Active surveillance of African swine fever in domestic swine herds in Georgia, 2014

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Summary

Since its introduction to the Republic of Georgia in 2007, African swine fever virus (ASFV) has spread across the Caucasus region, the Russian Federation, and some Eastern European countries. It is assumed that large populations of naïve, domestic, free-ranging and wild pigs are vital to the transmission of the disease. Since its epidemic emergence in the region in 2007, ASFV has continued to circulate, which suggests that an endemic cycle has been established

and is maintained by contact between free-ranging domestic pigs, wild pigs, and possibly native *Ornithodoros* ticks, the most likely reservoirs for the virus. In 2014, a survey was conducted across the Republic of Georgia to determine ASFV prevalence among domestic swine herds. All 1,231 samples collected for this survey tested negative for ASF. The probability of observing no reactors in a sample of this size (n = 1,231) from a population with an estimated disease prevalence of 1% is very low (<0.0001). Therefore, it is possible but very unlikely that ASFV was present among domestic swine during the span of this survey. These data suggest that, in 2014, domestic pig herds were not the source of the virus, and that the ASF endemic cycle may be supported by the circulation of ASFV among feral pigs, wild pigs, and possibly native *Ornithodoros* ticks.

Keywords

Active survey – African swine fever virus – Detection – Epidemiology – Georgia – Pig – Porcine – Surveillance – Swine.

Introduction

African swine fever (ASF) is endemic in more than 20 sub-Saharan African countries. In Africa, ASF virus (ASFV) infects and cycles between *Ornithodoros* ticks (*Ornithodoros moubata* complex*),* and warthogs (*Phacochoerus africanus)*, establishing a natural reservoir of endemic infection that can spill over into the domestic pig population. A domestic cycle between *O. moubata*-complex ticks that inhabit pigsties and feed on domestic pigs has also been described in Africa (1). A similar epidemiological scenario developed in the Iberian Peninsula after ASFV was introduced from Africa in the late 1950s; constant circulation among pigs and indigenous *Ornithodoros* ticks was probably a contributing factor in the persistence of the virus until the eradication of the disease in the mid-1990s. There is currently no published evidence to suggest that an ASFV wild cycle has been established in the Caucasus region or elsewhere in the Russian Federation or Eastern Europe.

African swine fever is a highly contagious viral disease of swine. The causative agent, ASFV, is a large enveloped virus containing a double-stranded (ds) DNA genome of approximately 190 kilobase pairs. ASFV shares aspects of its genome structure and replication strategy with other large dsDNA viruses, including *Poxviridae, Iridoviridae* and *Phycodnaviridae* (2). ASFV infections in domestic pigs are often characterised by high morbidity and high mortality, and clinical signs that include fever, haemorrhages, ataxia, and severe depression. The course of infection varies, ranging from highly lethal to subclinical, depending on host characteristics and the particular virus strain (3). The current epidemic virus in the Caucasus, ASFV Georgia 2007/1, is a highly virulent virus belonging to genotype II (4), which probably originated from south-east Africa.

On 22 April 2007, ASF was first reported in the Republic of Georgia. The available epidemiological data at that time suggested that the outbreak most likely originated in the Poti Harbour region on the Black Sea. The occurrence of a disease with clinical presentations similar to those of ASF was reported from different regions across the country. It was speculated that free-roaming pigs came into contact with contaminated, untreated food waste from ships.

By 9 July 2007, the outbreak of ASFV had spread across most of the country. Between April and July 2007, the Laboratory of the Ministry of Agriculture (LMA) in Tbilisi collected, through its regional laboratories, 56 clinical samples from seven different geographical locations. By the end of 2007, the Ministry of Agriculture had declared that 56 out of 61 districts (92%) in the country had swine herds infected with ASFV, although many of the cases were diagnosed based solely on clinical signs or post-mortem examination. At that time, confirmatory laboratory testing was performed on a limited number of clinical samples (Table I), through polymerase chain reaction (PCR) and ASFV antigen enzyme-linked immunosorbent assay (ELISA) (5). The disease re-emerged in Georgia in 2010 and 2011, resulting in outbreaks in several areas.

Seven years after ASF was first identified in the Republic of Georgia, an active survey was launched to gather field information on the epidemiological status of the disease throughout the country. The goal was to see if the modes of spread and persistence of ASFV in Georgia could be identified. In this paper, the authors report the results of this investigation of ASFV among domestic swine herds in seven regions across the Republic of Georgia.

