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REGULAR ARTICLES

Risk factors for rotavirus infection in pigs in Busia and Teso subcounties, Western Kenya

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Abstract We analysed data that were previously collected for molecular characterisation of rotavirus (RV) groups A and C in pigs from Teso and Busia subcounties in Kenya to determine risk factors for its infection. The data included records from 239 randomly selected piglets aged between 1 and 6 months raised in free range and backyard production systems. RV infection was confirmed by screening of fresh faecal samples by using reverse transcription polymerase chain reaction (RT-PCR); selected positive samples were subsequently sequenced and used for phylogenetic analysis. In this analysis, RV infection status was used as outcome variable, while the metadata collected at the time of sampling were used as predictors. A Bayesian hierarchical model which used integrated nested Laplace approximation (INLA) method was then fitted to the data. The model accounted for the spatial effect by using stochastic partial differential equations (SPDEs). Of the 239 samples screened, 206 were available for the analysis. Descriptive analyses showed that 27.7 % (57/206) of the samples were positive for rotaviruses groups A and C, 18.5 % were positive for group A rotaviruses, 5.3 % were positive for group C rotaviruses, while 3.9 % had co-infections from both groups of rotaviruses. The spatial effect was insignificant, and a simple (non-spatial) model showed that piglets (≤4 months) and those pigs kept in free range systems had higher risk of exposure to rotavirus infection as compared to older pigs (>4 months) and those tethered or housed, respectively. Intervention measures that will target these high-risk groups of pigs will be beneficial to farmers.

Keywords Rotaviruses A and C · Smallholder pigs · Western Kenya · INLA · Risk factors

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Introduction

A majority of pigs in Western Kenya are raised in free range or backyard production systems with low biosecurity standards and inadequate health and welfare standards (Kagira et al. 2010; Nantima et al. 2015). They often come in direct and indirect contact with other livestock, wildlife and people increasing risk of acquiring new infections and disseminating the pathogens they harbour. They also have the potential to act as important reservoirs for multiple zoonotic agents that are endemic in the region including tape worms, influenza viruses, trypanosomes and rotaviruses (RVs). Recent studies conducted in the area demonstrate that pigs often contaminate the local water bodies and food crops with RVs via their excreta (Kagira et al. 2010; Mbuthia et al. 2015; Mutua et al. 2012; Nantima et al. 2015; Wabacha et al. 2004). RV is also a common cause of diarrhoea and hospitalisation in children below 5 years especially in resource-poor countries (Othero et al. 2008; Parashar et al. 2006).

The virus is also associated with huge economic impact in livestock industry due to increased mortality in young animals, reduced growth in animals that recover and high cost of treatment. It causes diarrhoea in calves (Saif and Jiang 1994), nursing and post-weaned piglets (Kapikian and Shope 1996) and foals (Conner et al. 1983). RV infection often leads to diarrhoea in suckling and weaned pigs that usually

resolves in 2–3 days if not complicated by secondary bacterial infections (Chang et al. 2012). A recent study by (Amimo et al. 2015) reported a prevalence of 26.2 % for group A RV in asymptomatic pigs in Western Kenya and eastern Uganda. However, the prevalence of rotaviruses in other domestic animals in Kenya is largely unknown.

There is scanty information on the geographical distribution and burden of RV infections in both human and animals in developing countries including Kenya. A description of the distribution of the virus and processes involved in its transmission is required to inform interventions for the disease (Schærström 1996; Palombo 2002). Spatial epidemiological models offer an inclusive framework for identifying ecological, demographic and socioeconomic determinants of the virus. Methods for developing these models are rapidly evolving; those based on Bayesian statistics have been demonstrated to be more reliable in identifying determinants of a disease and predicting its distribution at high spatial scales (Diggle et al. 1998). This paper analyses risk factors that influence distribution of RVs in Western Kenya by using a Bayesian model developed in R-integrated nested Laplace approximation (INLA).

Materials and methods

Study area and sampling design

Data used in the present analysis were provided by the African swine fever (ASF) surveillance project that was implemented in Teso and Busia subcounties of Western Kenya. That project used locations with high pig population densities with a high proportion of free range and backyard production systems. Indigenous pigs were commonly raised by farmers, although other genotypes including exotic and crossbreds were also prevalent. The RV study was implemented under the ASF project as an additional subsidiary activity to assess ASF disease co-infections.

