**INTRODUCTION**

Swine erysipelas, or ‘diamond skin disease’, is an economically significant disease affecting all stages of pig production. The biggest losses may occur in growers-finishers because of sudden death or acute septicemia. Survivors often suffer from chronic lameness, arthritis and endocarditis leading to poor body growth or infertility. The causative agent is the ubiquitous bacterium *Erysipelothrix (E.) rhusiopathiae*, which is also able to enter the skin of people handling infected animals and meat and cause infection. In order to show the presence of *E. rhusiopathiae* in pigs, serum samples from 426 randomly selected pigs were collected in four subcounties (Bugelumbya, Butansi, Kitayunjwa and Namwendwa) in Kamuli District in Uganda, as part of a multipathogen survey conducted by the International Livestock Research Institute in 2013. Subsequently, 100 samples of fresh pork were collected from all 67-pork slaughterhouses operating in the same subcounties for isolation and bacterial culture. Overall, 308/460 (67%) of the pig sera carried antibodies against *E. rhusiopathiae* and 45/100 (45%) of the fresh pork samples were contaminated with *E. rhusiopathiae*. This is the first ever report of *E. rhusiopathiae* in pigs and pork in Uganda.


**Detection of *Erysipelothrix rhusiopathiae* in naturally infected pigs in Kamuli District, Uganda**

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Delia Grace4  Michel Dione3  Joseph Erume5

**Keywords**
Swine, *Erysipelothrix rhusiopathiae*, pork, smallholder, Uganda

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It is distributed worldwide and primarily considered as an animal pathogen. Turkeys and pigs are most commonly affected, followed by other birds, sheep, fish and reptiles. *E. rhusiopathiae* is zoonotic, and transmitted through scratches or small injuries of the skin. It is not commonly known among researchers, veterinarians, personnel of the health care sector in general, butchers, abattoir workers, farmers, fishermen, fish handlers and housewives. It has been referred to as an occupational disease by Wood (1975), as it affects these categories of people who work closely with infected animals or their products. In humans, it usually manifests itself as localized cellulitis known as erysipeloid. If infections get systemic, patients may have fever, joint pain and lymphadenopathy. Infections in otherwise healthy people are usually self-limited within three to four weeks (Reboli and Farrar, 1989). In rare cases, *E. rhusiopathiae* causes septicemia and potentially fatal endocarditis.

In Uganda, pig keeping has emerged as a popular income-generating activity over the past three decades (MAAIF/UBOS, 2009). It is the first East African country in terms of consumption per capita (FAOSTAT, 2011). Nevertheless, many of the potentially zoonotic pig diseases have never been researched in Uganda (Alonso et al., 2016) because generally pigs have played a negligible role in agricultural production in the past in the country (Blench, 2000).

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Preliminary information from participatory rural appraisals conducted in 2013 indicated the occurrence of diamond skin disease in pigs in Kamuli District, Eastern Uganda (Roesel et al., 2014). According to these appraisals, farmers from three subcounties had reported skin lesions in live pigs, which may have been caused by *E. rhusiopathiae*; the disease is locally referred to as ‘Okumyuka’. In the present study, we investigated the existence of *E. rhusiopathiae* in pigs and pork in the villages that reported signs of the disease.

**MATERIALS AND METHODS**

**Study area**

The study was conducted in Kamuli District, Eastern Uganda, and was part of a multidisciplinary program implemented by the International Livestock Research Institute (ILRI) and partners to identify constraints and opportunities to improve smallholder pig value chains in Uganda (Ouma et al., 2014). The district has an estimated population of 55,998 pigs and most pigs are kept under extensive or semi-extensive systems with free-ranging and tethering being the most practiced keeping types.

**Sample collection**

Serum aliquots from 426 randomly selected pigs in four subcounties, namely Bugulumbya, Butansi, Kitayunyu, Namwendwa, in Kamuli District (Figure 1), had been obtained in previous investigations (Erume et al., 2016) that were part of a multipathogen survey led by ILRI. Subsequently, 100 samples of fresh pork were collected from all 67 slaughterhouses operating in the same subcounties at the time of the study in March 2014. Using a sharp knife and gloves, approximately 250 grams of small pieces of pork from deep inside the muscle were cut. After collection, the pork was put in a stomacher bag, labelled and sealed with tape. It was put on ice in a cool box and transported to the College of Veterinary Medicine, Animal Resources and Biosecurity for microbiological analysis, which was carried out within ten days.

