Ebola Virus as a Foodborne Pathogen? Cause for Consideration, but Not Panic

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(See the article by Kobinger et al, on pages 200–8.)

In this issue of the *Journal*, Kobinger et al [1] report on experimental infection of common domestic pigs with Ebola virus (EBOV). What possessed them to perform this seemingly odd experiment? The answer lies in a closer look at various species of the virus and recent findings from the field. There are 5 species of EBOV with a range of pathogenicity in humans; on one end of the spectrum is Zaire EBOV (ZEBOV), the quintessential "hot zone" EBOV, associated with case fatality ratios up to 90%. On the other end is Reston EBOV (REBOV), which does not appear to be pathogenic to humans.

REBOV has always been the outlier among EBOVs, genetically distinct, with >40% nucleotide sequence divergence from ZEBOV for the glycoprotein gene [2, 3], which is thought to play a crucial role in pathogenesis [4]. Although ZEBOV and the other species are endemic in Africa, until 2008 all REBOV infections had been traced to monkeys in or imported from a single breeding

facility in the Philippines [5–8]. The site was finally shuttered in 1997, and REBOV disappeared until 2008, when it resurfaced in domestic pigs on 2 Philippine farms within a few hundred miles of the closed monkey breeding facility [9]. The pigs had a respiratory disease but were coinfected with porcine reproductive and respiratory syndrome virus, so it was not possible to definitely attribute their disease to REBOV. It is not known how the pigs became infected, but it is reasonable to suppose that is was from exposure to fruit bats, the likely reservoir for EBOV [10].

The REBOV epizootic in pigs effectively answers some lingering questions, including whether this virus is truly endemic in the Philippines (apparently so), rather than somehow introduced from Africa, and whether REBOV is pathogenic to humans (apparently not, with some caveats discussed below). As is usually the case in science, however, answers bring about new questions. The pig epizootic was the first recorded instance of any EBOV in livestock [11]. What does this finding mean about the safety of our food and the people providing it?

The work of Kobinger et al [1] provides some answers, showing that (1) domestic pigs can be infected with ZE-BOV via the nasogastric mucosa; (2) infection in pigs causes a respiratory disease that can be severe; (3) virus

replicates in cells of the upper airway and is shed in respiratory secretions; (4) sick pigs can transmit the virus to naive animals; and (5) histopathologic findings in the experimentally ZEBOV-infected pigs are very similar to those found in the pigs coinfected with REBOV and porcine reproductive and respiratory syndrome virus in the Philippines [9].

What are the ways in which EBOV might pose a threat to us through our food chain? Pigs are unlikely to be an integral part of the natural maintenance of EBOVs, but could they serve as accidental hosts transmitting REBOV or even ZEBOV to humans? Serologic evidence of past REBOV infection in 6 workers on the pig farms in the Philippines [9], all of whom denied any contact with bats or monkeys, strongly suggests this possibility [12]. The parallels are striking with the 1998–1999 outbreak of Nipah virus in Malaysia and Singapore, in which infected pigs developed a respiratory syndrome and transmitted the virus to humans, who developed the now well-described Nipah virus encephalitis [13]. The pigs were presumed to be infected through direct or indirect contact with *Pteropus* bats, later shown to be the Nipah virus reservoir [13].

What might be the risk from infected meat? ZEBOV transmission to humans has been clearly documented in central Africa through the hunting, butchering,

and consumption of "bushmeat," especially gorillas and chimps [14]. However, the EBOV lipid envelope renders it relatively unstable in the environment [15]. Infection in persons whose sole contact is with meat procured by others has not been reported and is probably rare, if it occurs at all, and there is no risk to eating cooked food. It should be noted, however, that these observations come from remote African settings where there is little or no cold storage. The more reliable cold storage conditions typically involved in commercial pig farming could help preserve the virus. Another potential concern is the many porcine subproducts used for medical or cosmetic purposes. The susceptibility of pigs to EBOV infection could also be seen as an opportunity for bioterrorists wishing to disrupt the food supply, infect humans, or both.

Finally, consideration must be given to the direct economic consequences of EBOV infection in pigs, which would almost certainly result in widespread culling, as it did in the Philippines. The Nipah virus outbreak in Malaysia and Singapore resulted in the culling of >1 million pigs, with millions of dollars lost [13]. Furthermore, consumer fears often have significant economic repercussions even when scientifically unfounded, illustrated by the avoidance of pork during the 2010 "swine flu" (H1N1 influenza) pandemic [16]. Certainly, news of EBOV in pigs would not go over well with the public.