A cross-sectional survey design was used to obtain samples for determining the distribution of RV infection. The sample size was estimated by using the formula described by Dohoo et al. (2003):

$$n = \frac{z_{\alpha}^2 pq}{L^2} \quad (1)$$

where n represents the desired sample size to estimate a sample proportion, Z_{α} is the value of the Z score for a 95 % confidence interval in a standard normal distribution, p is a priori estimate of the population proportion, which previous studies such as Martella et al. (2007) indicate that this is about 28 %, $q = 1 - p$ and L is the margin of error, assumed to be 5 %. The estimated sample size for pigs needed for this study was 315.

The sampling units were selected by using a multistage sampling technique. Random sampling techniques based on computer-generated random numbers were used at each stage with the assistance from the local administration and veterinary staff. From each subcounty, two sublocations were selected; in each of the selected sublocations, two villages were selected. From each village, 10 households which were keeping pig were selected for the study. The selected households kept between 1 and 25 pigs per farm. From these selected farms, between one and five piglets aged 6 months or less were selected for faecal sampling. A map of the study area showing the sampling sites is given in Fig. 1.

Sample collection and handling

Fresh faecal samples were collected from individual pigs, transferred into sterile 15-ml centrifuge tubes and buried in dry ice before being transported to the International Livestock Research Institute's (ILRI) satellite laboratory in Busia for storage at −70 °C. The samples were later shipped to BecA-ILRI laboratories in Nairobi, Kenya, on dry ice for processing and analysis. All the samples were assigned unique identifiers, and the metadata collected at the time of sampling included age, sex and breed of pigs and pig production system (rural and urban populations). All the sampling sites were georeferenced by using handheld Garmin eTrex® GPS gadgets.

Detection of rotaviruses

Methods used and the results from the detection of group A rotaviruses from the samples collected have been described by Amimo et al. (2015). Group C rotaviruses were detected by using conventional reverse transcription polymerase chain reaction (RT-PCR) with the following validated primer sets: VP6L-F (ACAGTATTTCAGCCAGGDTTTC) and VP6L-R (AGCCACATAGTTCACATTTCATC (Amimo et al. 2013a). The RT-PCR was done by using Promega reagents according to the manufacturers' instructions. The amplicons were analysed in 2.5 % agarose gel. In that study, a total of 239 samples were screened for group A and group C rotaviruses. The current study used 206 out of the 239 samples because 33 samples did not have required metadata and were excluded from the analysis. Of the 206 samples selected, 27.7 % (57/206) were positive for rotaviruses from groups A and C; 18.5 % were positive for those from group A alone, 5.3 % were positive for those from group C alone and 3.9 % had infections from group A and C rotaviruses. These results were combined to obtain one outcome that determined whether or not an animal had rotavirus exposure.

