VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 14, Number 8, 2014

DOI: 10.1089/vbz.2013.1521

Serological Anthrax Surveillance in Wild Boar (*Sus scrofa*) in Ukraine

Karoun H. Bagamian,[1,2] Artem Skrypnyk,[3] Yana Rodina,[4] Maksym Bezymennyi,[5] Oleg Nevolko,[6] Valeriy Skrypnyk,[4] and Jason K. Blackburn[1,2]

Abstract

Anthrax, caused by *Bacillus anthracis*, is an acute disease affecting wildlife, livestock, and humans worldwide, although its impact on these populations is underappreciated. In Ukraine, surveillance is passive, and anthrax is often detected in livestock. However, wildlife is not subject to surveillance, although anthrax deaths (such as in wild boar, *Sus scrofa*) have been documented. The wild boar is a plentiful and widespread species in Ukraine and is frequently hunted. We initiated a screening study testing Ukrainian wild boar blood samples for antibodies to *B. anthracis*. We mapped results relative to known livestock anthrax hotspots. We discovered evidence of exposure in wild boar up to 35 km from livestock anthrax hotspots and over 400 km from previous anthrax reports in boars. We make recommendations about using wildlife species as biosentinels for anthrax in Ukraine.

Key Words: Wild boar—*Sus scrofa*—Anthrax—*Bacillus anthracis*—Serological surveillance—Ukrainian wildlife—Biosentinel.

Introduction

ANTHRAX IS A SEVERE ZOONOSIS caused by the bacterium *Bacillus anthracis*. For susceptible hosts, infection can be rapid, with some animals succumbing within several hours of clinical signs (Turnbull 2008). Epizootics occur nearly worldwide, with human cases most often occurring in agrarian or developing nations (Turnbull 2008). Despite zoonotic risk, anthrax remains underreported and undervalued as a public health concern (Fasanella et al. 2010).

Ukraine reports sporadic anthrax outbreaks and has a national policy for passive surveillance for livestock anthrax that includes required vaccination and mandated decontamination measures during outbreak responses. Human and livestock interaction with wildlife is common because wildlife roam Ukrainian farms and hunting is a common pastime. However, there is no anthrax wildlife surveillance—a problem because free-roaming wildlife can be involved in anthrax, even in the absence of livestock (Hugh-Jones and Blackburn 2009). In the winters of 1995 and 1998, there were mass anthrax die-offs of wild and domestic piglets in Ukraine near the Romanian border (Volokh 2002), indicating that wild boar are exposed to anthrax. Boar may be exposed more regularly, but this may go undetected, especially as wildlife mortality, unless notable or opportunistically discovered, is often missed and is usually investigated only if severe (Stallknecht 2007). In addition to mortality reports, active surveillance using serological tests is needed to understand anthrax exposure risk fully (Bagamian et al. 2013).

Sentinel species are those that are more likely to be exposed and survive infection, because regular serological surveillance is more likely to detect a pathogen in infected or exposed but recovered animals. Suids are believed to be more resistant to anthrax than other species (Turnbull 2008). Thus, boars could serve as sentinels to identify whether anthrax is persisting in known areas or has spread to new areas where the disease may be emerging or re-emerging.

To better understand anthrax epizootiology in Ukraine, we tested wild boar serum samples collected across Ukraine for

[1]Spatial Epidemiology & Ecology Research Laboratory, Department of Geography, and [2]Emerging Pathogens Institute, University of Florida, Gainesville, Florida.
[3]Metabiota Inc., Kyiv, Ukraine.
[4]State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine.
[5]Institute for Veterinary Medicine, Academy of Agrarian Sciences, Kyiv, Ukraine.
[6]State Research Institute of Laboratory Diagnostics and Veterinary Sanitary Expertise, Kyiv, Ukraine.

antibodies to *B. anthracis* and determined whether exposed boars were associated with livestock anthrax hotspots.

Materials and Methods

Samples were collected from hunter-killed boar (*Sus scrofa scrofa*) during the 2011 and 2013 hunting seasons (January–February 2011 and April 2013). We used the nonspecies and immunoglobulin (Ig) subtype-specific Immunetics Quick-ELISA Anthrax PA-Kit (Boston, MA) for detection of antibodies to anthrax-protective antigen per the manufacturer's protocol following Lembo et al. (2011). Controls and samples were tested in duplicate. We tested three internal house controls of bison (*Bison bison*) sera (two unvaccinated, one vaccinated) confirmed at the University of Florida.

Rayon-level (district-level) seroprevalence was calculated as the number of antibody-positive samples divided by the rayon sample population. Exact 95% binomial confidence intervals (BCI) were calculated for seroprevalence estimates using the R epitools package (www.medepi.com/epitools/).

