Using MEGA 7.0 software (http://www.megasoftware.net) with the maximum-likelihood algorithm and a bootstrap of 1,000 replicates, we constructed a phylogenetic tree (online Technical Appendix Figure). All 8 sequences clustered within subtype 4d of HEV. The sequences were similar to each other (95.5%–99.8% similarity in nucleotide sequence) and similar to sequences reported for other cattle (83.3%–85.3%; online Technical Appendix Figure). Moreover, these sequences shared 96.1%–96.6% similarity with a human HEV strain (GenBank accession no. KC163335) from the Yantai Prefecture in 2012 and 95.7%–97.9% similarity with a swine strain (GenBank accession no. KF176351) isolated in Shandong Province the same year.

Our data strongly indicate that HEV infection occurs in yellow cattle and that they could also play a role as a reservoir of HEV. Because these animals serve mainly as a source of food, consumption of undercooked meat from yellow cattle, similar to pork, might also contribute to the transmission of HEV to humans. Additionally, we also detected HEV RNA in 8 of 70 sheep (online Technical Appendix Table 2). Eight sequences from yellow cattle had 95.1%–99.8% nt homology with 8 sheep-derived HEV strains, possibly because mixed raising of domestic livestock is popular in this region. Our finding of high sequence similarity between yellow cattle, sheep, swine, and human populations suggests a complicated interspecies transmission of HEV occurred in this province. Further studies are required to evaluate the contribution of the yellow cattle reservoir to human HEV infection.

Acknowledgments

References

1. Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, et al. Hepatitis E. Lancet. 2012;379:2477–88. http://dx.doi.org/10.1016/S0140-6736(11)61849-7
2. Pérez-Gracia MT, García M, Suay B, Mateos-Lindemann ML. Current knowledge on hepatitis E. J Clin Transl Hepatol. 2015;3:117–26. http://dx.doi.org/10.14218/JCTH.2015.00009
3. Xu F, Pan Y, Baloch AR, Tian L, Wang M, Na W, et al. Hepatitis E virus genotype 4 in yak, northwestern China. Emerg Infect Dis. 2014;20:2182–4. http://dx.doi.org/10.3201/eid2012.131599
4. Huang F, Li Y, Yu W, Jing S, Wang J, Long F, et al. Excretion of infectious hepatitis E virus into milk in cows imposes high risks of zoonosis. Hepatology. 2016;64:350–9. http://dx.doi.org/10.1002/hep.28668
5. Hu GD, Ma X. Detection and sequences analysis of bovine hepatitis E virus RNA in Xinjiang autonomous region [in Chinese]. Bing Du Xue Bao. 2010;26:27–32.
6. Li K, Zhang Y, Mao Y, Cornforth D, Dong P, Wang R, et al. Effect of very fast chilling and aging time on ultra-structure and meat quality characteristics of Chinese yellow cattle M. longissimus lumborum. Meat Sci. 2012;92:795–804. http://dx.doi.org/10.1016/j.meatsci.2012.07.003
7. Wang YC, Zhang HY, Xia NS, Peng G, Lan HY, Zhuang H, et al. Prevalence, isolation, and partial sequence analysis of hepatitis E virus from domestic animals in China. J Med Virol. 2002;67:516–21. http://dx.doi.org/10.1002/jmv.10131
8. Geng Y, Wang C, Zhao C, Yu X, Harrison TJ, Tian K, et al. Serological prevalence of hepatitis E virus in domestic animals and diversity of genotype 4 hepatitis E virus in China. Vector Borne Zoonotic Dis. 2010;10:765–70. http://dx.doi.org/10.1089/vbz.2009.0168
9. Li F, Meng JH, Dong C, Dai X, Yang YG, Zhou ZX. Design and application of a set of universal PCR primers for genotyping of hepatitis E virus [in Chinese]. Bing Du Xue Bao. 2009;25:9–16.
10. Smith DB, Simmonds P, Izopet J, Oliveira-Filho EF, Ulrich RG, Johne R, et al. Proposed reference sequences for hepatitis E virus subtypes. J Gen Virol. 2016;97:537–42. http://dx.doi.org/10.1099/jgv.0.000393

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Introgressed Animal Schistosomes *Schistosoma curassoni* and *S. bovis* Naturally Infecting Humans

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To the Editor: Schistosomiasis, a disease caused by infection with parasitic worms (schistosomes), is a neglected tropical disease across many parts of the world. Numbers of infected livestock are unknown, but >250 million persons are infected; the greatest number of cases are in sub-Saharan Africa (*1*). Schistosome eggs are excreted through urine or feces, depending on the species, and hatch into miracidia upon contact with freshwater. Larvae are transmitted to the mammalian host indirectly through a molluscan intermediate host. Goals to eliminate schistosomiasis by 2020 in select countries in Africa have

been proposed by the World Health Organization (http://www.who.int/neglected_diseases/NTD_RoadMap_2012_Fullversion.pdf) and a coalition of partners combating neglected tropical diseases (*2*).

