

Exploration of local transmission routes for African swine fever in pigs in the Russian Federation, 2007-2014

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Article summary line: The transmission of African swine fever from infected wild boar populations to domestic pigs in the Russian Federation played a marginal role in the epidemic as compared to the proximity to infectious farms.

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ABSTRACT

African swine fever (ASF) is a devastating disease of swine that was introduced into Georgia in 2007, and subsequently spread to the western part of the Russian Federation. This study analysed the spatial and spatio-temporal distributions of ASF cases that were reported in domestic pigs and wild boars for assessing the likelihood of wild boar-to-domestic pig and farm-to-farm transmission routes in the epidemic that occurred from 2007 to 2014 in two of the most affected regions of the Russian Federation. Results suggest that, in these two regions, the main driver of infection of a susceptible farm was the proximity to an infectious farm and that transmission from infected wild boar populations, if any, was only of marginal significance. These findings confirm the need to maintain high biosecurity standards on pig farms and justify strict control measures targeted at domestic pig production such as preventive culling and local movement restrictions.

INTRODUCTION

African swine fever (ASF) is a viral disease of swine associated with a case fatality rate that can reach 100% depending on the strain. Historically present in Africa, ASF was introduced into Georgia in April 2007 by international ships transporting infected swill that was fed to pigs in the region of Poti (Beltran-Alcrudo et al., 2009). Following introduction, the virus spread throughout the whole country, then crossed the Caucasus probably via infected wild boar (*Sus scrofa*) and entered the Russian Federation in late 2007. Whilst the first detection of an infected wild boar occurred in November 2007, the first report of an outbreak in domestic pigs was published seven months later, in June 2008. From 2007 to 2010, the virus circulated only in the southern regions of the Russian Federation affecting both domestic pigs and wild boars. In early 2011, the virus was introduced into central and northern regions where it subsequently spread locally resulting in new clusters of outbreaks and an increased risk of introduction for the European Union (Figure 1). In 2014, several infected wild boar

and domestic pigs were reported in Estonia, Latvia, Lithuania and Poland. So far, 333 outbreaks in domestic pigs and 137 wild boar cases have been detected and reported in the Russian Federation (source: EMPRES-i, accessed October 2014).

In Africa, the transmission cycle of the virus involves different types of hosts such as domestic pigs, wild suids (mainly warthogs and bushpigs), and soft ticks from the *Ornithodoros moubata* complex, with their relative role depending on locally relevant epidemiological conditions (Plowright et al., 1969; Jori and Bastos, 2009; Costard et al., 2012). In the Russian Federation, human activities appeared to play the most important role in ASF virus (ASFV) transmission across the country (Oganesyan et al., 2013), with illegal movements of swill containing infected pork products fed to pigs being responsible for 97% of the identified routes of introduction of ASFV into naive pig populations (FAO, 2013; Gogin et al., 2013). Because of the contagious aspect of the disease (Ferreira et al., 2013), secondary spread is likely to occur from farm to farm through the movement of infected pigs, contaminated humans or vehicles, etc. During the early stages of the epidemic, it was reported that infected wild boar contributed to the infection of domestic pigs in specific southern regions characterised by the presence of free-ranging pigs such as the Republic of South Ossetia (Gogin et al., 2013), but subsequent outbreak investigations in other parts of the country did not reveal strong evidence of wild boar-to-domestic pig transmission. As a consequence, the role of wild boars in the maintenance of ASF virus in domestic pig populations in the Russian Federation is still unclear (Oganesyan et al., 2013). In Eastern Europe, the distribution of *Ornithodoros* tick species, whether or not they feed on pigs and wild boar and their competence for transmitting ASF are also largely unknown (FAO, 2013).

At the local level, different transmission routes are likely to lead to different epidemic patterns. Movements of contaminated vehicles and humans and direct contacts between pigs from neighbouring farms are likely to result in local farm-to-farm transmission depending on spatial proximity, leading to space-time interaction between domestic pig outbreaks. In addition, direct and

indirect contacts between infected wild boar and susceptible domestic pigs may result in disease introduction to susceptible farms resulting in spatial clustering of pig outbreaks around wild boar cases.

