RESEARCH LETTERS

Therefore, the new H7N9 viruses were highly pathogenic to chickens when compared with the early H7N9 virus and could transmit among chickens by contact.

The biological features of H7N9 virus and its pandemic potential have caused global concern (8). The early H7N9 viruses lacked the basic HA cleavage site, exhibited low pathogenicity, and caused mild or no disease in poultry (9). The cleavage site in HA protein of the isolates we analyzed were KGKRTAR⁻G or KRKRTAR⁻G. They had high pathogenicity and replication in chickens and could transmit among chickens by contact. Therefore, these new H7N9 viruses could cause a pandemic among poultry and humans in China.

Molecular evolution showed that Q1 was a triple reassortant virus (H5, H7, and H9 subtypes) consisting of Yangtze River Delta A and B lineages of H7N9 and GSGD96 lineage of H5N1. The Q26 and Q39 viruses were both double reassortant avian influenza viruses (H7 and H9 subtype), as was the early H7N9 virus (Figure; online Technical Appendix Table 1, Figure). Therefore, the 3 H7N9 viruses we isolated have 2 kinds of insertions in the cleavage sites and were likely derived from different lineages of H7N9 viruses, or even from different subtypes that were co-circulating in southern China during 2016–2017.

Acknowledgments

We thank the Key Laboratory of Zoonosis, Ministry of Agriculture; the National and Regional Joint Engineering Laboratory for Medicament of Zoonosis Prevention and Control; the Key Laboratory of Animal Vaccine Development, Ministry of Agriculture; and the Key Laboratory of Zoonoses Control and Prevention of Guangdong.

This work was supported by grants from the National Key Research and Development Program of China (2016YFD0500207), the National Natural Science Foundation of China (U1501212), the Natural Science Foundation of Guangdong Province (2016A030308001), and Basic Research (Discipline Layout) of Shenzhen (JCYJ20160323163102764).

Mr. Nianchen Wang is a student in the Master of Science program in the College of Veterinary Medicine, South China Agricultural University. His primary research interest is the epidemiology and pathogenesis of avian influenza viruses.

References

- World Health Organization. Update on monthly risk assessment summary [cited 2017 Apr 20]. http://www.who.int/influenza/ human_animal_interface/Influenza_Summary_IRA_HA_ interface 04 20 2017.pdf?ua=1
- Wang D, Yang L, Zhu W, Zhang Y, Zou S, Bo H, et al. Two outbreak sources of influenza A (H7N9) viruses have been established in China. J Virol. 2016;90:5561–73. http://dx.doi.org/ 10.1128/JVI.03173-15
- 3. Kang M, He J, Song T, Rutherford S, Wu J, Lin J, et al. Environmental sampling for avian influenza A(H7N9) in

live-poultry markets in Guangdong, China. PLoS One. 2015; 10:e0126335. http://dx.doi.org/10.1371/journal.pone.0126335

- Shi J, Deng G, Liu P, Zhou J, Guan L, Li W, et al. Isolation and characterization of H7N9 viruses from live poultry markets implication of the source of current H7N9 infection in humans. Chin Sci Bull. 2013;58:1857–63. https://doi.org/10.1007/ s11434-013-5873-4
- Ramos I, Krammer F, Hai R, Aguilera D, Bernal-Rubio D, Steel J, et al. H7N9 influenza viruses interact preferentially with α2,3linked sialic acids and bind weakly to α2,6-linked sialic acids. J Gen Virol. 2013;94:2417–23. http://dx.doi.org/10.1099/vir.0.056184-0
- Li Z, Chen H, Jiao P, Deng G, Tian G, Li Y, et al. Molecular basis of replication of duck H5N1 influenza viruses in a mammalian mouse model. J Virol. 2005;79:12058–64. http://dx.doi.org/10.1128/JVI.79.18.12058-12064.2005
- Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. Science. 2001;293:1840–2. http://dx.doi.org/10.1126/science.1062882
- Zhou J, Wang D, Gao R, Zhao B, Song J, Qi X, et al. Biological features of novel avian influenza A (H7N9) virus. Nature. 2013;499:500–3. http://dx.doi.org/10.1038/nature12379
- World Health Organization. Update on analysis of recent scientific information on avian influenza A (H7N9) virus [cited 2017 Feb 10]. http://www.who.int/influenza/human_animal_interface/avian_ influenza/riskassessment_AH7N9_201702/en/