Selective pressures imposed by natural phenomena and human activities affect the dynamics and distribution of schistosomiasis. For example, ongoing changes in agricultural practices in some regions are creating new water bodies shared by humans and domestic livestock. These anthropogenic changes increase opportunities for mixing of and subsequent exposure to different *Schistosoma* species, especially those that infect humans and livestock. Such mixing could increase the potential for novel zoonotic hybrid parasites to emerge and become established (*3,4*).

Since the 1940s, researchers have suspected that natural hybridization within and between human and animal schistosome species occurs in definitive and intermediate hosts. In western Africa, hybrids, predominantly between *S. haematobium* (human schistosome) and *S. bovis* or *S. curassoni* (livestock schistosomes), have been isolated from children and snails (*5,6*). Hybrids between these livestock-only *Schistosoma* species have also been reported in cattle and sheep but not in other hosts (*7*). As demonstrated in the field and under experimental laboratory conditions, neither *S. bovis* nor *S. curassoni,* as single species, can fully develop in humans or nonhuman primates (*8,9*).

We report evidence of a child in Niger who was infected by livestock-specific schistosomes through the hybridization and introgression of *S. bovis* with *S. curassoni*. Samples were collected from a 10-year-old girl in Kokourou, Tillaberi region, Niger (14°20′61.50″N, 0°91′94.0″E), as part of longitudinal monitoring and evaluation of national disease control interventions in the area. Tillaberi has an ongoing high prevalence and infection intensity of schistosomiasis, despite more than a decade of high-coverage mass treatment with praziquantel.

Schistosoma eggs were recovered from the child's urine. Miracidia that hatched from the eggs were stored on a Whatman FTA card (Sigma-Aldrich, St. Louis, MO, USA), providing the opportunity to perform noninvasive molecular characterization on schistosome larvae without any necessity for laboratory passage. Multilocus analyses of mitochondrial and nuclear DNA regions (*6*) on all 42 larvae collected from the child identified 2 individual miracidia with a livestock *S. bovis* × *S. curassoni* hybrid profile with no genetic signal from human-specific *S. haematobium*. Although no species-specific markers exist for determining parental lineages, internal transcribed spacer (ITS), a powerful marker for detecting introgression, has been used successfully to detect hybridization events across the *Schistosoma* genus. A partial mitochondrial *cox*1 sequence for miracidia was identical to that for *S. bovis*, and the nuclear ITS and partial 18S rDNA sequences were identified as *S. curassoni*.

One miracidium from the child had a pure *S. haematobium* profile, 13 had *S. bovis* (*cox*1) × *S. haematobium* (ITS) profiles, and 5 had *S. bovis* (*cox*1) × *S. haematobium* × *S. curassoni* (ITS) profiles, suggesting the potential for repeated interactions and cross-pairings among these 3 species. No *S. curassoni* mitochondrial DNA was found in miracidia. The molecular data suggest that these hybrids were not first generation, but a result of parental and/or hybrid backcrosses. The data confirm the occurrence of bidirectional introgression between *Schistosoma* species that infect livestock and those that infect humans in Niger. Our data also show that hybrid livestock:livestock schistosomes can directly infect humans without combining with a *Schistosoma* species that infects humans.

Hybridization of parasites is an emerging public health concern at the interface of infectious disease biology and evolution (*3*). Our results raise several critical questions regarding the evolution, epidemiology, health effects, and ultimate control of schistosomiasis. Hybrid schistosomes, and, in particular, hybridized livestock:livestock schistosomes infecting humans, could potentially extend intermediate and definitive host ranges; confer altered infectivity, virulence, and drug efficacy; or even potentially replace existing single species (*3,4*). Hybrid vigor has been observed experimentally for schistosomes, and similar evidence is gathering from experiments with other parasites. Our results strongly indicate that hybrid livestock:livestock schistosomes can infect a human definitive host, even when neither of its single parental livestock species appears compatible. Such novel livestock schistosome hybrids infecting humans could be predicted to spread to wherever suitable intermediate snail hosts are endemic, as has recently been reported for zoonotic *Schistosoma* hybrids in Corsica (*10*).

It is imperative to identify and understand the transmission dynamics of introgressed *Schistosoma* species combinations. The detection of multiple introgressed hybrids with mixed ancestry in a single child suggests that *Schistosoma* species may be adapting to recent anthropogenic changes. If novel zoonotic hybrid species are playing a role in maintaining and exacerbating schistosome transmission, illness caused by infection, or both, treatments for humans and livestock may have to be adjusted accordingly within a One Health framework (*4*).