The objective of this study was to explore the spatial and spatio-temporal patterns of the observed ASF epidemic at local level in order to assess the importance of the farm-to-farm and the wild boar-to-domestic pig transmission routes. The study focused in two of the most affected regions of the Russian Federation, namely the Krasnodar and the Tver regions (Figure 1).

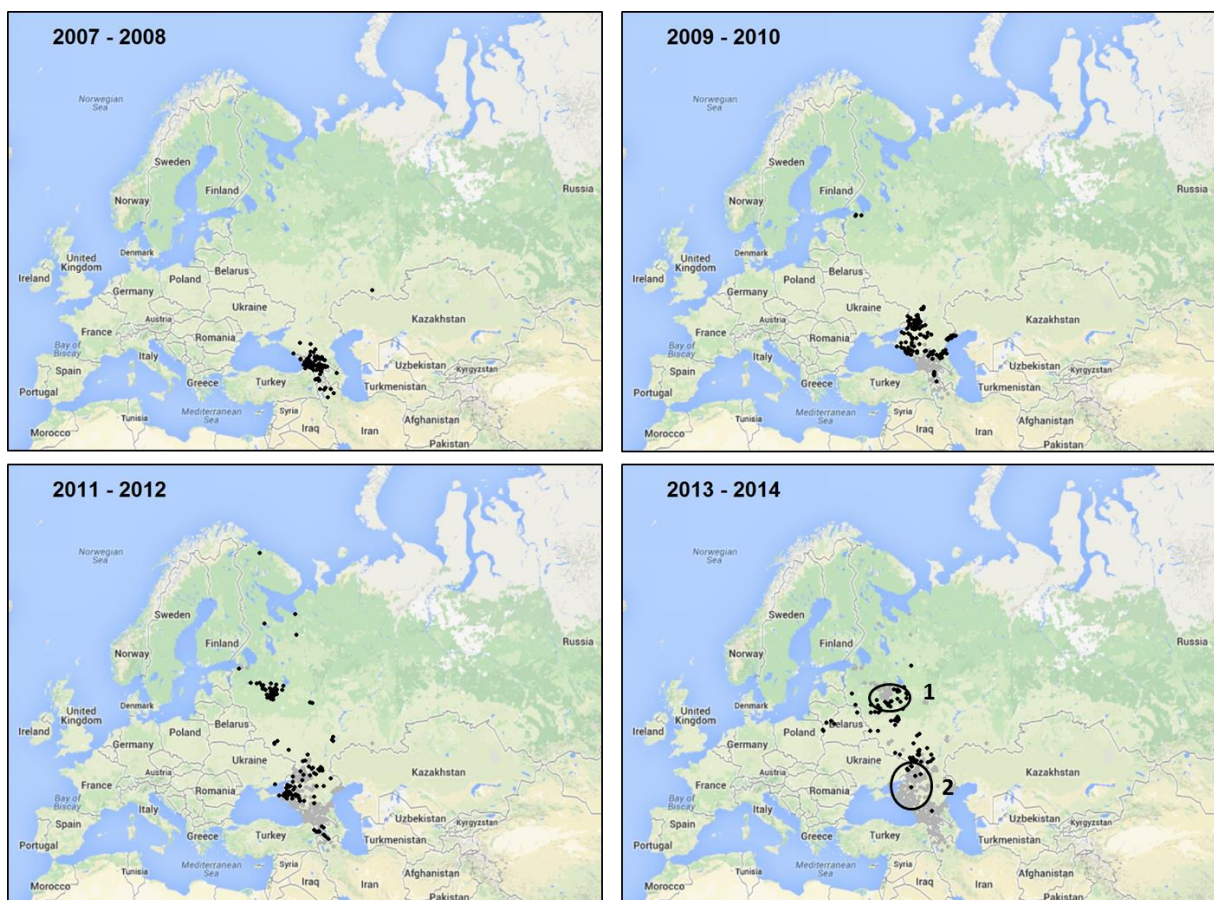


Figure 1: Dynamics of the African swine fever epidemic in Eastern Europe since its introduction in 2007. Black dots represent cases that were reported during the period of interest either in domestic pigs or in wild boar, whilst grey dots correspond to older cases; circles 1 and 2 identify the Tver region and the Krasnodar region, respectively. Last update: March 2014.