Materials and methods

Study area

Surveillance was conducted in 41 communities across seven regions in the Republic of Georgia (Table II), with a collective swine population of 191,000 pigs. These districts were selected for study based on their previous history of ASFV outbreaks, domestic pig density, the presence of soft-bodied ticks and, in some cases, proximity to international borders.

Sampling protocol

Sample collection

A total of 1,231 pigs were sampled from small-scale farms clustered in 41 communities across Georgia (Table III). Sera and whole blood were obtained using a Vacutainer system (Becton, Dickinson and Company, Franklin Lakes, NJ, United States of America [USA]). Ticks were collected manually from swine and from areas where swine were housed, as well as from domestic animals living in the same grounds (Table IV). Samples were submitted to regional laboratories and then to the LMA in Tbilisi for processing and testing.

Sample and data processing

African swine fever virus antibody detection using enzyme-linked immunosorbent assay

Serologic analysis was performed to detect ASF antibodies, using a blocking ELISA test (Ingezim PPA COMPAC; Ingenasa, Madrid, Spain), according to the manufacturer's instructions.

African swine fever virus antibody detection using an immunoperoxidase assay

A quantity of the ELISA-tested serum samples were also tested for ASFV antibodies by immunoperoxidase assay, according to protocols and recommendations provided by the Centre for Animal Health Research, which is the European Reference Laboratory for ASF, and part of the National Institute for Agricultural and Food Research and Technology (CISA-INIA), in Valdeolmos, Spain. In brief, serum samples were incubated in 96-well plates containing fixed ASFV-infected Vero cell monolayers, which were kindly provided by the Foreign Animal Disease Diagnostic Laboratory (FADDL), Animal Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA).

After repeated washes, alkaline phosphatase-conjugated protein A (Thermo Scientific, Grand Island, NY, USA) was added to the wells and incubated for one hour at 37°C. Cell monolayers were then washed repeatedly and exposed to a substrate solution in the presence of H_2O_2. Monolayers were examined for colour changes under a light microscope. An ASFV antibody-positive serum (FADDL, APHIS, USDA) was used as a positive control for the assay.

African swine fever virus detection in blood samples using quantitative polymerase chain reaction

Total DNA was obtained from blood samples using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA), according to the instructions provided. An ASFV real-time PCR (quantitative polymerase chain reaction or qPCR) kit (Tetracore, Rockville, MD,

USA) was used to detect viral DNA in extracted samples. Reactions were set according to the manufacturer's instructions and run in a Light Cycler 1.2 (Roche, Indianapolis, IN, USA).

Testing ticks for African swine fever virus

Collected ticks were identified morphologically. Total DNA was extracted from the ticks using a BeadBeater (Biospec Products, Bartlesville, OK, US) for grinding, and the DNeasy Blood and Tissue kit (Qiagen) for DNA extraction. Tick DNA was then tested by ASFV qPCR, as described above.

Data analysis

EpiTools (6, 7) was used to estimate the prevalence of ASFV and to analyse results for this structured survey.

Results

Communities surveyed for African swine fever in Georgia

In total, 434 ticks were collected, along with blood and serum samples from 1,231 pigs (Table II). None of the collected ticks was identified as being of the genus *Ornithodoros*. Samples were collected in the Guria, Mtskheta-Mtianeti, Kvemo Kartli, Racha-Lechkhumi Kvemo Svaneti, Kakheti, Samegrelo Zemo Svaneti and Imereti regions of Georgia (Fig. 1) (Tables III and IV). Samples were delivered to regional laboratory support stations and zonal diagnostic laboratories and submitted to LMA. All samples tested negative for ASFV and for antibodies against ASFV.

Estimating disease prevalence among domestic pigs

For this survey, the sample size was calculated in a way that allowed for an estimation of true prevalence while using imperfect tests (8). Inputs for the calculation assumed a true prevalence (1%); a desired level of confidence (95%); a desired precision of the estimate (2.5% acceptable error in the estimate), considering a population size of 191,000 for the country; and assumed values for sensitivity (80%) and

specificity (90%) for the chosen testing regimen. Based on this formulation, at least 1,199 samples were required to detect a disease prevalence of at least 1% (Table V). The tool also provided sample size estimations for a range of true prevalence and precision values for a population size of 191,000. An analysis of the results to demonstrate freedom from disease using imperfect tests and finite population size was also undertaken (9).