**Laboratory analysis**

**Indirect enzyme-linked immunosorbert assay**

A commercially available ELISA (CIVTest Suis SE/MR, Laboratorio Hipra, Amer, Spain) based on *E. rhusiopathiae*-specific antigen (*E. rhusiopathiae* R32E11, serotype B) was used to detect anti-Erysipelothrix spp. immunoglobulin G. According to the manufacturer’s instructions, the assay showed a sensitivity and specificity of 100%. The 96-well microplates were provided precoated with antigen, and the assay was performed as follows: 50 μl of the undiluted, ready-to-use controls and 1:200 diluted test samples were added to the appropriate wells and left to incubate for 60 min at 37°C to allow antigen-antibody binding. All wells were washed three times with reconstituted washing solution (300 μl) and, subsequently, 50 μl of a ready-to-use conjugate solution (MAb anti-porcine IgG/HIPRO) were added to each well and left to incubate for another 60 min at 37°C to bind any attached porcine antibody. Remaining unbound conjugate was washed three times (300 μl) in another step, before a peroxidase-specific chromogenic substrate solution (50 μl) was added to each well and left in the dark at room temperature for 15 min to allow a chromogenic reaction to develop. The reaction was stopped by adding 50-μl ready-to-use oxalic acid solution.

Optical density (OD) of the sample was measured by reading the test plate at 405 nm wavelength (ELx800, BioTEK; Gen5 2.00.18). For the interpretation of results, a relative index (IRPC) value was required (IRPC = [sample OD - mean negative control OD] / mean positive control OD - mean negative control OD) * 100, where an IRPC value above 40.0 was defined as positive, according to the manufacturer. All samples were analyzed in duplicates using the positive and negative controls provided by the manufacturer.

**Culture isolation and identification from pork samples**

Bacteriological culture was performed according to standard procedures (Reboli and Farrar, 1989). Briefly, pork samples were homogenized in a sterile stomacher bag. Five microliters of the resulting solution were streaked on sterile trypticase soy agar (Laboratorios-Conda) as reviewed by Wang et al. (2010) and subsequently incubated for 24 to 48 hours at 37°C. The plates were examined after incubation for suspect *E. rhusiopathiae* colonies which were pinpoint size (approximately 0.1 mm), convex, circular, translucent, smooth, mildly hemolytic colonies (Reboli and Farrar, 1989). The colonies with morphological characteristics of *E. rhusiopathiae* were subcultured on Erysipelothrix selective media (Laboratorios-Conda) as reviewed by Wang et al. (2010) and subsequently incubated for 24 hours at 37°C. Bacterial colonies that grew on the selective media were identified by their morphology: small (0.3-0.6 mm), convex, circular, translucent, smooth, mildly hemolytic colonies, and subsequently, they were biochemically confirmed by testing for catalase activity as well as their ability to hydrolyze esculin and break down protein gelatine (Forbes et al., 2007).

**Data management and analysis**

Laboratory data and data collected on demographics (location of the butcher, location of the pigs) were double entered in Excel and cross-checked for completeness and accuracy before descriptive analysis in STATA 12.0 (StataCorp). Confidence limits were computed with Ausvet EpiTools (http://epitools.ausvet.com.au/content.php?page=CIPportion) for a desired level of confidence of 95% using the binomial (Clopper-Pearson) ‘exact’ method based on beta distribution. Significance in prevalence between villages was assessed based on the overlap of confidence intervals (Tables I and II).
Ethics

This work involved animal sampling and interviewing of farmers. Ethical clearance was obtained from the Ethical Review Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University (No. VAB/REC/13/101) and ILRI Institutional Research Ethics Committee (Ref. IREC 2013–03). The farmers were duly informed about the study and their written consent was sought prior to the start of data collection. No blinding was done.

RESULTS

In the first step, ELISA revealed an overall seroprevalence of antibodies against *E. rhusiopathiae* in pig sera of 66.9%, with no significant differences between subcounties (Table I).

In the second step, results showed that *E. rhusiopathiae* was isolated from 45% of the 100 raw pork samples with the highest proportion found in Butansi subcounty (Table II). However, differences between subcounties were not statistically significant.