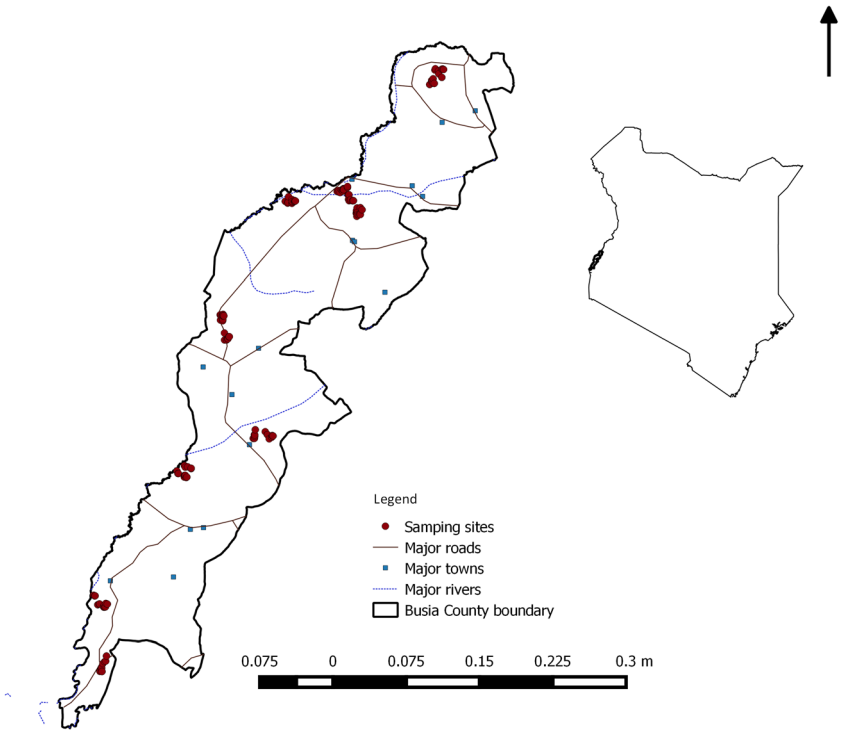

Fig. 1 A map of Busia County in Kenya showing the locations of the sampling sites. A map of Kenya is provided as an inset map to show the location of the county

Data management and analysis

Data generated from the study were stored in a relational database constructed by using MS Access. Independent variables were classified into animal-level, farm-level and spatial-level factors. The animal-level factors included sex, age in months and breed, while the farm-level factors were husbandry system (tethered versus free range), pig herd size, district and division where a farm was located. Spatial data were obtained from online databases as illustrated in Table 1. These included human and pig population densities in the selected sublocations, soil types, altitude and land cover types. Relevant values of these data were extraction based on the coordinates recorded at the time of sampling. In addition, Euclidean distances from the sampling sites to rivers, major roads and towns were derived and included in the analysis. All these data were concatenated into a single data frame.

Descriptive analyses examining the distribution of outcomes by the independent variables were done by using chi-squared tests of independence and unpaired *t* tests for categorical and continuous data types, respectively. These analyses were implemented by using R-INLA (Bivand et al. 2015). Univariate and multivariate models were fitted to the data to evaluate factors that influenced distribution of rotavirus infection in pigs in the study area. A description of the analytical model used and model fitting procedures are given below.

Table 1 Spatial data used with the metadata collected in the survey as predictors for RV occurrence in Busia, Kenya (2016)

Data	Description	Source
Towns	Major and small towns in Kenya	ILRI database
Altitude	Digital elevation model	Downloaded: 30 m ASTER GDEM Version 2 http://gdex.cr.usgs.gov/gdex/
Human population	Human population density based on 1999 population and housing census extracted using the sublocation shapefile	Data extracted to Busia-Teso sub locations
Pig population	Pig population density from the Department of Veterinary Services at sublocation level	Data extracted to Busia-Teso sublocations
Rivers	Kenya rivers	ILRI
Roads	Kenya roads	ILRI

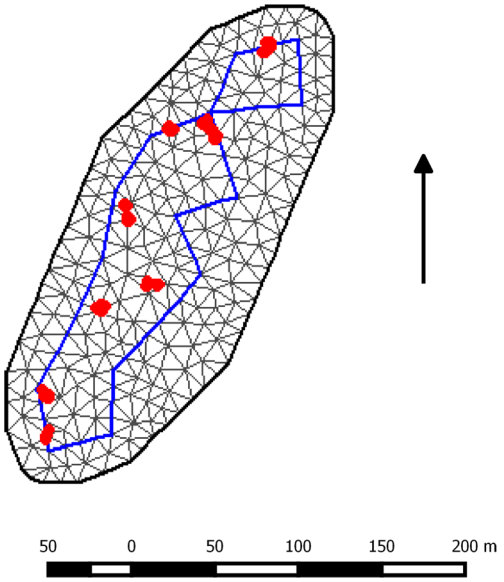

Fig. 2 A mesh constructed in R-INLA for approximating the mean of the Gaussian random field

Analytical framework

A Bayesian framework based on the R-INLA algorithm proposed by Rue et al. (2009) was used for analysis. The model used was of the form

$$\eta_i = \beta_0 + \sum_{m=1}^{M} \beta_m x_{mi} + f\left(z_i\right) \quad (2)$$

where

η_i is the linear predictor, linked to the original scale of the outcome; y_i is a binary dependent variable, through a binomial link; β_0 is a scalar representing the intercept; β_m represents the values of the coefficients quantifying the linear effect of covariates x_m and $f(z_i)$ is a function used to account for the spatial random effect.