Discussion

The goal of this survey was to generate knowledge about the extent of ASF spread in the Republic of Georgia by estimating ASFV prevalence in domestic swine herds across the country. It was hoped that this may eventually lead to the identification of the source and reservoirs of the disease. To this end, the objectives of this study were to:

– estimate the prevalence of ASFV in domestic swine herds

– survey biologically plausible vectors to determine if ASFV is present in soft ticks collected from pigs and pig housing, and

– describe the spatial-temporal distribution of disease and vector surveillance data collected from seven regions in Georgia (Imereti, Kakheti, Guria, Samegrelo Zemo Svaneti, Qvemo Qartli, Racha-Lechkhumi Kvemo Svaneti and Mtckheta-Mtianeti).

It was estimated that ASFV prevalence is less than 1% among domestic swine herds in Georgia.

Pork is widely consumed in Georgia. Commercial pig farming in the country is represented mainly by small pig holdings. According to national statistics provided by the Ministry of Agriculture, Georgia has a total pig population of approximately 190,000 animals, which is probably an underestimation, given the lack of more accurate indicators of livestock populations (e.g. animal identification systems).

Georgia is a net importer of pork (fresh and frozen), which is needed to meet consumer demand for this product. Most pig holdings (over

90%) only have a small number of animals (two to three sows) for the farmer's own meat supply (subsistence farming). Most of these animals are reared under free-ranging conditions. This particular husbandry practice may increase the likelihood of disease transmission, through frequent unconstrained contact between domestic pigs of diverse origins and by providing more opportunities for contact with wild boar and feral swine. Wild boar are present in Georgia, mainly in the eastern part of the country and along the border with Turkey. The precise size of these populations and their possible role in ASFV transmission are unknown.

Under this production system, pig slaughtering typically occurs at the farm level, with or without proper methods of waste disposal. Pork that is not consumed on the farm is usually sold at local markets; live animal trade mainly occurs in and among individual villages. Pork production systems, as observed in pork-producing countries, with defined standards of biosecurity, food safety, and commercialisation, represent only a very small proportion of the pig holdings in Georgia.

Since the re-emergence of the disease outside Africa in 2007, and according to the World Organisation for Animal Health (OIE), ASFV outbreaks have been confirmed in Ukraine (2012), Belarus (2013), Lithuania, Latvia, Estonia and Poland (2014). Nearly all of those outbreaks occurred in wild boar. In the context of the epidemic caused by ASFV Georgia 2007/1, the roles played by wild boar, feral swine, and tick vectors in virus eco-epidemiology and transmission are unknown. Given the uncertainty surrounding ASF maintenance and transmission in Georgia, field information was gathered to assess the epidemiological status of ASF. Particular emphasis was given to understanding the modes of spread and persistence of ASFV in this new environment, especially among domestic, feral and wild swine, and potential soft tick vectors. The results of this survey suggest that the prevalence of ASFV is low among domestic swine in Georgia. It is probable that ASFV has established an endemic cycle supported by the circulation of the virus among free-ranging domestic pigs (feral pigs), wild pigs and/or native *Ornithodoros* ticks. Domestic swine,

although at risk of an epidemic, do not appear to be essential to maintaining the virus in this environment.

It should be stated that, in this study, the method used to search for *Ornithodoros* ticks was limited in scope and not effective. The involvement of *Ornithodoros* ticks in ASFV transmission in Georgia needs to be thoroughly investigated, using more appropriate and effective methods for collecting these ticks. Although this particular study cannot assess the role of ticks in transmitting ASFV in Georgia, it is important to note that surveys done in the Iberian Peninsula (10, 11) and Africa (1) have shown a low rate of infection among ticks that can, nevertheless, contribute significantly to maintenance of the virus.

Changes in the virus may lead to changes in the way that the disease is transmitted and spread. ASFV isolates obtained from different regions in the Caucasus, ASFV Armenia 2008 and ASFV Chechen Republic 2009, have been used to challenge European wild boars via intranasal and intramuscular routes. Both routes of inoculation resulted in 100% mortality of inoculated wild boar (12), which suggests that, since its introduction into the Caucasus region, circulating ASFV has retained a virulent phenotype similar to that of the original Georgia 2007/1 isolate. Given this high lethality, it is likely that the circulating virus has a limited capability to persist in wild boar populations.