References

1. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis. 2006;6:411–25 http://dx.doi.org/10.1016/S1473-3099(06)70521-7
2. Uniting to Combat Neglected Tropical Diseases. London declaration on neglected tropical diseases [cited 2016 Jan 22]. http://unitingtocombatntds.org/resource/london-declaration
3. King KC, Stelkens RB, Webster JP, Smith DF, Brockhurst MA. Hybridization in parasites: consequences for adaptive evolution, pathogenesis, and public health in a changing world. PLoS Pathog. 2015;11:e1005098 http://dx.doi.org/10.1371/journal.ppat.1005098
4. Webster JP, Gower CM, Knowles SC, Molyneux DH, Fenton A. One Health—an ecological and evolutionary framework for tackling neglected zoonotic diseases. Evol Appl. 2016;9:313–33 http://dx.doi.org/10.1111/eva.12341
5. Huyse T, Webster BL, Geldof S, Stothard JR, Diaw OT, Polman K, et al. Bidirectional introgressive hybridization between a cattle and human schistosome species. PLoS Pathog. 2009;5:e1000571 http://dx.doi.org/10.1371/journal.ppat.1000571
6. Webster BL, Diaw OT, Seye MM, Webster JP, Rollinson D. Introgressive hybridization of Schistosoma haematobium group species in Senegal: species barrier break down between ruminant and human schistosomes. PLoS Negl Trop Dis. 2013;7:e2110. http://dx.doi.org/10.1371/journal.pntd.0002110
7. Rollinson D, Southgate VR, Vercruysse J, Moore PJ. Observations on natural and experimental interactions between Schistosoma bovis and S. curassoni from West Africa. Acta Trop. 1990;47:101–14 http://dx.doi.org/10.1016/0001-706X(90)90072-8
8. Taylor MG, Nelson GS, Smith M, Andrews BJ. Comparison of the infectivity and pathogenicity of six species of African schistosomes and their hybrids. 2. Baboons. J Helminthol. 1973;47:455–85 http://dx.doi.org/10.1017/S0022149X00027462
9. Vercruysse J, Southgate VR, Rollinson D. Schistosoma curassoni Brumpt, 1931 in sheep and goats in Senegal. Journal of Natural History. 1984;18:969–76 http://dx.doi.org/10.1080/00222938400770851
10. Moné H, Holtfreter MC, Allienne JF, Mintsa-Nguéma R, Ibikounlé M, Boissier J, et al. Introgressive hybridizations of Schistosoma haematobium by Schistosoma bovis at the origin of the first case report of schistosomiasis in Corsica (France, Europe). Parasitol Res. 2015;114:4127–33 http://dx.doi.org/10.1007/s00436-015-4643-4

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Rickettsia raoultii in *Dermacentor reticulatus* Ticks, Chernobyl Exclusion Zone, Ukraine, 2010

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To the Editor: The Chernobyl Exclusion Zone (CEZ) surrounds the center of the 1986 Chernobyl nuclear power plant disaster. Preliminary study shows predominance of *Dermacentor reticulatus* ticks in the CEZ; ticks of other species, such as *Ixodes ricinus*, are surprisingly rare, even in habitats where they should be relatively common (*1*). A few reports document presence of *Ix. trianguliceps* ticks (*2,3*). Prevalence of pathogens (*Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Babesia* spp.) in these ticks is higher in the CEZ than in other regions (*3,4*). One pathogen transmitted by *Dermacentor* spp. ticks is *Rickettsia raoultii*, which has been isolated from species of *Dermacentor* ticks found in Asia (*5,6*) and since 1999 has also been detected in Europe.

In our study, *D. reticulatus* ticks were collected by use of the flagging method (*1*) in the CEZ in September 2010. Ticks were collected from areas where they were known to occur, around the former villages of Korohod (51°16′02″N; 30°01′04″E) and Cherevach (51°12′44″N; 30°07′45″E) and around Chernobyl city (51°17′04″N; 30°13′25″E). The habitats investigated included open areas and the remnants of farmlands. A total of 201 *D. reticulatus* ticks, 87 males and 114 females, were collected and investigated (Table).

DNA was extracted by use of the ammonium hydroxide method (*7*). Isolated DNA was examined for the presence of the *Rickettsia* sp. citrate synthase gene (*gltA*) by use of PCR with *Rp*CS.409d and *Rp*CS.1258n primers (*8*). Positive amplicons were sequenced, and sequences were edited by using AutoAssembler software (Applied Biosystems, Foster City, CA, USA) and compared with GenBank entries by using blastn version 2.2.13 (http://www.ncbi.nlm.nih.gov/blast/download.shtml). All obtained sequences were submitted to GenBank (accession nos. KX056493 and KX056494).