

- 2024;96:e29559. <https://doi.org/10.1002/jmv.29559>
3. Gurley ES, Montgomery JM, Hossain MJ, Bell M, Azad AK, Islam MR, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerg Infect Dis*. 2007;13:1031–7. <https://doi.org/10.3201/eid1307.061128>
  4. Gokhale MD, Sreelekshmy M, Sudeep AB, Shete A, Jain R, Yadav PD, et al. Detection of possible Nipah virus infection in *Rousettus leschenaultii* and *Pipistrellus pipistrellus* bats in Maharashtra, India. *J Infect Public Health*. 2021;14:1010–2. <https://doi.org/10.1016/j.jiph.2021.05.001>
  5. Plowright RK, Becker DJ, Crowley DE, Washburne AD, Huang T, Nameer PO, et al. Prioritizing surveillance of Nipah virus in India. *PLoS Negl Trop Dis*. 2019;13:e0007393. <https://doi.org/10.1371/journal.pntd.0007393>
  6. Reynes JM, Counor D, Ong S, Faure C, Seng V, Molia S, et al. Nipah virus in Lyle's flying foxes, Cambodia. *Emerg Infect Dis*. 2005;11:1042–7. <https://doi.org/10.3201/eid1107.041350>
  7. Ching PK, de los Reyes VC, Sucaldito MN, Tayag E, Columna-Vingno AB, Malbas FF Jr, et al. Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis*. 2015;21:328–31. <https://doi.org/10.3201/eid2102.141433>
  8. Taniguchi S, Maeda K, Horimoto T, Masangkay JS, Puentes-pina R Jr, Alvarez J, et al. First isolation and characterization of pteropine orthoreoviruses in fruit bats in the Philippines. *Arch Virol*. 2017;162:1529–39. <https://doi.org/10.1007/s00705-017-3251-2>
  9. Kaku Y, Noguchi A, Marsh GA, Barr JA, Okutani A, Hotta K, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. *J Virol Methods*. 2012;179:226–32. <https://doi.org/10.1016/j.jviromet.2011.11.003>
  10. Tong S, Chern SW, Li Y, Pallansch MA, Anderson LJ. Sensitive and broadly reactive reverse transcription-PCR assays to detect novel paramyxoviruses. *J Clin Microbiol*. 2008;46:2652–8. <https://doi.org/10.1128/JCM.00192-08>

Address for correspondence: Shumpei Watanabe, Department of Microbiology, Faculty of Veterinary Medicine, Okayama University of Science, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan; email: s-watanabe@ous.ac.jp

## Crimean-Congo Hemorrhagic Fever Virus Africa 1 Lineage in *Hyalomma dromedarii* Ticks, Algeria, 2023

Marbouha Temani, Aissam Hachid, Rafik Garni, Amir Abderezzak Guessoum, Mohammed Hocine Benaissa, Ahmed Fayed Khardine, Abdelhakim Kimouche, Ahcène Hakem, Idir Bitam, Kamal Eddine Benallal,<sup>1</sup> Ismail Lafri<sup>1</sup>

Author affiliations: Institut Pasteur d'Algérie, Algiers, Algeria (M. Temani, A. Hachid, R. Garni, A.A. Guessoum, A.F. Khardine, K.E. Benallal, I. Lafri); Université Alger 1 Faculté de Pharmacie, Algiers (A. Hachid); Centre de Recherche Scientifique Et Technique Sur Les Régions Arides, Touggourt, Algeria (M.H. Benaissa); Inspection Vétérinaire, Direction des Services Agricoles de la Wilaya d'Illizi, Illizi, Algeria (A. Kimouche); Centre de Recherche en Agropastoralisme, Djelfa, Algeria (A. Hakem, I. Bitam); Université de Blida 1, Blida, Algeria (I. Lafri)

DOI: <https://doi.org/10.3201/eid3108.250123>

We conducted a Crimean-Congo hemorrhagic fever virus (CCHFV) survey of *Hyalomma* spp. ticks collected from camels in southeastern Algeria. Of 138 tick pools, 1 was CCHFV positive; the sequenced strain belonged to the Africa 1 genotype. Healthcare professionals in Algeria should be aware of this detection of a circulating pathogenic CCHFV genotype.