MATERIALS AND METHODS

Data

The disease reporting database collated by the National Research Institute for Veterinary Virology and Microbiology of Russia was used for these analyses. It included the list of all diagnosed cases (in domestic pigs and in wild boar), the species involved, their geographic location (latitude and longitude), the assumed start date of the outbreaks in domestic pigs and the date of diagnostic confirmation. The Tver region (defined here as the Eastern part of Tver oblast) and the Krasnodar region (defined here as the association of Krasnodar krai and the Republic of Adygea) were selected for this study as they were amongst the two most affected regions in the Russian Federation since the start of the epidemic (Figure 1). Assuming that the curvature of the Earth can be ignored given the relatively small size of each region, latitude and longitude were converted into planar coordinates for the analyses.

Whilst testing for space-time interaction amongst domestic outbreaks did not require data on the population at risk, testing for spatial clustering of outbreaks in domestic pigs around wild boar cases required the spatial distribution of the population at risk, i.e. the locations where outbreaks in domestic pigs could have occurred in the two regions. The population at risk was based on locations of high biosecurity farms and of rural settlements. A settlement is a cluster of rural households in which small numbers of pigs are raised under low biosecurity conditions. When an outbreak occurs in backyard pig populations, the settlement represents the scale at which the outbreak is reported and registered. The location of the settlements in the two regions of interest was extracted from the 2011 database 'digital model of the Russian Federation' distributed by the Russian Environmental Systems Research Institute (ESRI-CIS; <http://www.esri-cis.ru>);).

Table 1 presents the observed incidence of ASF in the three main compartments (rural settlements, high biosecurity farms and wild boar) for the two regions of interest from 2008 to 2013. Figure 2 illustrates their spatial distribution.

	2008	2009	2010	2011	2012	2013	Total
Krasnodar region							
Outbreaks reported in rural settlements	1	1	19	15	9	–	45
Outbreaks reported in high biosecurity farms	1	–	–	3	20	–	24
Infected wild boars identified	–	6	11	1	1	1	20
Tver region							
Outbreaks reported in rural settlements	–	–	–	8	21	1	30
Outbreaks reported in high biosecurity farms	–	–	–	–	1	3	4
Infected wild boars identified	–	–	–	4	36	8	48

Table 1. Number of African swine fever (ASF) events reported in each compartment as the beginning of the epidemic in the Krasnodar and Tver regions, Russian Federation

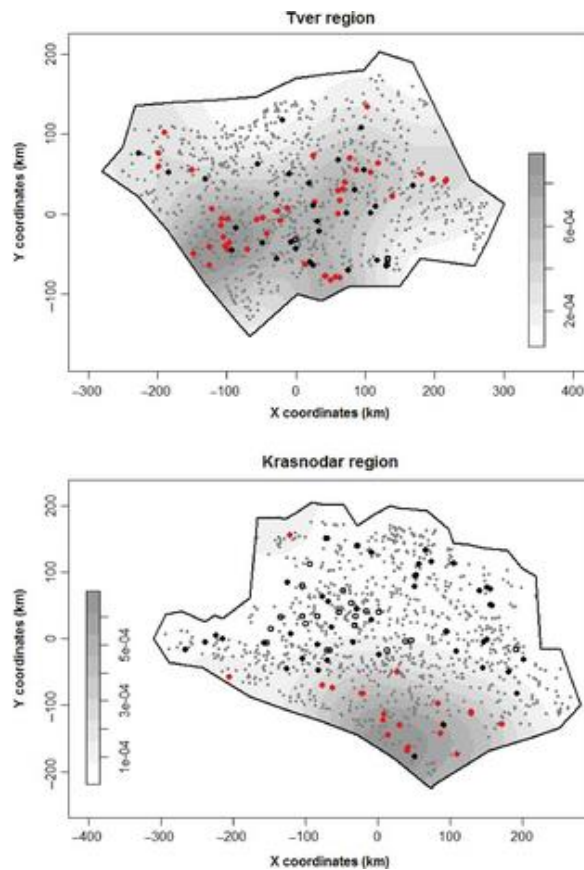


Figure 2: Spatial distribution of the wild boar cases (red dots), outbreaks in low biosecurity farms (black dots), outbreaks in high biosecurity farms (black circles) and settlements (grey dots) superimposed on the intensity surface (grey-scale image) calculated from the observed distribution of wild boar cases using a Gaussian kernel with a standard deviation of 50 km. [Colour figure can be viewed at wileyonlinelibrary.com].