Spatial effect was accounted for by using stochastic partial differential equations (SPDEs) which are suited for modelling geostatistical data. SPDE model assumes that random effect at each point (location) is a stochastic process with a Gaussian distribution. Its mean is assumed to be zero and variance approximated by Matérn correlation function. To estimate the spatial effects in the Gaussian random field, u, SPDE uses computation properties of Gaussian Markov random fields (GMRFs), often used in Besag and autoregressive processes, to replace continuously indexed random field to piecewise random field specified by GMRF. This is partly achieved through triangulation of the spatial domain. Key steps followed in the development of the spatial model included (i) construction of a mesh, (ii) setting up of a projector matrix, (iii) construction of a data stack, (iv) fitting the model and (v) generating posteriors/predictions.

Mesh construction

The extent of the spatial domain was defined by using the shape file of the study area obtained from the ILRI GIS database. The mesh was constructed by setting maximum length of the edge of triangles to 0.05, both within and outside the domain, and a cut-off of 0.1 to avoid building too many triangles around the observed locations. The extension of the mesh to a region outside the target domain was intended to avoid boundary problems associated with SPDE model. The mesh constructed had 269 vertices (Fig. 2).

Projector matrix

A projector matrix was designed to link the latent field, represented by the processes modelled at the mesh vertices, to the locations of the response based on the coordinates collected during the survey. The matrix created was of the dimension 206 records × 269 mesh vertices. This linked the SPDE model to the data.

Data stack

A data stack combining the outcome, the predictor variables and the projector matrix was developed to improve coding

Table 2 Cross tabulation between categorical variables—sex, breed and housing type—and the outcome

Variable	Levels	Number of records	Proportion infected (95 % CI)	Chi-sq., P
Age	Less or equal to 4 months	114	35.09 (26.38, 44.59)	6.21, P = 0.01
	More than 4 months	92	18.48 (11.15, 27.93)	
Sex	Male	42	30.95 (17.62–47.09)	0.11, P = 0.74
	Female	61	27.87 (17.15–40.83)	
Breed	Local	174	28.16 (21.62–35.47)	0.13, P = 0.71
	Crossbreed	32	25.00 (11.46–43.40)	
Husbandry	Free range with tethering/housing	91	35.16 (25.44–45.88)	4.58, P = 0.03
	Full-time housing/tethering	115	21.74 (14.59–30.40)	

Table 3 Results of two-sample *t* test used to analyse the distribution of continuous variables against rotavirus infection

Variable	Mean (95 % CI)		*t* test, *P*
	Positive	Negative	
Herd size	2.75 (2.30–3.21)	3.02 (2.63–3.41)	0.77, $P = 0.44$
Pig population	37.48 (32.32–42.65)	36.99 (33.63–40.35)	−0.16, $P = 0.88$
Human population	3544.67 (3151.86–3937.47)	3551.31 (3270.82–3831.80)	0.03, $P = 0.98$
Altitude	1166.26 (1152.08–1180.44)	1173.99 (1163.99–1183.98)	0.83, $P = 0.41$
Distance to main roads	1.78 (1.37–2.21)	1.80 (1.56–2.03)	0.04, $P = 0.97$
Distance to rivers	24.77 (20.69–28.86)	24.10 (21.32–26.88)	−0.26, $P = 0.80$
Distance to towns	3.98 (3.40–4.56)	3.74 (3.39–4.08)	−0.72, $P = 0.47$

efficiency. The stack was then called into the main model command as an object.

Multivariable analysis

The linearity assumption was tested for all the continuous variables by creating quadratic terms and testing for their significance in crude models that had the factors of interest as predictors and the outcome. Only age measured in months did not satisfy this assumption, and so, it was classified into a binary variable: less or equal to 4 months and over 4 months.