DISCUSSION

To the knowledge of the authors, this is the first report of the occurrence of *E. rhusiopathiae* in pigs and pork in Uganda, and the bacterium has been isolated from all subcounties in the study where farmers had described clinical signs of acute swine erysipelas during systematically conducted participatory research (Roesel et al., 2014) therefore offering potential for increased income through small-scale pig production and marketing. A multi-disciplinary value chain assessment conducted by ILRI and partners aimed to identify constraints and opportunities for value chain actors as well as shortcomings in the safety of pork products in three districts in Uganda. Prior to quantitative surveys and biological sampling at various nodes of the chain, participatory rural appraisals and focus group discussions were held with about 1,400 smallholder pig farmers to map out qualitative aspects including the various actors involved in pig rearing (e.g. input and service providers). In the East African Community, *E. rhusiopathiae* has been previously isolated from pigs in Kenya (Wabach et al., 1998, Friendship and Bilkei, 2007), where it caused high mortality suggesting a virulent serovar affecting pigs in the region. Pigs have been shown to harbor both avirulent and virulent serotypes in their tonsils (Takahashi et al., 1987). Although clinical cases of swine erysipelas are predominantly caused by serotypes 1a, 1b, or 2, less common serotypes typically have lower virulence for swine (Opriessnig and Wood, 2012). The current aim of the study was to reveal the presence of *E. rhusiopathiae* in pigs but not to serotype the isolates. However, given the unexpectedly high prevalence in raw pork, the serotypes circulating in Kamuli District should be determined in future research.

Infection with *E. rhusiopathiae* in pigs can be present under three known forms of the disease, depending on the virulence of the serotype and the host’s immunity status: acute, subacute and chronic. The acute form is characterized by a septicemia with sudden fever, abortion, lethargy, lying down, stiffness of joints, partial or complete lack of appetite, characteristic pink, red or purple, raised, rhomboid or squared skin lesions around the snout, ears, jowls, throat, abdomen and thighs, and death (Opriessnig and Wood, 2012). Especially in dark-skinned pigs, where the characteristic shape of the skin lesion may not be fully visible, many of the symptoms may be confused with signs of African swine fever which is perceived to be a major health constraint to smallholder pig production in the region (Dione et al., 2014). On the other hand, the subacute form of the disease may result in infertility in pigs, whereas the chronic form may cause lameness; both conditions can cause severe production losses. However, there are both treatment and immunization available for the control of

<table>
<thead>
<tr>
<th>Subcounty</th>
<th>Village</th>
<th>Sample size</th>
<th>ELISA+</th>
<th>Prevalence (%)</th>
<th>Confidence interval* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitayunjwa</td>
<td>Butabala</td>
<td>38</td>
<td>27</td>
<td>71.1</td>
<td>54.1–84.6</td>
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<td>Bugulumbya</td>
<td>Bukyonza, Baluboinewa</td>
<td>118</td>
<td>81</td>
<td>68.6</td>
<td>59.5–76.9</td>
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<tr>
<td>Namwenda</td>
<td>Isingo A</td>
<td>78</td>
<td>49</td>
<td>62.8</td>
<td>51.1–73.5</td>
</tr>
<tr>
<td>Butansi</td>
<td>Ntansi, Kanyu zone</td>
<td>192</td>
<td>128</td>
<td>66.7</td>
<td>59.5–73.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>426</td>
<td>285</td>
<td>66.9</td>
<td>62.2–71.4</td>
</tr>
</tbody>
</table>

* Calculated at p = 0.05, EpiTools Exact Binomial Clopper-Pearson

<table>
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<tr>
<th>Subcounty</th>
<th>Village</th>
<th>Sample size</th>
<th>Culture+</th>
<th>Prevalence (%)</th>
<th>Confidence interval* (%)</th>
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<td>5</td>
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<td>12.8–64.9</td>
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<tr>
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<td>25.6–67.2</td>
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<tr>
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<td>Ntansi</td>
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<td>6</td>
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<tr>
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<td>45</td>
<td>45.0</td>
<td>35.0–55.3</td>
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</tbody>
</table>

* Calculated at p = 0.05, EpiTools Exact Binomial Clopper-Pearson
Swine erysipelas in smallholder pig farms in Uganda

**E. rhusiopathiae** infection in pigs, but the latter requires knowledge on the circulating serotypes. In humans as in pigs, the disease can easily be treated but only if detected in time.

**E. rhusiopathiae** is a ubiquitous bacterium that can survive in swine feces at different ambient temperatures (Conklin and Steele, 1979), in soil (Weaver, 1985), water, sewage, abattoir effluent and fertilizer (Woodbine, 1950; Cottral, 1978; Reboli and Farrar, 1992). In pork it survives chilling, freezing, and curing (Wood, 1975) as well as decomposing to some extent (Conklin and Steele, 1979).

## CONCLUSION

As clinical signs of swine erysipelas had been reported by smallholder pig farmers in Kamuli District, the study aimed at showing the prevalence of the disease in the area. We showed that a 66.7% seroprevalence was found among the 426 pigs investigated, and a 45% prevalence was found in pork samples from the 67 slaughterhouses.