Statistical analyses

Exploration of farm-to-farm transmission

The hypothesis of local farm-to-farm transmission of ASFV by contaminated fomites, humans or movement of pigs was tested by evaluating the significance of space-time interaction of the reported outbreaks in the domestic pig population. To do so, we used the space-time K -function (Diggle et al., 1995) which tested whether ASF outbreaks that were close in space were also close in time. This approach has already been applied to characterise the transmission process of a variety of animal infectious diseases including foot-and-mouth disease in Great Britain (Wilesmith et al., 2003; Picado

et al., 2007), bovine tuberculosis in New Zealand (Porphyre et al., 2007), highly pathogenic avian influenza in Vietnam (Minh et al., 2011) or Rift Valley fever in South Africa (Métras et al., 2012).

The bivariate space–time K-function $K(s,t)$ measures the expected number of ASF outbreaks occurring within a distance s and a time t from a randomly selected outbreak, relative to the average number of outbreaks occurring per space–time unit. When outbreaks occur independently in space and time (i.e. when there is no space–time interaction), $K(s,t)$ equals the product of the space K-function $K_1(s)$ and the time K-function $K_2(t)$ so that the difference $D(s,t) = K(s,t) - K_1(s).K_2(t)$ is null. The test statistic is defined as the sum of $D(s,t)$ over s and t . The estimate $D_0(s,t)$, defined as $D(s,t)/[K_1(s).K_2(t)]$, corresponds to the excess risk attributable to space–time interaction (Diggle et al., 1995). Therefore, a value of $D_0(s,t)$ equal to 1 indicates that the risk of an outbreak in a farm located within a distance s and a time window t of a randomly selected outbreak is 100% greater than the risk expected under the assumption of no space–time interaction. Because the average distance between consecutive outbreaks in domestic pigs was shown to be around 150 km (Korennoy et al., 2014) and because delays in reporting suspicions and implementing intervention measures could last several weeks (EFSA, 2014), it was considered that if a spatio-temporal interaction of the outbreaks was present, it would occur within a maximum space–time window of 150 km (representing around 50% of the full-spatial window of each study area) and 20 weeks (corresponding to around 10–20% of the periods of interest).

For testing the null hypothesis of no space–time interaction, 9999 Monte Carlo simulations were performed with the onset date of the ASF outbreaks randomly permuted on the fixed geographical locations, therefore generating a distribution of the test statistic under the null hypothesis of no space–time interaction. The P-value of the test was defined as the proportion of simulated test statistic values that were larger than the observed value. If the P-value was <5%, it could be concluded that there was <5% chance to observe the spatio-temporal pattern of the ASF epidemic in

the absence of space–time interaction. The null hypothesis of the absence of local farm-to-farm transmission would therefore be rejected (Diggle et al., 1995).

Exploration of the dependence between epidemics in wild boar and in domestic pigs

If the ASF epidemics in wild boar and in domestic pigs occurred independently from each other (i.e. if there was no substantial transmission of the virus from one population to the other), the location of outbreaks in domestic pigs would be independent from the location of wild boar cases. Therefore, the hypothesis of independence of the two epidemics was explored in each region by testing whether the mean intensity of wild boar cases at the location of outbreaks in domestic pigs was greater than what would be expected if the null hypothesis was true. To do so, the intensity function of wild boar cases was estimated using an isotropic Gaussian kernel with fixed standard deviations of 30, 50 or 100 km (Diggle, 1985), and the mean value of the intensity function at the location of outbreaks in low biosecurity farms was calculated. Figure 2 shows the intensity function of wild boar cases using an isotropic Gaussian kernel with a fixed standard deviation of 50 km.

For testing the null hypothesis of independence of the epidemics, a one-sided Monte Carlo hypothesis testing procedure was used (Dwass, 1957). To do so, 9999 Monte Carlo simulations were performed with the location of the outbreaks in low biosecurity farms (i.e. in settlements) randomly permuted amongst the settlements at risk within the region. This resulted in 9999 values of the average intensity of wild boar cases at the location of settlements under the null hypothesis of independence of the location of wild boar cases and outbreaks in domestic pigs. The P-value was calculated as the proportion of simulated test statistic values that were larger than or equal to the observed value. If the P-value was equal to or less than 5%, it was concluded that there was <5% chance to observe the joint spatial distribution of the wild boar cases and of outbreaks in low biosecurity farms given that the two epidemics were independent from each other. The null hypothesis would therefore be rejected.