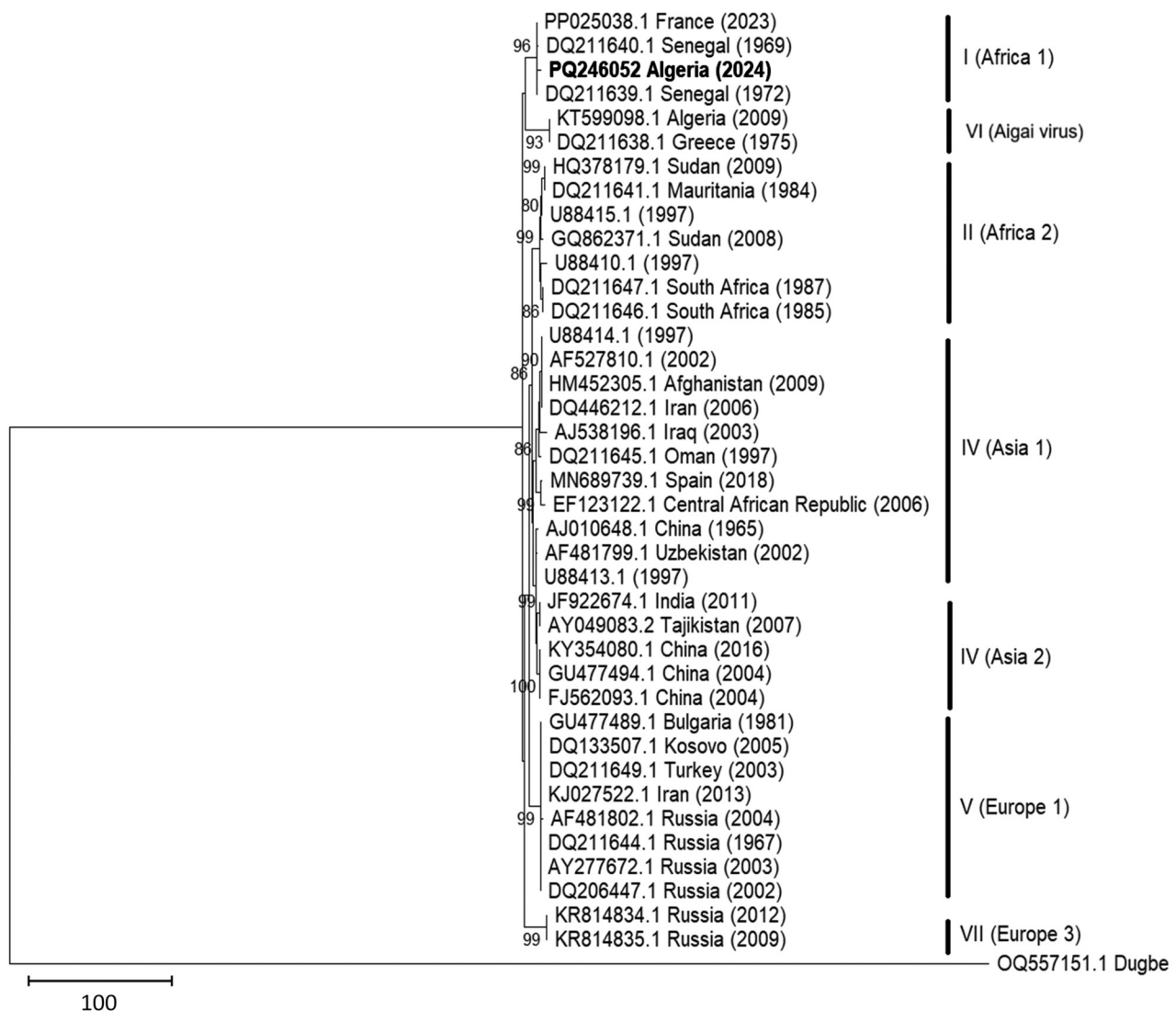
Infection with Crimean Congo hemorrhagic fever virus (CCHFV; *Orthonairovirus hemorrhagiae*; *Nairoviridae*: *Bunyavirale*) provokes fever and hemorrhagic manifestations in humans but results in asymptomatic infections in animals (1). CCHFV is maintained in nature through wild and domestic animals serving as amplification hosts and ticks as reservoirs. CCHFV is endemic to Africa, the Middle East, Asia, and Europe (2). However, knowledge of CCHFV in North Africa is limited to few serologic surveys and molecular characterization in ticks.