A full model was fitted to the data before a reduced parsimonious model was generated. Variables extracted from spatial data layers including distance to major roads, distance to rivers, human and pig population densities and altitude were first rescaled by dividing them by 100 in order to provide realistic parameter estimates. The significance of the spatial effect was determined by using deviance information criterion (DIC) statistic. For this analysis, two hierarchical models—with and without the spatial effect—were fitted to the data and the model that provided a smaller DIC estimate was preferred. The significance of independent factors was assessed by using credible (5–95 %) intervals generated as part of the posterior distributions of the model parameters.

Results

Descriptive analyses

All pigs that were used in the study appeared clinically healthy at the time of sampling though a few had loose stool. Fifty seven out of 206 pigs (27.7 %; 95 % confidence interval [CI] 21.7–34.3) were positive for either of the two genotype groups of rotavirus (groups A and C). The distribution of infected pigs by age, sex, breed and husbandry type is given in

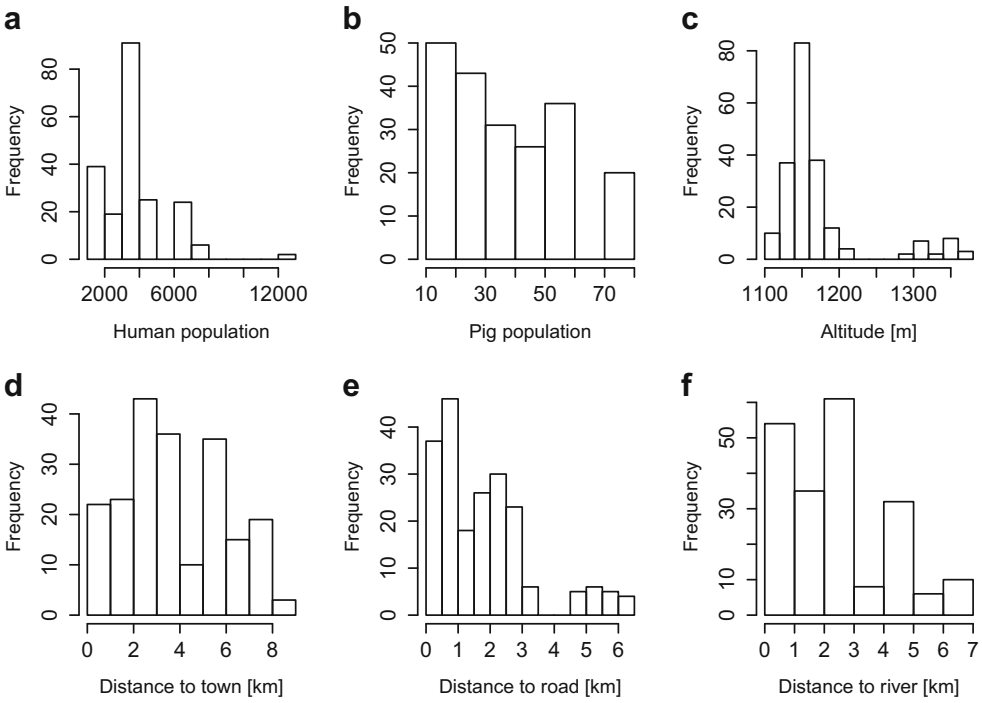

Fig. 3 Frequency distributions of the predictors estimated from the spatial data sets included in the regression analysis

Table 4 Outputs from saturated spatial and non-spatial multivariable models used to analyse association between rotavirus infection in pigs and animal-level, farm-level and area-level factors

Variable	Level	Spatial model			Non-spatial model		
		Mean	Percentile interval[a]		Mean	Percentile interval[a]	
			Lower limit	Upper limit		Lower limit	Upper limit
Age	>4 months	−0.89	−1.66	−0.14	−0.89	−1.66	−0.14
	≤4 months	0.00			0.00		
Sex	Male	−0.18	−1.05	0.67	−0.17	−1.03	0.68
	Female	0.00			0.00		
Husbandry system	Housed	−0.66	−1.34	0.01	−0.65	−1.33	0.01
	Free range	0.00			0.00		
Herd size		−0.05	−0.22	0.10	−0.05	−0.22	0.10
Pig population		−0.32	−2.90	2.37	−0.42	−2.75	1.88
Human population		−0.01	−0.04	0.02	−0.01	−0.04	0.02
Altitude		0.01	−0.14	0.18	0.01	−0.13	0.16
Distance to road		−0.01	−0.04	0.03	−0.01	−0.04	0.02
Distance to river		−0.01	−0.04	0.02	−0.01	−0.03	0.02
Distance to town		11.64	−11.54	34.74	11.22	−9.91	32.39
DIC		250.2			249.8		