Similar conclusions can be drawn from a recent study aimed at determining the genetic changes in ASFV isolates collected in the Russian Federation from 2007 to 2011. Using partial genome sequencing of these isolates, Malogolovkin *et al.* (13) observed 100% identity for B646L and E183L genes and determined that all examined viruses formed a genetic cluster within genotype II. These findings led the authors to conclude that only one ASFV virus variant caused the outbreaks from 2007 to 2011 in the territory of the Russian Federation and throughout the Caucasus. Although virulent and genetically stable, the virus continues to re-emerge in the Caucasus region, which suggests that still-unidentified factors contribute to its persistence in the environment.

Most field strains of ASFV can persistently infect *Ornithodoros* ticks, which can act as an ASFV reservoir. *Ornithodoros* is a genus in the soft-bodied tick family, Argasidae. The pathogenesis of ASFV in *O. moubata* ticks is characterised by a low infectious dose, lifelong infection, efficient transmission to both pigs and ticks, and low tick mortality until after the first oviposition (14).

At least one European species of *Ornithodoros*, *O. erraticus,* is known to be capable of transmitting ASFV (15), which can persist in these ticks for up to 655 days (10). Furthermore, it has been proposed that *O. erraticus* ticks surviving from 1993 were the source of Portugal's ASF outbreak in 1999 (11). Coincidentally, the last provinces in Spain to eradicate the disease were also the ones where *O. erraticus* was present. Diaz *et al.* (16) have shown that ASFV Georgia 2007/1 can replicate effectively in *O. erraticus* ticks, heightening the risk for this species. This means that this species could become a reservoir for ASFV in regions where the geographical range of the virus overlaps that of the ticks. Since *Ornithodoros* ticks also inhabit the Caucasus region, with an unknown geographical range, it will be crucial to assess the role that they may play in ASFV transmission and persistence.

Conclusion

The results of this large and geo-diverse survey demonstrate that ASFV was either absent, undetectable, or present at an extremely low prevalence (less than 1%) in domestic pigs in Georgia in 2014. This suggests that domestic pigs may be epidemiologically irrelevant for ASFV transmission or as maintenance reservoirs in Georgia at this time. Since sporadic ASFV outbreaks in domestic pigs continue to occur in the Caucasus region, feral swine and especially wild boar (combined with ASFV's capacity for long-term survival in a protein environment) are the most likely reservoirs and drivers of ASFV transmission spillover into domestic swine in Georgia.

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References

1. Jori F., Vial L., Penrith M.L., Pérez-Sánchez R., Etter E., Albina E., Michaud V. & Roger F. (2013). – Review of the sylvatic cycle of African swine fever in sub-Saharan Africa and the Indian Ocean. *Virus Res.*, **173** (1), 212–227. doi:10.1016/j.virusres.2012.10.005.

2. Costard S., Wieland B., de Glanville W., Jori F., Rowlands R., Vosloo W., Roger F., Pfeiffer D.U. & Dixon L.K. (2009). – African swine fever: how can global spread be prevented? *Philos. Trans. Roy. Soc. Lond., B, Biol. Sci.*, **364** (1530), 2683–2696 doi:10.1098/rstb.2009.0098.

3. Borca M.V., Gay C.G., Risatti G.R., O'Toole D., Li N., Kuhn J.H., Lewis C.E., Loiacono C.M. & White D. (2016). – Viral hemorrhagic fevers of animals caused by DNA viruses. *In* Global

virology. I: Identifying and investigating viral diseases (P. Shapshak, J.T. Sinnott, C. Somboonwit & J. Kuhn, eds), 1st Ed. Springer Science, New York, 319–343. doi:10.1007/978-1-4939-2410-3_12.

4. Chapman D.A.G., Darby A.C., Da Silva M., Upton C., Radford A.D. & Dixon L.K. (2011). – Genomic analysis of highly virulent Georgia 2007/1 isolate of African swine fever virus. *Emerg. Infect. Dis.*, **17** (4), 599–605. doi:10.3201/eid1704.101283.

5. World Organisation for Animal Health (OIE) (2007). – African swine fever, Georgia. OIE, Paris. Available at: www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=5720 (accessed on 6 May 2016).

6. AusVet Animal Health Services (2016). – EpiTools epidemiological calculators. Available at: http://epitools.ausvet.com.au (accessed on 6 May 2016).

7. Humphry R.W., Cameron A. & Gunn G.J. (2004). – A practical approach to calculate sample size for herd prevalence surveys. *Prev. Vet. Med.*, **65** (3–4), 173–188. doi:10.1016/j.prevetmed.2004.07.003.