In Algeria, Agai virus (*Orthonairovirus parahemorrhagiae*), previously known as AP92-like CCHFV, has been detected in *Hyalomma aegyptium* ticks collected from tortoises (3). In addition, 2 seroprevalence studies of CCHFV conducted on dromedary camels (*Camelus dromedarius*) in different regions from southern Algeria showed a high rate of IgG against CCHFV (2,4). We aimed to detect CCHFV among ticks in southern Algeria, where serologic evidence of the virus was reported among camels.

<sup>1</sup>These authors contributed equally to this article.

During September–November 2023, we conducted surveillance for CCHFV in ticks collected from camels in the Wilayates (provinces) of Ouargla, Illizi, and Djanet, located in southeastern Algeria (Appendix, <https://wwwnc.cdc.gov/EID/article/31/8/25-0123-App1.pdf>). We morphologically identified ticks by using taxonomic keys and pooled specimens on the basis of species, sex, developmental stage, feeding status, and collection sites; we stored pools at  $-80^{\circ}\text{C}$  until analysis. We cleaned ticks with 70% ethanol and then crushed them by using a Retsch MM 400 Mixer Mills (<https://www.retsch.com>). We extracted nucleic acid material (RNA and DNA) from supernatants by using NucleoSpin Virus kits (Macherey-Nagel, [https://](https://www.mn-net.com)

[www.mn-net.com](https://www.mn-net.com)), according to the manufacturer's instructions. We screened tick extracts for CCHFV by using real-time reverse transcription PCR (RT-PCR) targeting the small (S) segment of CCHFV (5) and confirmed positive pools by using an endpoint RT-PCR targeting the S segment of the *Nairovirus* group (6), followed by Sanger sequencing. We molecularly confirmed positive pools by sequencing the mitochondrial cytochrome oxidase I gene (7). We constructed a maximum-likelihood tree with 1,000 bootstrap replicates using a Tamura 1992 with gamma distribution substitution model (8) using different CCHFV sequence genotypes (Figure). We deposited the sequence obtained in this study into GenBank (accession no. PQ246052).



**Figure.** Phylogenetic analysis of the small segment sequence of Crimean-Congo hemorrhagic fever virus Africa 1 lineage detected in ticks collected from camels in southeastern Algeria, 2023. Bold indicates the strain detected in Algeria; other sequences are labeled by GenBank accession number, geographic origin, and sampling year. Only bootstrap values  $>80$  are shown. Scale bar indicates substitutions per site.

**Table.** Description of CCHFV in tick pools collected from camels in southeastern Algeria, 2023\*

Wilayates (province)	Tick species	No. pools	No. ticks/pool, by sex	Positive pool no./Ct/sex
Ouargla	<i>Hyalomma dromedarii</i>	45	20 M, 25 F	
	<i>Hy. rufipes</i>	2	1 M, 1 F	
Illizi	<i>Hy. dromedarii</i>	60	23 M, 37 F	13/39.04/M; 22/26.91/M
	<i>Hy. rufipes</i>	4	1 M, 3 F	
	<i>Hy. impeltatum</i>	6	1 M, 5 F	19/36.24/M
	<i>Hy. impressum</i>	2	2 F	
Djanet	<i>Hy. dromedarii</i>	13	4 M, 9 F	
	<i>Hy. impeltatum</i>	4	4 F	
	<i>Hy. impressum</i>	2	2 M	

\*Pools of ticks were tested with quantitative reverse transcription PCR for the small segments of CCHFV RNA. A total of 138 pools, comprising 346 ticks collected from 103 camels, were tested for CCHFV. Only 3 pools of *Hy. dromedarii* ticks from Illizi were CCHFV positive; only the pool in bold, containing 5 male *Hy. dromedarii* ticks, generated a 465-bp fragment of the small segment using the endpoint RT-PCR. CCHFV, Crimean-Congo hemorrhagic fever virus; Ct, cycle threshold.

We grouped a total of 346 ticks collected from 103 camels into 138 pools. Tick species consisted of 290 (83.81%) *Hy. dromedarii*, 26 (7.51%) *Hy. rufipes*, 19 (5.49%) *Hy. impeltatum*, and 11 (3.17%) *Hy. impressum* (Table). Each pool contained 1–5 ticks grouped by feeding status, species, locality, and sex. Three pools tested positive for CCHFV by the first real-time RT-PCR: pool 22 (cycle threshold [Ct] value = 26.91), pool 19 (Ct = 36.24), and pool 13 (Ct = 39.04). Only pool 22, containing 5 male *Hy. dromedarii* ticks, generated a 465-bp fragment of the S segment using the endpoint RT-PCR. A maximum-likelihood tree showed that the Algeria sequence formed a monophyletic group or cluster with strains from Senegal and France belonging to the Africa 1 genotype (GenBank accession nos. DQ211639, DQ211639, and PP025038) with 95% bootstrap support (Figure). Molecular identification of ticks in positive pools using cytochrome oxidase I gene confirmed the presence of *Hy. dromedarii* and *Hy. impeltatum* ticks (Table), both species are known as competent vectors for CCHFV.