Exploration of wild boar-to-domestic pig transmission

If wild boar-to-domestic pig transmission played a substantial role in the local spread of the disease, the risk of outbreaks in low biosecurity farms would be increased in the vicinity of wild boar cases. Therefore, the hypothesis of local wild boar-to-domestic pig transmission was explored by testing for the presence of spatial clustering of domestic outbreaks in low biosecurity farms around wild boar cases. Testing whether cases are clustered around pre-specified sources has been intensively used in public health for investigating whether the risk of diseases increases around sources of pollution such as incinerators or nuclear installations (Lawson and Waller, 1996; Tango, 2010).

The null hypothesis of no spatial clustering of outbreaks in low biosecurity farms around wild boar cases was tested by calculating the probability of infection $R(i)$ for a rural settlement located in the i^{th} region around wild boar cases (the i^{th} region was defined as the geographical area located between $10*(i-1)$ and $10*i$ km from wild boar cases).

To define the probability of infection $R(i)$, the region was divided into several 10 km-width regions centred around wild boar cases (Tango, 2010). For the i^{th} region, the risk of infection $R(i)$ for a rural settlement located in that region was calculated as follows:

$$R(i) = \frac{n_{\text{inf}}(i)}{n_{\text{tot}}(i)}$$

with $n_{\text{inf}}(i)$ and $n_{\text{tot}}(i)$ being, respectively, the number of outbreaks in low biosecurity farms and the number of settlements in the i^{th} region.

For testing the null hypothesis of the absence of focused clustering, a one-sided Monte Carlo hypothesis testing procedure was also used and P-values were calculated for each region i . If, for small values of i , the P-values associated with $R(i)$ were equal to or less than 5%, it could be concluded that there was <5% chance to observe the spatial pattern of the ASF epidemic in the absence of

focused clustering. The null hypothesis of the absence of focused clustering would therefore be rejected. Note that, the reciprocal hypothesis whether there is focused clustering of wild boar cases around domestic cases cannot be tested as the distribution of the wild boar population is not known.

All analyses were conducted in the statistical software R (R-Development-Core-Team, 2011) using the SPLANCS and the SPATSTAT libraries when necessary (Rowlingson and Diggle, 1993; Bivand and Gebhardt, 2000; Baddeley and Turner, 2005).

RESULTS

Space-time interaction of the outbreaks in domestic pigs was detected both in the Krasnodar region ($P\text{-value} < 10^{-3}$) and the Tver region ($P\text{-value} = 0.002$). Figure 3 shows the perspective plot of the excess risk attributable to space-time interaction (D_0) for the two regions. Comparison between the maximum excess risks (MER) suggests that the spatio-temporal scale of the interaction was similar between the Krasnodar (MER = 5.69) and the Tver regions (MER = 5.33). However, in the Tver region, the intensity of the space-time interaction decreased abruptly with increasing distance and time (although it remained larger than 1 during more than 20 weeks for very short distances), whilst in the Krasnodar region, it remained larger than 1 for both relatively long distances and long periods of time. Figure 3 shows evidence of space-time interaction for more than five months over a distance of up to 20 kilometres in the Krasnodar region.