8. AusVet Animal Health Services (2016). – Sample size to estimate a true prevalence with an imperfect test. Available at: http://Epitools.ausvet.com.au/content.php?page=PrevalenceSS (accessed on 6 May 2016).

9. AusVet Animal Health Services (2016). – FreeCalc: analyse results of freedom testing. Available at: http://Epitools.ausvet.com.au/content.php?page=FreeCalc1 (accessed on 6 May 2016).

10. Endris R.G., Hess W.R. & Caiado J.M. (1992). – African swine fever virus infection in the Iberian soft tick, *Ornithodoros* (Pavlovskyella) *marocanus* (Acari: Argasidae). *J. Med. Entomol.*, **29** (5), 87–88. doi:10.1093/jmedent/29.5.874.

11. Boinas F.S., Wilson A.J., Hutchings G.H., Martins C. & Dixon L.J. (2011). – The persistence of African swine fever virus in field-infected *Ornithodoros erraticus* during the ASF endemic period in Portugal. *PLoS ONE*, **6** (5), e20383. doi:10.1371/journal.pone.0020383.

12. Gabriel C., Blome S., Malogolovkin A., Parilov S., Kolbasov D., Teifke J.P. & Beer M. (2011). – Characterization of African swine fever virus Caucasus isolate in European wild boars. *Emerg. Infect. Dis.*, **17** (12), 2342–2345. doi:10.3201/eid1712.110430.

13. Malogolovkin A., Yelsukova A., Gallardo C., Tsybanov S. & Kolbasov D. (2012). – Molecular characterization of African swine fever virus isolates originating from outbreaks in the Russian Federation between 2007 and 2011. *Vet. Microbiol.*, **158** (3–4), 415–419. doi:10.1016/j.vetmic.2012.03.002.

14. Kleiboeker S.B. & Scoles G.A. (2001). – Pathogenesis of African swine fever virus in *Ornithodoros* ticks. *Anim. Hlth Res. Rev.*, **2** (2), 121–128. Available at: www.cambridge.org/core/journals/animal-health-research-reviews/article/pathogenesis-of-african-swine-fever-virus-in-ornithodoros-ticks/23663499DE9B36B2E5F6ADBA6A017CBD (accessed on 6 May 2016).

15. Sanchez-Botija C. & Badiola C. (1966). – Presencia del virus de la peste porcina Africana en el *Haematopinus suis*. *Bull. Off. Int. Epiz.*, **66**, 699–705. Available at: http://doc.oie.int:8080/dyn/portal/index.seam?page=alo&aloId=34154 (accessed on 6 May 2016).

16. Diaz A.V., Netherton C.L., Dixon L.K. & Wilson A.J. (2012). – African swine fever virus strain Georgia 2007/1 in *Ornithodoros erraticus* ticks. *Emerg. Infect. Dis.*, **18** (6), 1026–1028. doi:10.3201/eid1806.111728.

Table I
African swine fever virus outbreaks detected in the Republic of Georgia in 2007

Confirmed by the Laboratory of the Ministry of Agriculture in Georgia, as reported to the World Organisation for Animal Health (4)

Date	District/village	Number of collected samples	ASFV–PCR		ASFV Ag–ELISA	
			+	–	+	–
24/05/2007	Ckaltubo	7	7			
08/06/2007	Gurjaani	10	10			
09/06/2007	Kvareli/Eniseli	3	3			
12/06/2007	Tbilisi/Gldani	1	1			
19/06/2007	Tbilisi/Gldani	1	1			
20/06/2007	Kakheti/Dedoplisckaro	10		10	9	1
22/06/2007	Tbilisi/Vashijvari	1	1			
22/06/2007	Samçkhe Javakheti/Sakuneti	6		6		6
22/06/2007	Samçkhe Javakheti/Tkemlana	13	11	2		12
25/06/2007	Tbilisi/Vashijvari	2		2		
03/07/2007	Tbilisi/Vashijvari	2	1	1		2

ASFV–PCR: African swine fever virus polymerase chain reaction

ASFV Ag–ELISA: African swine fever virus antigen detection enzyme-linked immunosorbent assay

Table II
Location of communities and number of pigs sampled in Georgia during this 2014 survey