Therefore, **E. rhusiopathiae** is present in pigs in Kamuli District, posing an economic risk to smallholder pig farmers, and a potentially public health risk to pig and pork handlers in the area. Moreover, we were able to confirm that community-based participatory disease reporting is helpful in identifying diseases in livestock species that are historically recent in developing countries. **E. rhusiopathiae** infection has not been reported in humans nor in pigs in Uganda. Fortunately, the disease is preventable and relatively treatable when diagnosed in time. Further research should include serotyping of isolates to identify control strategies, especially vaccination, that could be implemented in Kamuli District. It should also attempt to assess the burden of **E. rhusiopathiae** infection in raw pork handlers such as butchers and cooks, and identify practices that might increase the risk of human infection. Public health practitioners in Eastern Uganda such as physicians, nurses, veterinarians and meat inspectors should be sensitized on the presence of the pathogen as well as its diagnosis and control. Much remains to be elucidated on the epidemiology and transmission cycle of **E. rhusiopathiae** in animals, people and the environment in Uganda. Future investigations should focus on the One Health approach.

## Acknowledgments

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Résumé

Musewa A., Roesel K., Grace D., Dione M., Erume J. Détection d’Erysipelothrix rhusiopathiae chez des porcs infectés naturellement dans le district de Kamuli en Ouganda

L’érysipèle des porcs est une maladie économiquement importante qui affecte toutes les étapes de la production de porc. Les pertes les plus importantes peuvent survenir chez les producteurs de porc d’engraissement, suite à une mort subite ou à une septicémie aiguë. Les porcs survivants souffrent souvent de boiteries chroniques, d’arthrite et d’endocardite, entraînant une croissance corporelle médiocre. L’agent causal est la bactérie ubiquitaire Erysipelothrix (E.) rhusiopathiae. Elle est également capable d’entrer dans la peau des personnes qui manipulent des animaux et de la viande infectés, et ainsi causer une infection. Afin de montrer la présence de l’agent responsable chez les porcins, des échantillons de sérum provenant de 426 porcs sélectionnés au hasard ont été recueillis auprès d’élevages dans quatre sous-comtés (Bugulumbya, Butansi, Kitayunwa et Namwendwa) dans le district de Kamuli en Ouganda, dans le cadre d’une grande étude multipathogène menée par l’International Livestock Research Institute en 2013. Par la suite, 100 échantillons de viande de porc fraîche ont été prélevés auprès de 67 boucheries opérant dans les mêmes sous-comtés pour isolement et culture bactérienne. Dans l’ensemble, des anticorps contre E. rhusiopathiae ont été détectés dans 308 sur 460 (67%) séums, et 45 sur 100 (45%) échantillons de viande de porc fraîche étaient contaminés par E. rhusiopathiae. C’est la première étude rapportant l’occurrence d’E. rhusiopathiae chez les porc et dans la viande de porc en Ouganda.

Mots-clés : porcin, Erysipelothrix rhusiopathiae, viande porcine, exploitant agricole, Ouganda

Resumen

Musewa A., Roesel K., Grace D., Dione M., Erume J. Detección de Erysipelothrix rhusiopathiae en cerdos infectados naturalmente en el distrito de Kamuli, Uganda

La erisipela porcina es una enfermedad económicamente significativa, que afecta todos los estadios de producción porcina. Las mayores pérdidas se observan en los criadores de engorde debido a muertes súbitas o septicemia aguda. Los sobrevivientes a menudo sufren de laminitis, artritis y endocarditis, llevando a un pobre crecimiento corporal. El agente causal es la bacteria ubiquitous Erysipelothrix (E.) rhusiopathiae, capaz también de infiltrarse por la piel de las personas que manejan animales y carne infectados, causando infección. Con el fin de mostrar la presencia de E. rhusiopathiae en cerdos, se recolectaron muestras de suero en 426 cerdos seleccionados al azar en cuatro sub condados (Bugulumbya, Butansi, Kitayunwa y Namwendwa) en el distrito de Kamuli en Uganda, como parte de una encuesta sobre patógenos múltiples llevadas a cabo por el Instituto Internacional de Investigación Ganadera (ILRI) en 2013. Subsecuentemente, se recolectaron 100 muestras de carne de cerdo fresco de cada uno de los 67 mataderos operando en los mismos sub condados, para aislamiento y cultivo bacteriano. En total, 308/460 (67%) de los sueros de cerdo presentaron anticuerpos contra E. rhusiopathiae y 45/100 (45%) de las muestras de carne de cerdo fresco estuvieron contaminadas con E. rhusiopathiae. Este es el primer reporte de E. rhusiopathiae en cerdos y en carne de cerdo en Uganda.

Palabras clave : cerdo, Erysipelothrix rhusiopathiae, carne de cerdo, pequeño agricultor, Uganda