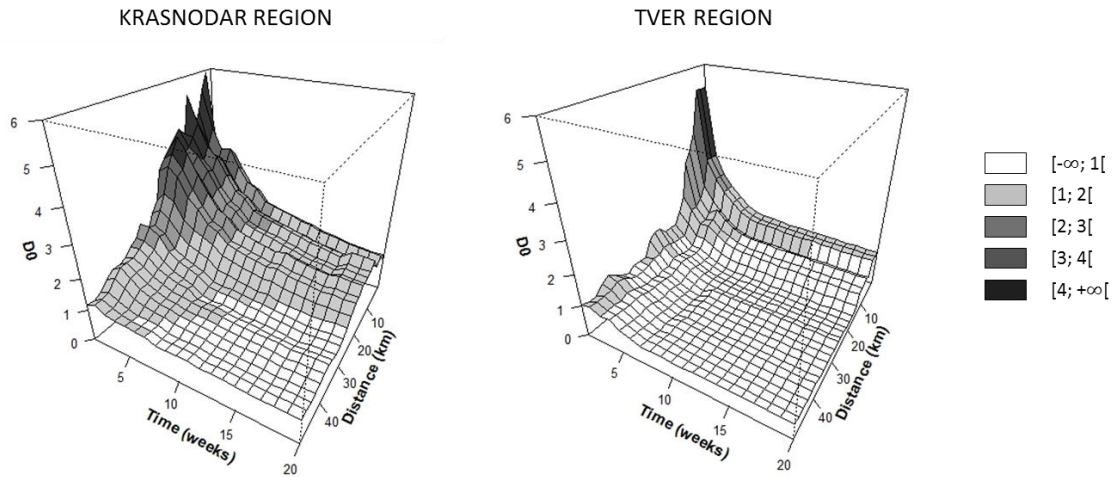


Figure 3: 3D plots of excess risk attributable to space-time interaction (D_0) for the Krasnodar (left) and Tver regions (right).

In the Krasnodar region, independent from the values for the standard deviation of the Gaussian kernel, the mean intensity of wild boar cases at the location of outbreaks in low biosecurity farms was not statistically significantly greater than what would be expected under the null hypothesis.

However, in the Tver region, independent from the values for the standard deviation of the Gaussian kernel, this mean intensity was statistically significantly greater than would have been expected under the null hypothesis (Table 2).

Standard deviations of the Gaussian kernel	Krasnodar region	Tver region
30 km	$5.6e-5$ (P -value = 0.977)	$5.9e-4$ (P -value = 0.018)
50 km	$7.1e-5$ (P -value = 0.976)	$5.4e-4$ (P -value = 0.008)
100 km	$1.0e-4$ (P -value = 0.970)	$4.7e-4$ (P -value = 0.023)
The P -values inform about the probability that this mean intensity would have been at least as great as the one observed if the null hypothesis of no focused clustering was true.		

Table 2. Mean intensity of wild boar cases at the location of outbreaks in low biosecurity farms, using a Gaussian kernel with different standard deviation

In both the Krasnodar and the Tver regions, the probability of infection of rural settlements located close to wild boar cases was not statistically significantly greater than the probability of infection of epidemiological units located further away (Figure 4).

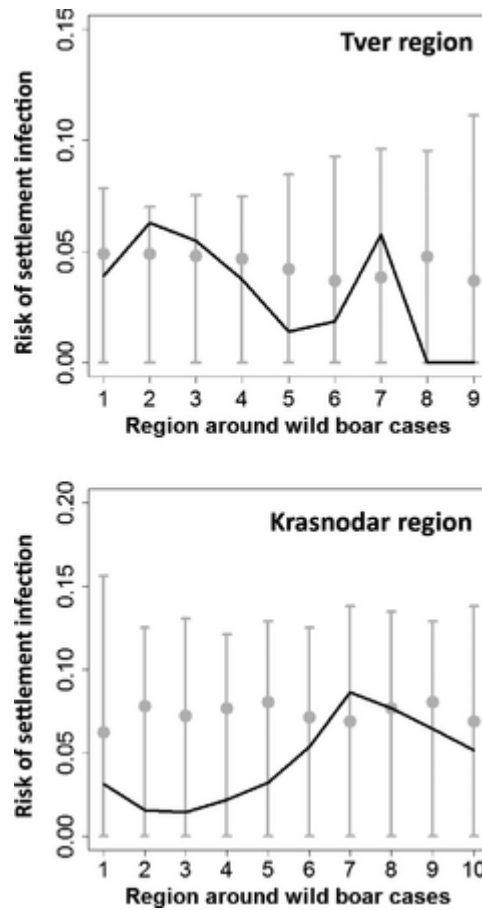


Figure 4. Probability of infection of rural settlements for the different regions around wild boar cases (black line). The i^{th} region corresponds to the geographical area located between $10^{*}(i-1)$ and $10^{*}i$ km from wild boar cases. The grey bars and grey dots represent, respectively, the likely range and the most likely value of the probability of settlement infection if the null hypothesis of no focused clustering was true.