We estimated anthrax hotspot locations for domestic livestock within the country using kernel density estimation (Fotheringham et al. 2000) and a database of 479 livestock anthrax mortality events. Kernel density estimation was performed with the Spatial Analyst Extension for ArcGIS 10 using the optimal bandwidth function and a 1-km output grid cell size. Anthrax hotspots were defined as the upper 25%, 10%, and 5% of estimated density values (Nelson and Boots 2008).

Results

We tested 124 sera samples from 39 rayons (Fig. 1A). Two positive samples (1.61%; BCI, 0.20–5.70%) were detected in Reshetylivskyi (50.0% [1/2]; BCI, 1.26–98.74%) and

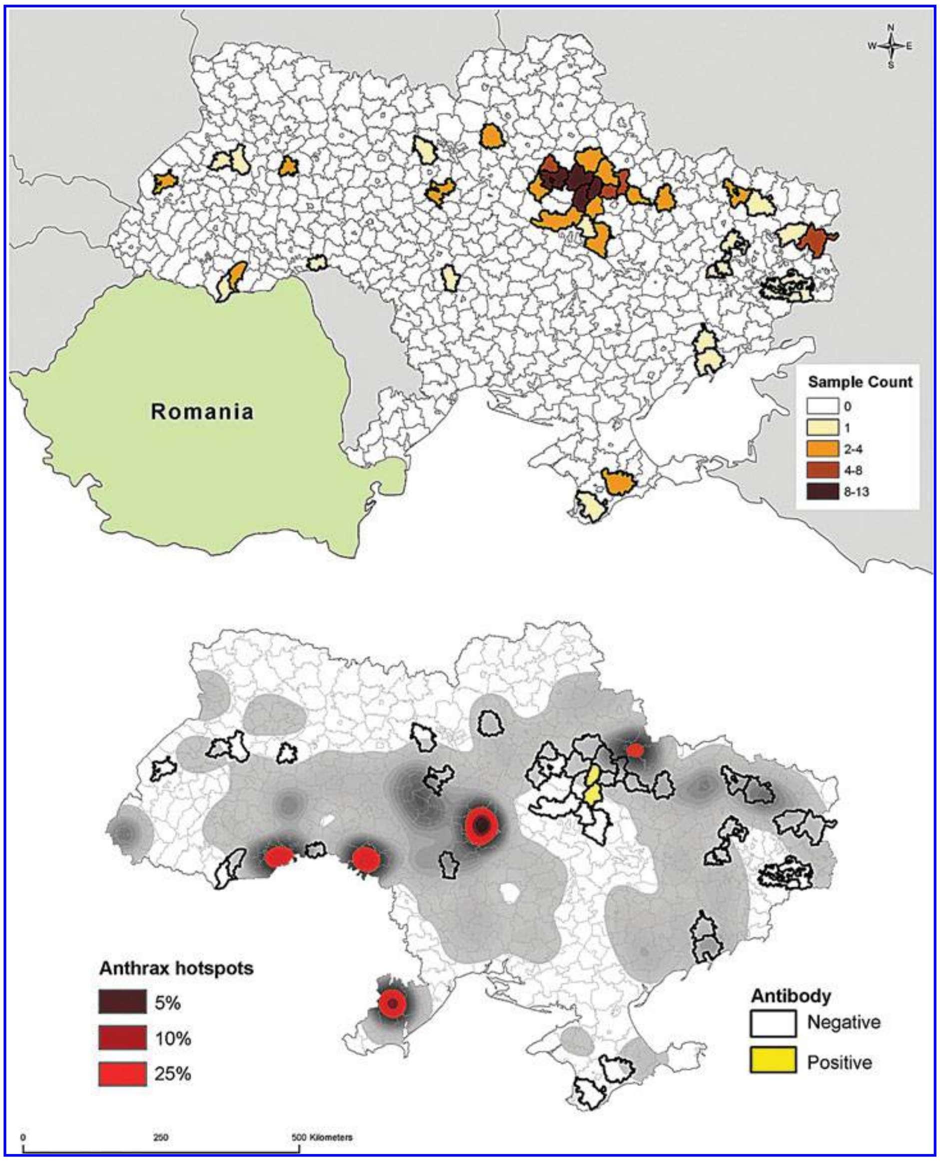

FIG. 1. Maps of Ukraine comparing number of wild boar samples (*S. scrofa*) tested per rayon (**A**) and rayons with anthrax antibody–positive and –negative boar samples relative to historical livestock anthrax hotspots derived from kernel density estimation at three different thresholds (**B**) (upper 5%, 10%, 25% of density values). Gray color ramp represents anthrax density estimates below the hotspot cutoff values.

Shyshatskyi (9.09% [1/11]; BCI, 0.23–41.28%) rayons (Fig. 1B). Both were collected on January 27, 2011.