Address for correspondence: Peirong Jiao or Ming Liao, College of Veterinary Medicine, South China Agricultural University, 483 Wushan Rd, Guangzhou 510642, China; email: prjiao@scau.edu.cn or mliao@scau.edu.cn

Rabies and Distemper Outbreaks in Smallest Ethiopian Wolf Population

Jorgelina Marino, Claudio Sillero-Zubiri, Asefa Deressa, Eric Bedin, Alemayehu Bitewa, Fekadu Lema, Gebeyehu Rskay, Ashley Banyard, Anthony R. Fooks

Author affiliations: University of Oxford, Oxford, UK (J. Marino, C. Sillero-Zubiri, E. Bedin); International Union for Conservation of Nature Species Survival Commission Canid Specialist Group, Oxford (C. Sillero-Zubiri); Ethiopian Public Health Institute, Addis Ababa, Ethiopia (A. Deressa); Environmental, Forest, Wildlife Development and Protection Authority, Bahir Dar, Ethiopia (A. Bitewa); Ethiopian Wolf Conservation Programme, Bale Robe, Ethiopia (J. Marino); Ethiopian Wolf Conservation Programme, Bahir Dar (F. Lema, G. Rskay); Animal and Plant Health Agency, Weybridge, UK (A. Banyard, A.R. Fooks)

DOI: https://doi.org/10.3201/eid2312.170893

Widespread deaths recently devastated the smallest known population of Ethiopian wolves. Of 7 carcasses found, all 3 tested were positive for rabies. Two wolves were subsequently vaccinated for rabies; 1 of these later died from canine distemper. Only 2 of a known population of 13 wolves survived.

Anine diseases pose a growing threat to wildlife species of conservation concern worldwide. Although extensive oral vaccinations have eliminated rabies virus (RABV) from wild carnivore populations in some developed countries (1), elsewhere, the challenges to controlling diseases in endangered wildlife are many and persistent. Massive outbreaks of rabies and, more recently, canine distemper have repeatedly decimated populations of Ethiopian wolves (Canis simensis) in the Bale Mountains of Ethiopia, where more than half of a global population of ≈ 500 wolves live (2,3). Extensive efforts to control RABV in the reservoir population of sympatric domestic dogs have proved insufficient. Therefore, reactive vaccination of Ethiopian wolves, carried out in response to an outbreak in wolves, has been the primary mechanism to curtail mortality in the affected wolf populations in the Bale Mountains (4).

The fragile status of the Bale population highlights the conservation value of the other remaining, much smaller, wolf populations scattered throughout the highlands of Ethiopia. Models predict these small populations to be particularly vulnerable to disease outbreaks (5); however, no outbreaks had been detected outside Bale, either because they went unnoticed, because in small populations outbreaks die out before causing a major epizootic event, or both. We report consecutive rabies and canine distemper outbreaks among Ethiopian wolves in Delanta, in the Wollo highlands.

This group of wolves is the smallest extant wolf population; 13 wolves in 3 family packs lived in the remaining 20 km² of Afroalpine habitat in 2015. The first wolf carcass was detected in late June 2016; by early September, 7 deaths had been confirmed. RABV infection was identified as the cause of death in all 3 of the carcasses tested, as well as in samples from 1 domestic dog concurrently found dead within wolf habitat (Table). A vaccination intervention was initiated in September 2016, when only 3 wolves were known to be alive; 1 adult male (>2 years of age) and 1 subadult female (1–2 years of age) were trapped (7) and parenterally inoculated with Nobivac Rabies (Merck Animal Health, Madison, NJ, USA) (4). In December 2016, the female wolf was found dead and tested positive for canine distemper virus (CDV) (Table); CDV was also detected in a dog carcass found concurrently in the vicinity of the wolf range. In late May, the vaccinated male was still alive and was observed until at least April 2017 with an unknown adult female.