We detected and characterized a pathogenic strain of CCHFV in local tick populations collected from camels in southern Algeria, underscoring circulation of the virus in this region. Camels play a vital economic and cultural role in the region, especially through transhumance. However, movements between Algeria and endemic areas in neighboring countries through legal and illegal cross-border trade increase the likelihood of encountering viremic animals and tick vectors. Moreover, migratory birds from the Trans-Saharan Flyway carrying *Hyalomma* spp. ticks are likely a major source of CCHFV strains circulating between Africa and Europe, as reported in Morocco and France (9,10). Our findings suggest that the possible pathway of CCHFV dissemination to Algeria from endemic areas could involve migratory birds, considering that the CCHFV Africa 1 strain identified in this study is phylogenetically closely related to the strains previously reported in Corsica

(France) and Senegal. The potential for the continuous spread of CCHFV across Algeria and North Africa is substantial. Indeed, Algeria's large territory harbors various tick species known for their CCHFV transmission competence, increasing the likelihood of CCHFV circulation among ticks and animals. This study, limited to 3 provinces in the Sahara, serves as a starting point for broader epidemiologic studies across the country; expanding surveillance to other regions, animals, humans, and tick vectors is crucial for informing policy-makers and enabling a comprehensive risk assessment of CCHFV exposure in Algeria. Using next-generation sequencing technologies for whole-genome sequencing of CCHFV will enable detailed genomic characterization and clarify spatio-temporal transmission dynamics.

In summary, our results document detection of a CCHFV pathogenic genotype among camels in Algeria, carried by *Hyalomma* spp. ticks. Healthcare professionals should be aware of CCHFV circulation in this region and the resulting potential for human infection.

### Acknowledgments

We thank the local veterinary authorities for their technical and administrative supports and all camel breeders for their collaboration during sample collection.

This study was carried out under the auspices of the Ministry of Higher Education and Scientific Research of Algeria, as part of 2 approved research projects. Informed consents were obtained from all the camel owners at the time of tick sampling. The procedure for tick collection was performed by qualified entomologists of Institut Pasteur of Algeria. To ensure the well-being and safety of both the staff and the animals, appropriate physical restraint measures were taken before conducting tick collection.

This study was financially supported by the project LABEX-TA 2019 (EVARBO) and the project Programme

National de Recherche, supervised by I.L. and funded by the Ministry of Higher Education and Scientific Research of Algeria, The Directorate-General for Scientific Research and Technological Development, registered in 2021 and 2023.

## About the Author

Dr. Temani is scientific microbiologist at Institut Pasteur d'Algérie, Algiers, Algeria. Her research interest are arboviruses and seroneutralization techniques.