DISCUSSION

The aim of the study was to explore the relative importance of the routes of transmission of ASF (including farm-to-farm and wild boar-to-domestic pig transmissions) that could be responsible for the local spread of the disease as observed in the Krasnodar and the Tver regions. There was strong statistical evidence of space–time interaction between the outbreaks that were reported in domestic pigs in both regions. However, the space–time interaction appeared to be more spread out in space in

the Krasnodar than in the Tver region. In the Krasnodar region, there was no statistical spatial dependence between the epidemics in wild boar and in domestic pigs (Table 2) and, consistently, the risk of infection of settlements did not increase with decreasing distance from wild boar cases (Figure 4). This suggests that wild boar-to-domestic pig transmission played a marginal role, if any, in the ASF epidemic that occurred in that region. In the Tver region, there was a statistically significant association between the locations of outbreaks in domestic pigs and those of wild boar cases (Table 2) suggesting a substantial transmission from domestic pigs to wild boar or vice versa. However, the risk of infection of settlements was shown not to increase statistically significantly with decreasing distance from wild boar cases (Figure 4), supporting the hypothesis that the direction of transmission was from domestic pigs to wild boar, rather than the reverse.

At the time of the epidemic, the density of pigs was much higher in the Krasnodar than in the Tver region, although the ratio of backyard to commercial pigs was fairly similar (FAO, 2010). In addition, extensive outdoor breeding of pigs was more common in the Krasnodar than in the Tver region (Oganesyanyan et al., 2013). As a consequence, direct and indirect contacts between farms and settlements were much more likely to occur in the Krasnodar region. This might explain why the space-time interaction of the outbreaks in domestic pigs was found to occur over much longer spatial distances in the Krasnodar region. In addition, it is worth noting that the epidemic in Tver started almost three years after the first outbreak in domestic pigs was reported in the Krasnodar region. The surveillance and intervention in Tver may therefore have been more effective than in Krasnodar where cooperation between local and federal authorities was initially poor (Gogin et al., 2013). This may have also contributed to the rapid decrease of the space–time interaction with increasing space and time windows in the Tver region.

Human behaviour is likely to have influenced the results presented here and their interpretation. Indeed, the surveillance data are very likely to represent only a subset of all the outbreaks that occurred in the two regions. In addition, as emphasized above, this reporting bias is likely to have

varied in space and time as a result of the diversity of human behaviours associated with reporting of ASF suspicions (Vergne et al., 2016). Spatial and temporal heterogeneity of the under-reporting rate are known to potentially decrease the statistical power of the space-time K -function. But this should not influence the likelihood of a Type I error, provided there is no dependence between the spatial and temporal variations (Fenton et al., 2004; French et al., 2005). Therefore, assuming that the probability for an outbreak to be reported did not increase after an outbreak had been reported in its vicinity is necessary to conclude that the detected space-time interaction of the outbreaks in domestic pigs represents an actual epidemiological effect. Sociological research focusing on the reporting attitude of Russian farmers in affected areas would be of great value to assess the validity of this assumption.

Another possible limitation of the space-time K -function is that, although it remains valid for a heterogeneous spatial distribution of the at-risk population and any temporal change of the population size, it requires the temporal change to be of similar magnitude for the entire study region. A violation of this assumption increases the likelihood of a Type I error of the test (Diggle et al., 1995; Kulldorff, 1998). In our context, preventive culling measures were implemented in the control zones defined as 5-to-20-kilometre-radius circles centred on detected outbreaks. As a consequence, all settlements located in this spatial window were no longer able to become infected and detected. In addition, some farmers may try to sell their animals as soon as they find out that a neighbouring farm has become infected, as has been observed for highly pathogenic avian influenza in Thailand (Paul et al., 2013). For these reasons, the population at risk is expected to have varied at different rates at different locations. However, because the areas where the population at risk was depleted were centred on the detected outbreaks themselves, the likelihood of a Type I error should not have been affected (the power of the test decreases though). It is worth noting that because the infection status of the pig herds that were culled in the control zones was unknown (they therefore could not be included in the analyses), the true value of D_0 for small distances was likely to be underestimated.

Considering the high contagiousness of the disease (Ferreira et al., 2013) and the ability of the virus to survive in the environment for extended periods (EFSA, 2010), we are confident that the detected space-time interaction of outbreaks in domestic pigs is a true reflection of the epidemiological process in these two regions, despite the bias resulting from potential modification of the reporting effectiveness in the vicinity of detected outbreaks. The mechanisms for local farm-to-farm transmission can include spread through contaminated vehicles, people from holdings nearby or the introduction of an infected animal from a neighbouring farm (FAO, 2013).