Evidence indicates a first outbreak of rabies, overlapping or followed soon after by a canine distemper outbreak. Confirmation of disease in contemporarily recovered dog carcasses is consistent with a pattern of transmission from reservoir domestic dogs to their wild relatives (as observed in the Bale Mountains [8]), with disastrous consequences for the small Delanta population, which harbored <20 wolves before the epizootic events. Although the larger Bale wolf population has recovered from epizootic events in the past (2,9), smaller populations are expected to be less resilient, a factor exacerbated by their virtual isolation from other wolf populations. Modeling has predicted a high extinction risk if Ethiopian wolf populations are affected by consecutive epizootic events over a short period of time (5). The combined exacerbated effects of RABV and CDV infection were first described in 2010 in the Bale Mountains (3).

Although the loss of Afroalpine habitats is bound to determine the fate of Ethiopian wolf populations (2 extinctions were recorded in areas of a similar size to that of Delaware during 1999 and 2010) (2), incursions of infectious diseases can drive local extinctions. Preemptive vaccination, in combination with actions to protect the habitat of this specialized predator, could greatly reduce the risk of populations becoming extinct, even if a relatively low proportion of the wolves is successfully vaccinated (4). Recently, SAG2, an oral rabies vaccine, was successfully tested in Ethiopian wolves (10), and a CDV parenteral vaccination trial is ongoing, with positive

Date found	Species	Age and sex	Postmortem	Tested for RABV ⁺	Tested for CDV
Jun 26	Ethiopian wolf	Juvenile female	No	NA	NA
Jun 28	Ethiopian wolf	Juvenile male	Yes	No	No
Jul 07	Ethiopian wolf	Adult male	Yes	Positive	No
Jul 11	Ethiopian wolf	Juvenile female	Yes	Positive	No
lul 18	Ethiopian wolf	Juvenile female	No	NA	NA
Nug 12	Ethiopian wolf	Adult male	No	NA	NA
Sep 01	Domestic dog	Adult male	Yes	Positive	Negative
Sep 07	Ethiopian wolf	Adult female	Yes	Positive	Negative
Nov 27	Domestic dog	Adult male	Yes	Negative	Positive
Dec 27	Ethiopian wolf	Adult female	Yes	Negative	Positive

*CDV, canine distemper virus; NA, not applicable; RABV, rabies virus.

†Rabies diagnostic reverse transcription PCR was performed as described previously (6).

‡CDV diagnostic reverse transcription PCR was performed as described previously (3).

RESEARCH LETTERS

preliminary results. We propose proactive vaccination of Ethiopian wolves across their distribution as an effective and urgently needed strategy to protect the species from extinction. This program should be part of an integrated disease control plan that also includes controlling disease in domestic dogs, limiting contact between dogs and wolves, and conducting policy and education interventions to reduce the size and roaming behavior of local dog populations (2).

Acknowledgments

We thank the Ethiopian Wildlife Conservation Authority and Environment and Forest and Wildlife Protection and Development Authority (Amhara National Regional State) for support and permission to work in Delanta. We thank Delanta and Gubalaftu Waredas and Wolf Ambassadors, and Leigh Thorne and Daisy Jennings for excellent technical assistance.

The work was funded by the Born Free Foundation and the Wildlife Conservation Network. The work undertaken by Animal and Plant Health Agency is funded by a grant (SEV3500) from the UK Department for Environment, Food and Rural Affairs, Scottish and Welsh Governments.

The animal care and use protocols for the ethical handling of Ethiopian wolves were approved by the Ethiopian Wildlife Conservation Authority and the University of Oxford's Local Ethical Review Process (Zoology ERC; case no. ZERC040905) and adhere to the United Kingdom's ASPA regulations (1986).

Dr. Marino is a conservation ecologist with the University of Oxford's Wildlife Conservation Research Unit (WildCRU), Oxford, UK, and is science director of the Ethiopian Wolf Conservation Programme. She is interested in the ecology and conservation of threatened carnivores, and coordinates Ethiopian Wolf Conservation Programme monitoring efforts, looking at the demography of the rare and endangered Ethiopian wolf, interactions with free-ranging dogs, and the impact of viral diseases.