## References

1. Sánchez-Seco MP, Sierra MJ, Estrada-Peña A, Valcárcel F, Molina R, de Arellano ER, et al.; Group for CCHFv Research. Widespread detection of multiple strains of Crimean-Congo hemorrhagic fever virus in ticks, Spain. *Emerg Infect Dis.* 2021;28:394–402. <https://doi.org/10.3201/eid2802.211308>
2. Degui D, Hachid A, Derrar F, Messahel NE, Bia T, Mockbel Y, et al. A survey of the tick-borne disease Crimean-Congo hemorrhagic fever in southern Algeria: first serological evidence in the dromedary camel population. *Vet Parasitol Reg Stud Reports.* 2024;54:101089. <https://doi.org/10.1016/j.vprsr.2024.101089>
3. Kautman M, Tiar G, Papa A, Široký P. AP92-like Crimean-Congo hemorrhagic fever virus in *Hyalomma aegyptium* ticks, Algeria. *Emerg Infect Dis.* 2016;22:354–6. <https://doi.org/10.3201/eid2202.151528>
4. Guidoum KA, Carrera-Faja L, Espunyes J, Pailler-García L, Benallou B, Bouabdelli S, et al. Crimean-Congo hemorrhagic fever virus seropositivity among dromedary camels, Algeria, 2020–2021. *Emerg Infect Dis.* 2023;29:2546–8. <https://doi.org/10.3201/eid2912.230587>
5. Atkinson B, Chamberlain J, Logue CH, Cook N, Bruce C, Dowall SD, et al. Development of a real-time RT-PCR assay for the detection of Crimean-Congo hemorrhagic fever virus. *Vector Borne Zoonotic Dis.* 2012;12:786–93. <https://doi.org/10.1089/vbz.2011.0770>
6. Lambert AJ, Lanciotti RS. Consensus amplification and novel multiplex sequencing method for S segment species identification of 47 viruses of the *Orthobunyavirus*, *Phlebovirus*, and *Nairovirus* genera of the family *Bunyaviridae*. *J Clin Microbiol.* 2009;47:2398–404. <https://doi.org/10.1128/JCM.00182-09>
7. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol.* 1994;3:294–9.
8. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Mol Biol Evol.* 2021;38:3022–7. <https://doi.org/10.1093/molbev/msab120>
9. Palomar AM, Portillo A, Santibañez P, Mazuelas D, Arizaga J, Crespo A, et al. Crimean-Congo hemorrhagic fever virus in ticks from migratory birds, Morocco. *Emerg Infect Dis.* 2013;19:260–3. <https://doi.org/10.3201/eid1902.121193>
10. Kiwan P, Masse S, Piorkowski G, Ayhan N, Gasparine M, Vial L, et al. Crimean-Congo hemorrhagic fever virus in ticks collected from cattle, Corsica, France, 2023. *Emerg Infect Dis.* 2024;30:1036–9. <https://doi.org/10.3201/eid3005.231742>

Address for correspondence: Ismail Lafri, Institut Pasteur d'Algérie, Route du Petit Staoueli, Dely-Ibrahim, Algiers 16000, Algeria; email: lafrismail@gmail.com

# Emergence of Novel Fluoroquinolone Resistance Mutations in *Mycoplasma bovis*, China, 2008–2023

Shimei Lan, Shuang Liu, Wenjing Cui, Zhangcheng Li, Huafang Hao, Ahmed Adel Baz, Jinjia Liang, Xiangrui Jin, Xinmin Yan, Pengcheng Gao, Fuying Zheng, Shengli Chen,<sup>1</sup> Yuefeng Chu<sup>1</sup>

Author affiliations: State Key Laboratory for Animal Disease Control and Prevention, College of Veterinary Medicine, Lanzhou University, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China (S. Lan, S. Liu, W. Cui, Z. Li, H. Hao, A.A. Baz, J. Liang, X. Jin, X. Yan, P. Gao, F. Zheng, S. Chen, Y. Chu); Gansu Province Research Center for Basic Disciplines of Pathogen Biology, Lanzhou (S. Lan, S. Liu, Z. Li, H. Hao, A.A. Baz, J. Liang, X. Jin, X. Yan, P. Gao, F. Zheng, S. Chen, Y. Chu); Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Ruminant Disease Prevention and Control (West), Ministry of Agricultural and Rural Affairs, Lanzhou (S. Lan, S. Liu, Z. Li, H. Hao, A.A. Baz, J. Liang, X. Jin, X. Yan, P. Gao, F. Zheng, S. Chen, Y. Chu)

DOI: <https://doi.org/10.3201/eid3108.241137>

We investigated quinolone resistance in *Mycoplasma bovis* samples isolated in China during 2008–2023. Sequence type 52 was the dominant genotype; GyrA (S83F/Y) and ParC (S80R) protein double mutations caused high resistance to fluoroquinolones. Increased vigilance and surveillance of *M. bovis* infections in cattle will be needed to prevent disease.

Diseases in cattle caused by *Mycoplasma bovis* include bronchopneumonia, mastitis, and arthritis (1,2). *M. bovis* was first isolated in 1961 (3) and, over the past >6 decades, it has become widespread worldwide. Bovine mycoplasmosis caused by *M. bovis* is an emerging disease in China. Since the first isolation of *M. bovis* strains in China's Hubei region in 2008, those strains have spread rapidly and extensively to most provinces in China (4–6). However, the epidemiologic features of *M. bovis* in China are unknown. Antimicrobial drugs are currently a critical means of controlling *M. bovis* infections (7,8). Fluoroquinolones have a substantial bactericidal effect against *Mycoplasma* spp.; however, their effectiveness has been gradually declining (9,10). Fluoroquinolone resistance in *Mycoplasma* spp. relies primarily on gene point mutations (7).

<sup>1</sup>These senior authors contributed equally to this article.