A recent study showed that the spatio-temporal pattern of ASF cases found in wild boar in the Southern region is more compatible with an epidemic than an endemic situation, as wild boar cases were rarely detected in previously infected areas (Lange et al., 2014). In addition, it was suggested that the basic reproduction number in wild boar in their natural environment is relatively high between 1.13 and 3.77 (Iglesias et al., 2016). Based on the analyses presented in the current study, the null hypothesis of independence of the location of outbreaks in domestic pigs from the location of wild boar cases could not be rejected in the Krasnodar region. Therefore, in that region, the combination of these three results suggest that although the virus circulated extensively in the wild boar populations, the two epidemics were independent from each other, confirming hypotheses formulated previously in other publications (FAO, 2013, Gogin et al., 2013). In the Tver region, epidemiological data provide evidence of a spatial dependence between outbreaks in domestic pigs and wild boar cases (Table 2). In this region, wild boar swill feeding and the illegal disposal of ASFV-infected pig carcasses are practised at times (FAO, 2013, Gogin et al., 2013) supporting the hypothesis of transmission of the ASF virus from domestic pigs to wild boar.

It is worth noting that human behaviour may have also influenced the outcome of the statistical analysis exploring the association between wild boar cases and outbreaks in domestic pigs. These analytical methods assume that the reporting probability of outbreaks in farms is independent of the location of reported wild boar cases. Indeed, the absence or the presence of evidence of dependence

between the epidemics in wild boar and in domestic pigs and of focused clustering of outbreaks in domestic pigs around wild boar cases may simply be explained by a decrease or an increase in the reporting probability of suspicions in pig farms in the neighbourhood of detected wild boar cases or vice versa. This would increase the likelihood of a Type II or a Type I errors, respectively. To assess this assumption and the validity of the test outcomes, field studies are necessary to better understand pig farmers' reporting behaviours in response to ASFV circulation in wild boar.

Unlike in most applications where focused tests are used to assess an increased risk around point sources such as incinerators (Tango, 2010), the source of infection in our application was not permanent. Therefore, because wild boar-to-domestic pig transmission implies that outbreaks in domestic pigs occur after the disease begins to circulate in the wild boar population, one could argue that the test should focus on the risk of an outbreak occurring around detected wild boar cases and after their detection. However, diseases are known to start circulating in wildlife populations much before the first infected individual from that population is identified. Therefore, incorporating the temporal dimension in the focused test would have decreased the power of the test. As a consequence, using the focused test without including the temporal dimension was considered to be more appropriate for the current analysis. We did have to make the assumption that the locations where the infected wild boars were found represented the centres of the areas where the virus was circulating amongst wild boars.

Implications for control

This study highlighted that in the two regions of interest the main driver of infection was the proximity to an infectious farm and that transmission from infected wild boar populations, if any, was only marginal. As a direct consequence, increasing the compliance of farmers with standard biosecurity measures such as restricted human access to pigs, cleaning and disinfection, etc. should be given top priority for preventing infectious contacts between infectious and susceptible farms.

Overall, in the two regions, around 27% of the reported outbreaks occurred in high-biosecurity farms

(Table 1). Although a positive reporting bias is very likely (FAO, 2013), this relative high figure is worrying. In these farms, ensuring that the biosecurity measures are appropriately applied (for example that workers really take a shower when entering and leaving the farm, swill feeding is not practiced, etc.) is of major importance.

Given the role of farm-to-farm transmission on the epidemics, interventions such as preventive culling and movement control are of major importance for preventing further spread between farms. In the past, these measures have been shown to be highly effective if applied appropriately, even in the absence of vaccination (Arias and Sanchez-Vizcaino, 2002). However, they are effective only if suspicions are reported and laboratory diagnosis results are generated in a timely fashion (Sanchez-Vizcaino et al., 2012). Delayed reporting would allow the disease to spread farther thereby compromising the cost-effectiveness of such drastic control methods. Therefore, optimizing early detection and reporting of outbreaks by increasing disease awareness combined with adequate financial compensation for culled animals is essential for successful control of outbreaks of this disease.

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