References

- Mähl P, Cliquet F, Guiot A-L, Niin E, Fournials E, Saint-Jean N, et al. Twenty year experience of the oral rabies vaccine SAG2 in wildlife: a global review. Vet Res (Faisalabad). 2014;45:77. http://dx.doi.org/10.1186/s13567-014-0077-8
- Strategic planning for Ethiopian wolf conservation. Oxford (UK): International Union for the Conservation of Nature, Canid Specialist Group; 2011. p. 79 [cited 2017 May 25]. https://portals.iucn.org/library/efiles/documents/2011-090.pdf
- Gordon CH, Banyard AC, Hussein A, Laurenson MK, Malcolm JR, Marino J, et al. Canine distemper in endangered Ethiopian wolves. Emerg Infect Dis. 2015;21:824–32. http://dx.doi.org/10.3201/ eid2105.141920
- Haydon DT, Randall DA, Matthews L, Knobel DL, Tallents LA, Gravenor MB, et al. Low-coverage vaccination strategies for the conservation of endangered species. Nature. 2006;443:692–5. http://dx.doi.org/10.1038/nature05177
- 5. Haydon DT, Laurenson MK, Sillero-Zubiri C. Integrating epidemiology into population viability analysis: managing the risk

posed by rabies and canine distemper to the Ethiopian wolf. Conserv Biol. 2002;16:1372–85. http://dx.doi.org/10.1046/j. 1523-1739.2002.00559.x

- Johnson N, Mansfield KL, Marston DA, Wilson C, Goddard T, Selden D, et al. A new outbreak of rabies in rare Ethiopian wolves (*Canis simensis*). Arch Virol. 2010;155:1175–7. http://dx.doi.org/ 10.1007/s00705-010-0689-x
- Sillero-Zubiri C. Field immobilization of Ethiopian wolves (*Canis simensis*). J Wildl Dis. 1996;32:147–51. http://dx.doi.org/ 10.7589/0090-3558-32.1.147
- Laurenson K, Sillero-Zubiri C, Thompson H, Shiferaw F, Thirgood S, Malcolm J. Disease as a threat to endangered species: Ethiopian wolves, domestic dogs and canine pathogens. Anim Conserv. 1998;1:273–80. http://dx.doi.org/10.1111/j.1469-1795. 1998.tb00038.x
- Marino J, Sillero-Zubiri C, Gottelli D, Johnson PJ, Macdonald DW. The fall and rise of Ethiopian wolves: lessons for conservation of long-lived, social predators. Anim Conserv. 2013;16:621–32. http://dx.doi.org/10.1111/acv.12036
- Sillero-Zubiri Č, Marino J, Gordon CH, Bedin E, Hussein A, Regassa F, et al. Feasibility and efficacy of oral rabies vaccine SAG2 in endangered Ethiopian wolves. Vaccine. 2016;34:4792–8. 10.1016/j.vaccine.2016.08.021 http://dx.doi.org/10.1016/ j.vaccine.2016.08.021

Address for correspondence: Jorgelina Marino, Wildlife Conservation Research Unit, University of Oxford, The Recanati-Kaplan Centre, Tubney House, Tubney, Oxford OX13 5QL, UK; email: jorgelina.marino@zoo.ox.ac.uk

High Abundance and Genetic Variability of Atypical Porcine Pestivirus in Pigs from Europe and Asia

Alexander Postel, Denise Meyer, Gökce Nur Cagatay, Francesco Feliziani, Gian Mario De Mia, Nicole Fischer, Adam Grundhoff, Vesna Milićević, Ming-Chung Deng, Chia-Yi Chang, Hua-Ji Qiu, Yuan Sun, Michael Wendt, Paul Becher

Author affiliations: University of Veterinary Medicine, Hannover, Germany (A. Postel, D. Meyer, G.N. Cagatay, M. Wendt, P. Becher); Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy (F. Feliziani, G.M. De Mia); University Medical Center Hamburg-Eppendorf, Hamburg, Germany (N. Fischer); Heinrich Pette Institute, Hamburg (A. Grundhoff); Institute of Veterinary Medicine of Serbia, Belgrade, Republic of Serbia (V. Milićević); Animal Health Research Institute,