Livestock anthrax outbreaks were concentrated in a series of foci stretching east to west from Kramatorsk to Chernivtsi, with a separate focus near Galati in the south. Reshetylivskyi and Shyshatskyi rayons are proximate to outbreak hotspots (Fig. 1B).

Discussion

Results suggest that Ukrainian wild boars have been infected with anthrax in areas far (~400 km) from a previous wild boar outbreak (Volokh 2002). This indicates that wild boars in Ukraine are likely exposed over a much larger range than previously thought. The positive boars were from two separate rayons. Shyshatskyi (northern yellow rayon) directly borders a livestock anthrax hotspot. Reshetylivskyi (southern yellow rayon) is ~35 km away from the livestock hotspot, indicating some evidence of a larger area of anthrax risk than previously documented and possibly new or expanding anthrax foci (see Fig. 1B). Because no livestock anthrax outbreaks have been reported since 1966 for Shyshatskyi and 1958 for Reshetylivskyi, our results indicate that ongoing, undetected anthrax transmission continues in the general region.

Suids, omnivorous scavengers, are most likely exposed to anthrax by eating infected carcasses, similar to other scavengers (for review, see Bagamian et al. 2013). Rooting in vegetation may also lead to infection by spore ingestion or inhalation. In two large anthrax epizootics in Texas in 2009–2010, free-ranging feral hogs scavenged confirmed *B. anthracis*–positive deer and cattle carcasses (Blackburn, unpublished data). Wild boars in Ukraine likely engage in similar behaviors, and our report supports that wild boars are exposed to anthrax more often than documented mortality events. These observations reinforce that reliance on passive detection of zoonotic outbreaks remains insensitive to the true risks for pathogen activity and emergence. North American feral hogs have also been recently identified as potential anthrax biosentinels by the National Wildlife Disease Program (Scmit 2013).

This preliminary study was intended to evaluate the feasibility of active surveillance built on current infrastructure (hunting stations). As such, it was limited geographically and in sampling effort. Although samples were not collected at the peak anthrax season (summer; Hugh-Jones and Blackburn 2009), they were collected within the several-month period that anthrax antibodies are thought to last. We cannot rule out the possibility of reporting bias influencing our results. Future widespread and regular anthrax wildlife surveillance that integrates serology and movement data can help identify at-risk areas at the wildlife–livestock interface, the range of affected Ukrainian wildlife, and the geographic extent of anthrax in Ukraine. This information can help plan anthrax intervention and control strategies for wildlife and livestock and better define human risk.

Acknowledgments

This work was funded by the US Defense Threat Reduction Agency's Cooperative Biological Engagement Program in Ukraine through the UP-2 Project. G. Glass generously edited this manuscript. A. Barro assisted with the kernel density estimation.

Author Disclosure statement

No competing financial interests exist.

References

Bagamian KH, Alexander KA, Hadfield TL, Blackburn JK. Ante- and postmortem diagnostic techniques for anthrax: Rethinking pathogen exposure and the geographic extent of the disease in wildlife. J Wildlife Dis 2013; 49:786–801.

Fasanella A, Galante D, Garofolo G, Jones MH. Anthrax undervalued zoonosis. Vet Microbiol 2010; 140:318–331.

Fotheringham AS, Brunsdon C, Charlton M. *Quantitative Geography: Perspectives on Spatial Data Analysis.* Sage Publications Ltd., 2000.

Hugh-Jones M, Blackburn J. The ecology of *Bacillus anthracis*. Mol Aspects Med 2009; 30:356–367.

Lembo T, Hampson K, Auty H, Beesley CA, et al. Serologic surveillance of anthrax in the serengeti ecosystem, tanzania, 1996–2009. Emerg Infect Dis 2011; 17:387–394.

Nelson TA, Boots B. Detecting spatial hot spots in landscape ecology. Ecography 2008; 31:556–566.

Scmit BS. *Feral Swine: Useful Biosentinels for* Bacillus anthracis. North American National Wildlife Disease Program, 2013.

Stallknecht D. Impediments to wildlife disease surveillance, research, and diagnostics. In: *Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission*. Springer, 2007:445–461.

Turnbull PCB. *Anthrax in Humans and Animals.* Geneva, Switzerland: World Health Organization, 2008.

Volokh AM. Some ecological characteristics of southern marginal wild boar population in Ukraine. Zoologichesky Zhurnal 2002; 81:6.

Address correspondence to:
Jason K. Blackburn
Spatial Epidemiology & Ecology Research Laboratory
Department of Geography & the Emerging Pathogens Institute
University of Florida
3141 Turlington Hall
P.O. Box 117315
Gainesville, FL 32611-7315

E-mail: jkblackburn@ufl.edu