mazuelas D, Roncero L, Arizaga J, Crespo A, et al. Molecular analysis of Crimean-Congo hemorrhagic fever virus and *Rickettsia* in *Hyalomma marginatum* ticks removed from patients (Spain) and birds (Spain and Morocco), 2009–2015. *Ticks Tick Borne Dis*. 2016;7:983–7.
70. Spiegelaar N: The role of birds in disseminating ticks and tick-borne diseases of veterinary and medical importance. Thesis Utrecht University, The Netherlands; 2015.
71. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev*. 2005;18:719–56.
72. Alberdi MP, Dalby MJ, Rodriguez-Andres J, Fazakerley JK, Kohl A, Bell-Sakyi L. Detection and identification of putative bacterial endosymbionts and endogenous viruses in tick cell lines. *Ticks Tick Borne Dis*. 2012;3:137–46.
73. OIE. WAHIS Interface World Animal Health Disease Information.
74. Butler CM, Sloet van Oldruitenborgh-Oosterbaan MM, Stout TAE, van der Kolk JH, Wollenberg Lvd, Nielen M, et al. Prevalence of the causative agents of equine piroplasmiasis in the South West of The Netherlands and the identification of two autochthonous clinical *Theileria equi* infections. *Vet J*. 2012;193 2:381–5.
75. Jongejan F, Ringenier M, Putting M, Berger L, Burgers S, Kortekaas R, et al. Novel foci of *Dermacentor reticulatus* ticks infected with *Babesia canis* and *Babesia caballi* in the Netherlands and in Belgium. *Parasit Vectors*. 2015;8:232.
76. Onyiche TE, Sukanuma K, Igarashi I, Yokoyama N, Xuan X, Thekisoe O. A Review on Equine Piroplasmiasis: Epidemiology, Vector Ecology, Risk Factors, Host Immunity, Diagnosis and Control. *Int J Envir Res Public Health*. 2019;16.

## Figures



**Figure 1**  
 Location of Hyalomma ticks found in The Netherlands (stars), and equestrian centres visited for the horse survey in 2019 (triangles) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

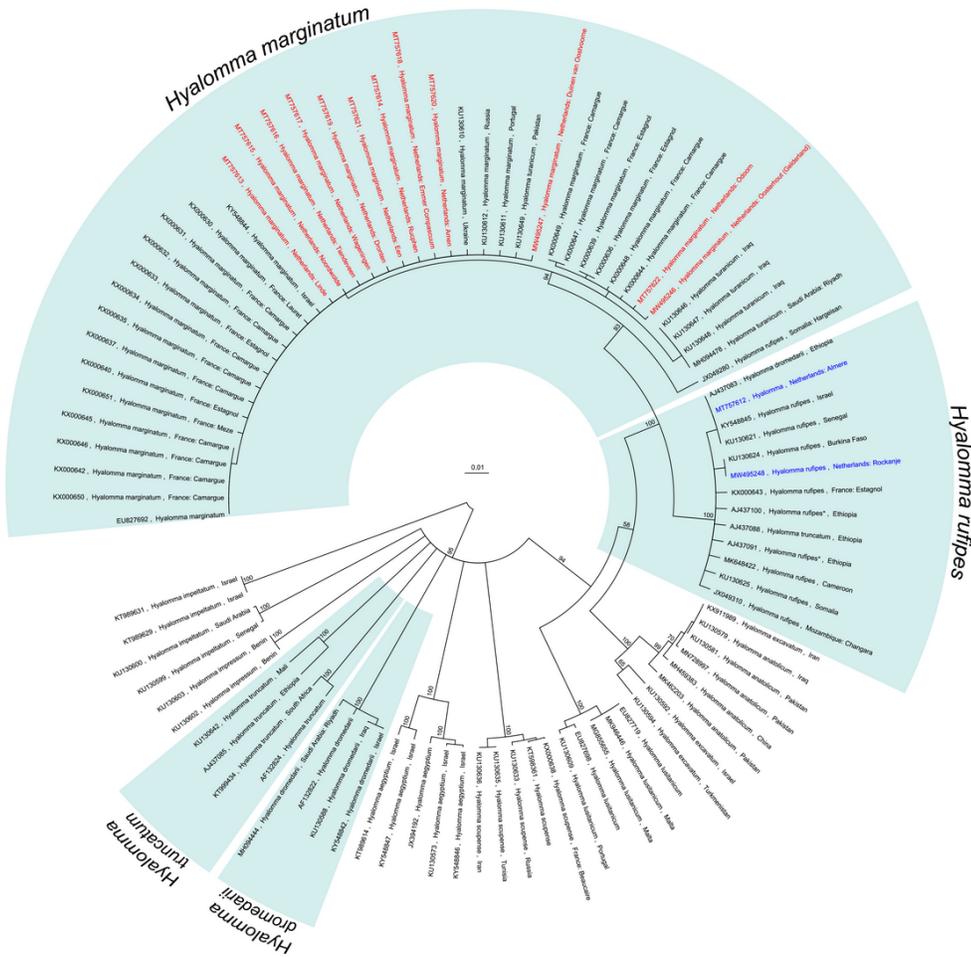


Figure 2

Cluster analysis of *Hyalomma* specimens. Cladogram of a fragment of the mitochondrial *cox1* gene based on 77 selected NCBI accessions and 12 *Hyalomma* specimens found during this study. Organism names of sequences indicated with “\*” have been altered relative to the NCBI record based on information provided in the original paper.

### Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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