[a] A percentile interval of 5–95 % is used in the analysis

Table 2. This analysis shows that the proportion of infected pigs among the younger pigs (less or equal to 4 months) and those kept in free range system with or without tethering was significantly higher than those in older groups (of more than 4 months) and pigs kept indoors or those tethered, respectively. The other independent factors (sex and breed), including all variables extracted from spatial data layers (Table 3), were not significant.

Figure 3 shows the distributions of the spatial datasets included in the analysis. These include human population, pig

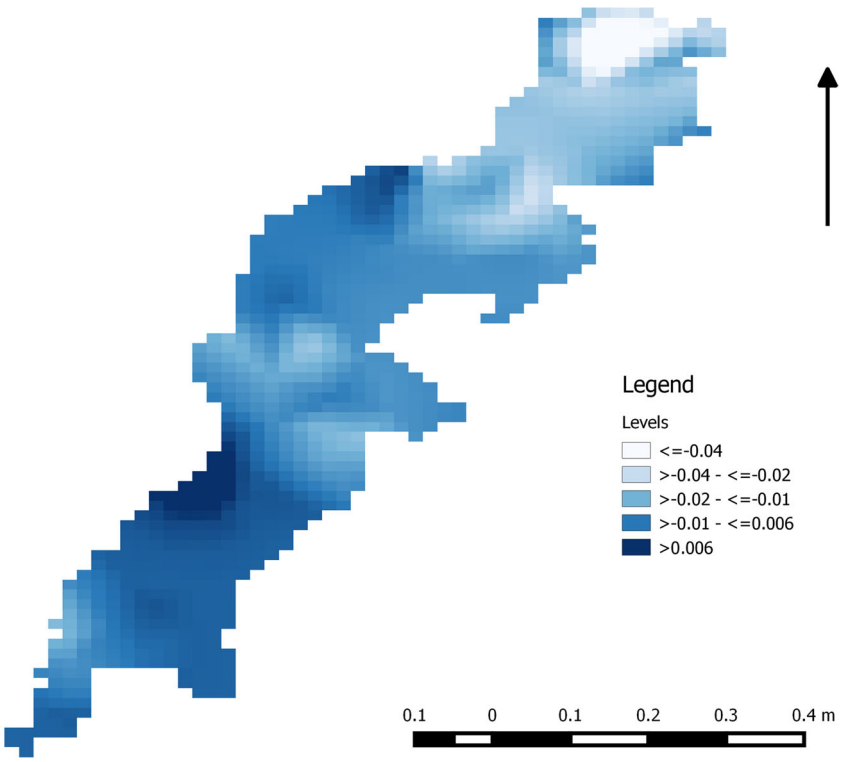

Fig. 4 Posterior mean log odds of the random field

Table 5 Outputs from a multivariable model showing factors that were significantly associated with rotavirus infection in pigs

Variable	Level	Parameter estimates		
		Mean	Percentile interval[a]	
			Lower limit	Upper limit
Age	>4 months	−0.97	−1.59	−0.38
	≤4 months	0.00		
Husbandry system	Housed/ tethered	−0.84	−1.37	−0.34
	Free ranging	0.00		
DIC		238.95		

[a] A percentile interval of 5–95 % is used in the analysis

population, altitude and Euclidean distances to major towns, roads and rivers. Analyses from unpaired *t* tests involving these variables and the outcome were not significant.