Region	District	Village	Number of pigs sampled
Racha-Lechkhumi Kvemo Svaneti	Ambrolauri	Likheti	23
	Oni	Chiora	33
		Glola	15
		Qvedi	2
	Lentekhi	Jakhunderi/Lexsura	31
		Rtskhmeluri/Khopuri/ Nanari/Lentekhi	61
Samegrelo Zemo Svaneti	Zugdidi	Ganmukhuri	29
		Rikhe	43
	Tsalenjikha	Pakhulani	29
		Mujava	31
		Chule	29
	Poti	Poti/Chaladidi	30
Imereti	Sachkhere	Perevi	6
		Jria	30
		Darkha	80
Guria	Ozurgeti	Natanebi	29
		Meria	22
	Lanchkuti	Nigoeti	36
		Qviani	19
	Chokhatauri	Khidistavi	27
Kvemo Kartli	Dmanisi	Dmanisi/Gantiadi	34
		Gomareti	33
	Marneuli	Akherpi	33
		Opridi/Khokhmeli	31
		Shaumiani	36
		Tseraqvi	31
Mtskheta-Mtianeti	Khazbegi	Arsha	40
		Kobi	30
Kakheti	Telavi	Lechuri	30
		Artana	24
		Lapankhuri	38
		Kisiskhevi/Fshaveli	30
	Akhmeta	Overno/Alvani	24
		Babaneuri	19
		Atskuri	28

	Argokhi	14
Kvareli	Shilda	30
	Eniseli	31
	Shaqriani	30
	Akhalsofeli	38
	Mtisdziri	22

Table III
Proportion of the regional domestic pig population sampled, and estimated age of sampled pigs, in the Republic of Georgia, 2014

Region	Pig population (2013)	Number of pigs sampled	Percentage of regional pig population	Age of sampled pigs (months)				
				1–3	3–6	6–12	>12	Unknown
Mckheta-Mtianeti		70	0.12%	16	10	35	9	0
Guria	58,000	133	0.23%	40	25	33	34	1
Racha		165	0.28%	46	32	51	31	5
Imereti	31,400	116	0.37%	35	26	16	38	1
Kakheti	35,300	358	1.01%	24	73	173	83	5
Kvemo Kartli	11,600	198	1.71%	19	74	76	27	2
Samegrelo/Zemo Svaneti	54,300	191	0.35%	4	49	48	77	13
Total	191,400	1,231	0.64%	184	289	432	299	27

Table IV
Number of ticks and tick species collected from selected domestic pig holdings in the Republic of Georgia in 2014

Region	Collection method	Time and weather conditions	Number of samples	Ticks collected	Tick species
Kakheti-Telavi-Artana	Forceps	Noon, sunny	25 pigs	2	Not determined
Kakheti-Telavi-Pshaveli	Dry ice trap, forceps	AM, cloudy	3 pigs, sheep, chickens	6	*Argas persicus*
Kakheti-Telavi-Pshaveli	Dry ice trap, forceps	AM, sunny	3 pigs, sheep, chickens	1	*A. persicus*
Kakheti-Telavi-Pshaveli	Forceps	AM, sunny	Abandoned farm	0*	*Allothrombium* spp.
Kakheti-Telavi-Pshaveli	Forceps	AM, sunny	3 pigs, sheep, chickens	369	*A. persicus*
Kakheti-Telavi-Pshaveli	Forceps	PM, sunny	16 pigs, chickens	0*	*A. persicus*
Kakheti-Telavi-Lafankuri	Forceps	AM, cloudy	Abandoned farm	11	Not determined
Kakheti-Kvareli-Shida	Forceps	Noon, sunny	5 pigs	1	Not determined
Kakheti-Kvareli-Akhalsopeli	Soil sieve	AM, sunny	6 pigs, chickens	14	Not determined
Racha-Oni-Chiora	Forceps	AM, sunny	1 pig	5	*A. persicus*
Racha-Oni-Glola	Forceps	Noon, sunny	1 pig	3	*A. persicus*
Mtskheta-Mtianeti-Kazbegi-Stepantsminda	Forceps	Noon, cloudy	1 pig	4	Not determined

0* No *Ornithodorus* spp. ticks were found

Table V
Estimation of sample size for calculating the true prevalence of African swine fever among domestic swine herds in the Republic of Georgia

Input	
Assumed true prevalence	0.1
Sensitivity	0.8
Specificity	0.9
Population size	191,000
Confidence	0.95
Desired precision	0.025
Sample size required	1,199

Fig. 1

Number of domestic swine sampled and geographical distribution of sampling sites across the Republic of Georgia in 2014

Sampled regions are shown in dark grey, including the total number of individual samples collected