But let's not panic yet. First, regarding REBOV, an array of field and laboratory data suggest that this virus is not a human pathogen, or at least not one that causes severe disease; of >800 persons potentially exposed, only 15 (2%) have developed antibody (including 4 in whom seroconversion was documented), and none have developed symptoms [9, 12]. Furthermore, in the laboratory, relative to ZEBOV, REBOV exhibits delayed replication and a milder cytopathogenic effect [17]; a decreased rate of cleavage of the glycoprotein

(considered an important step in virus activation and pathogenicity) [18]; and less infectivity [19], endothelial cell damage [4], lymphocyte apoptosis [20], down-regulation of the immune response [21], and antibody-dependent enhancement [22]. As for ZEBOV and the other known pathogenic EBOVs, there has been no suspicion of pigs playing a role in any outbreaks to date. Domestic pigs are occasionally found in parts of sub-Saharan Africa, but pigs and pork generally play a much smaller agricultural role than in most other parts of the world.

The aforementioned findings should assuage any sense of panic regarding EBOV as a potential foodborne pathogen, but the possibility cannot yet be dismissed; the 15 recorded REBOV-seropositive persons were all adult males [12]. The consequences of infection in pregnant women, the very young and old, and the immunocompromised or malnourished could be different. Furthermore, there is some degree of genetic diversity in REBOV strains [3], and minor sequence changes have sometimes been associated with enhanced pathogenicity in monkey models (Thomas Geisbert, personal communication). Furthermore, EBOV pathogenicity can be enhanced by serial passage in animals and cell culture [23–25]. A similar result through unintentional serial passage in pigs is not out of the question.

What are the next steps in terms of both scientific discovery and disease prevention? First, it will be important to confirm that REBOV infection alone causes disease in pigs. It would also be illustrative to test other types of livestock manifesting respiratory disease. Assuming that REBOV indeed causes disease in pigs, syndromic surveillance could be established in the Philippines, and perhaps in areas of Africa where EBOV is endemic and pig farming is practiced, with laboratory testing of sick animals. Experience from surveillance systems developed after the Nipah virus

outbreak in Malaysia and Singapore should be helpful.

The repeated emergence of REBOV within an area restricted to a few hundred miles of the Philippines might suggest that its geographic distribution is limited. However, respiratory syndromes in pigs are not uncommon, and EBOV infections are unlikely to be detected through present surveillance systems. Furthermore, assuming that bats are the reservoir for all species of EBOV, and considering the long-range migratory habits of some species, the area at risk for both human and animal infection could be vast. An example of this is the serologic evidence of EBOV infection in bats caught in Ghana, hundreds of miles from the nearest reported human case [26]. It would be illustrative to test bats in the Philippines for EBOV infection, targeting the areas of the implicated pig farms and then ultimately expanding the search to other regions of Asia and Africa. The best bet is that a thorough hunt will turn up evidence of infection over a wide geographic range, again noting the parallel with Nipah virus, which caused epidemics in India and Bangladesh [13] a few years after its discovery in Malaysia, with recent evidence of infected bats in Africa [27].

Poor animal care practices, such as reuse of needles, may have increased the spread of REBOV between monkeys in the breeding facility in the Philippines [28, 29]. The specific conditions on the implicated pig farms have not been reported, but poor animal care practices are unfortunately not uncommon in livestock facilities the world over. Reinforcing sound practices, including measures to limit exposure of livestock to bats and their excreta in the known endemic areas for EBOV, would seem prudent.

The REBOV infections in monkeys imported into the United States prompted revised regulations for the transit and import of nonhuman primates, including a period of quarantine and importer facility compliance inspections

[30]. It is premature to advocate widespread strict regulations for the movement of pigs or other livestock to prevent EBOV infection, but targeting exporters in endemic and high-risk areas could be warranted if continued surveillance indicates a risk.

Finally, on the basic science research side, an important question and opportunity arises: Why are very similar syndromes apparently produced by ZEBOV and REBOV infection in pigs and monkeys, but drastically different outcomes seen with these two virus infections in humans? Minigenome systems recently developed for EBOV should be valuable tools to tease apart the precise genetic determinants of pathogenicity [31, 32].

In conclusion, the laboratory findings by Kobinger et al [1] combined with the previous results of field investigations in the Philippines [9] highlight the possibility of EBOV as a foodborne pathogen. This, is cause for consideration, further scientific study, and prudent surveillance and prevention measures in the livestock industry in implicated areas of the world.

Acknowledgments

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