Multivariable analysis

Table 4 shows outputs from two saturated multivariable models fitted to the rotavirus data. One model accounted for the spatial effect by using the SPDE model, while the other did not. Based on the results from the DIC statistic, the analyses demonstrate that there is no significant spatial effect on rotavirus distribution in the study area. A prediction of the response on a map was therefore not generated since this was considered as being redundant. The posterior mean of the random field was however generated to study the distribution of the latent mean (Fig. 4). A reduced non-spatial model fitted to the data showed that age and husbandry system were significantly associated with rotavirus infection (Table 5). Pigs that were older than 4 months had low risk of infection compared to those that were aged less or equal to 4 months, while pigs that were housed or tethered had low risk of exposure to RV infections as compared to those raised under free range system.

Discussion

This analysis used outputs from an initial study which characterised RV by using molecular techniques reported by Amimo et al. (2015). Geostatistical models were used to determine the effects of animal-level, farm-level and area-level factors on the distribution of RV in pigs. These models were preferred because they offer a more robust framework for estimating the effect of multiple predictors on the outcome. RV infections have a lot of zoonotic potential, and knowledge on epidemiological factors that influence its distribution would be critical for its prevention and control. Although, rotavirus infection is endemic in pig herds worldwide (Steele et al. 2004), there is a limited number of comparable studies throughout Africa focusing on porcine rotavirus infection and risk factors associated with RV disease in pigs.

One of the key findings from this study is that animal (age) and farm (husbandry system) level factors had substantial effect on the distribution of the virus, while the effect of geographic factors was insignificant. Age resistance to clinical rotavirus disease occurs in swine and is associated with factors other than development of an age-dependent resistance (Dewey et al. 2003). In this study, the proportion of infected pigs among the young pigs (less or equal to 4 months) was significantly higher than that in the older group (of more than 4 months). This indicates that older pigs are more likely to have developed active immunity due to natural exposure. Our results is consistent with prior reports which have reported the relationship between age of the pig and RV infections (Amimo et al. 2013a,b; Chang et al. 2012; Collins et al. 2010).

Rotaviruses have epidemiological feature of persistence outside the pig, where they are resistant to environmental changes and many disinfectants. This indicates why RV infection is widespread and represents a constant risk to pigs in the tropics. Based on husbandry management systems in the study area, free ranging and tethering type of husbandry system presented higher risk of RV infection, than fully housed and/or tethered husbandry system. This could be a result of free ranging pigs acquiring infections from faecal contamination of watering points, interaction with wild pigs/warthogs and interaction with other domestic animals as well as humans.

Our analyses demonstrate that the spatial effect does not significantly influence the distribution of RV in the area. This is an important insight in its own right. First, the relative importance of non-spatial (animal and husbandry) factors compare to spatial ones gives a strong indication that RV control efforts would be more beneficial if they were implemented at the animal and husbandry level. The results suggest that RV is an important enteric pathogens in younger pigs (≤4 months) and the intervention strategies towards the control of this disease in the study region should target this age group. In addition, practicing husbandry systems that require the confinement of pigs such as housing and tethering would substantially reduce the risk of RV exposure. There was no interaction between these two factors indicating that their individual effects on the outcome would be additive.

Secondly, there has been a lot of interest on disease mapping given that tools generated (e.g. maps) can be used to guide risk-based surveillance and control. However, not much has been published on the minimum requirements for an effective disease or pathogen mapping, and many scientists have aspired to generate maps even for those diseases that are not amenable for mapping. Our findings can be used to demonstrate one of the key requirements for a disease/risk map. The unobserved latent effect (which might influence the

distribution of the outcome) predicted from the spatial model (in Fig. 4) had a more or less constant mean across the spatial domain, and hence, the outcome analysed in this study could not produce a meaningful risk map. We however expected RV to be more prevalent in areas with higher pig population densities, and so, more studies are required to determine why this was not so. One plausible reason could be that free ranging pigs aided the spread of the virus, and the small spatial scale used in the study further limited the ability of the analysis to identify differences in space.

Additional research should expand from current study to a national scale to avoid oversimplifying the geospatial impact on rotavirus transmission. Besides, future epidemiological studies should consider collection of both human and livestock samples from households to test the hypothesis on potential zoonotic link within these farming communities. Better understanding of the rotavirus distribution and risk factors for both human and animal exposure may be crucial for the planning of an efficient prevention and control programmes for rotavirus-associated gastroenteritis.

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Compliance with ethical standards

Conflict of interests None

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