

## High Prevalence of *Erysipelothrix rhusiopathiae* Infection Among Abattoir Workers in Kamuli District, Eastern Uganda

Angella Musewa<sup>1\*</sup>, Kristina Roesel<sup>2</sup>, Damalie Nakanjako<sup>1</sup>, Ismael Kawoya<sup>1</sup>, Ronald Ssenyonga<sup>1</sup>, Joanita Nangendo<sup>1</sup>, Ali Sam<sup>1</sup>, Joseph Erume<sup>4</sup> and Delia Grace<sup>3</sup>

<sup>1</sup>College of Health Sciences, Clinical Epidemiology Unit, Makerere University P.O., Kampala Uganda

<sup>2</sup>Freie Universität Berlin, Institute of Parasitology and Tropical Veterinary Medicine

<sup>3</sup>International Livestock Research Institute, Nairobi Kenya,

<sup>4</sup>College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Uganda

**\*Corresponding Author:** Angella Musewa, College of Health Sciences, Clinical Epidemiology Unit, Makerere University P.O., Kampala Uganda.

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### Abstract

**Introduction:** *Erysipelothrix (E.) rhusiopathiae* is a ubiquitous gram-positive bacterium, which causes erysipelas in swine, mammals, birds and erysipeloid in humans. A previous study conducted in Kamuli district, identified serotypes 1 and 2 in pigs thus a follow up study in humans aimed at establishing prevalence and factors associated with *E. rhusiopathiae* infection.

**Methods:** We conducted a cross-sectional community-based epidemiological study using quantitative methods of data collection. The study enrolled 302 raw pork handlers (butchers, abattoir workers and cooks) in Kamuli district, Eastern Uganda. *Erysipelothrix rhusiopathiae* infection among the handlers was determined by collecting whole blood for culture. Bacteria were isolated and infection confirmed using biochemical tests and gram staining. In addition, a semi-structured questionnaire was administered to establish the risk factors of *E. rhusiopathiae* infection.

**Results:** The overall prevalence of *E. rhusiopathiae* infection was 9.9 % (95% CI: 7.4-13). Being a raw pork handler and self-reported alcohol consumption increased the risk of acquiring the infection. Among butchers, the prevalence was 15% (95% CI: 5.9 - 25), abattoir workers 37% (95% CI: 21 - 52) and cooks 3.4% (95% CI: 1.0 - 5.9). Working in the abattoir and butchery made infection 26 times (OR = 26; 95% CI: 5.3 - 129) and 8 times (OR = 8.4; 95% CI: 1.8 - 39) more likely, respectively. Alcohol consumption was associated with *E. rhusiopathiae* infection (OR = 4.0; 95% CI: 1.1 - 15).

**Conclusion:** *E. rhusiopathiae* infections exist in Kamuli district, Eastern Uganda affecting all categories of raw pork handlers. We recommend that the isolates be serotyped to identify the circulating serotypes and inform the appropriate control measures for the disease in humans.

**Keywords:** Pork Handler; *Erysipelothrix rhusiopathiae*; Uganda

### Introduction

*Erysipelothrix (E.) rhusiopathiae* is a zoonotic agent infecting various mammals, birds and humans worldwide [1]. Infection in humans is usually acquired from birds and mammals, particularly swine [2]. If clinical, the infection often causes significant human suffering [3]. The most common form of *E. rhusiopathiae* infection in humans is erysipeloid, and this is characterized by cutaneous manifestations but

some succumb to generalized and systemic forms of the disease [4]. *E. rhusiopathiae* infection is considered an occupational hazard [5]. The populations at risk include people handling infected animal tissues who are often exposed due to their occupation and include veterinarians, butchers, abattoir workers and cooks. Nearly, 31% of all erysipeloid cases have serious complications requiring surgical debridement, reconstruction surgery, or amputation [6-8]. The complications may present in the form of abscesses, septic arthritis, osteomyelitis, and necrotizing fasciitis which if not treated can lead to even more debilitating conditions such as septicemia, endocarditis or even death [9]. *E. rhusiopathiae* serotype 1a and 2 have been associated with clinical disease in immunocompromised people. Underlying conditions such as alcohol and drug dependence, poor hygiene and chronic liver disease lead to immunosuppression which facilitates erysipeloid to progress into systemic infection [3]. There is lack of information about the status of *E. rhusiopathiae* infection in vulnerable populations in Uganda.

In 2012/13, during participatory appraisals conducted by the International Livestock Research Institute's (ILRI) Safe Food, Fair Food project with pig farmers in Masaka, Mukono and Kamuli districts, pig keepers in four villages in Kamuli district described skin lesions characteristic of diamond skin disease in their pigs [10]. In light of this, our preliminary screening study in Namwendwa, Kitayunjwa and Bugulumbya sub counties, Kamuli district, where these four villages are located, found a sero-prevalence of *E. rhusiopathiae* infection in live pigs of 67%, and the bacteria isolated in 45% of fresh pork samples sold in pork butchereries and from slaughter abattoirs [11]. Since *E. rhusiopathiae* is a zoonotic bacterium, this high prevalence in the pig population and the pork therefore implied that butchers, pork handlers, pig owners and other individuals frequently handling raw pork are at risk for developing clinical disease caused by infections with *E. rhusiopathiae*.

This study therefore sought to determine the prevalence and factors associated with *E. rhusiopathiae* infection among raw pork handlers in Namwendwa, Kitayunjwa and Bugulumbya sub counties, Kamuli district, Eastern Uganda.

## Materials And Methods

### Study area and population

The study was carried out in three sub counties of Kamuli district, including Namwendwa, Kitayunjwa and Bugulumbya, the sub counties where *E. rhusiopathiae* infections were found to be present at 67% and 40% prevalence in pigs and pork, respectively. At the time of the study, the area had three slaughter abattoirs, one in each of the sub counties. Kamuli district forms part of the Busoga sub region of Eastern Uganda, a multi-ethnic and multi-cultural region. It has an estimated population of 972,638 humans and 55,998 pigs [12]. In 2012, the district was selected by a research-for-development program to improve the performance of smallholder pig value chains because pigs are considered one of the livelihood activities that contribute to poverty alleviation [13].

### Study design

We conducted a cross-sectional community-based study from December 2015 to February 2016 in Kamuli district, Eastern Uganda. Thirty-eight (38) abattoir workers and fifty-nine (59) butchers from Namwendwa, Kitayunjwa and Bugulumbya sub counties were enrolled. Convenient sampling was employed to select 205 cooks from the three sub counties who participated in the study. The average number of customers who buy raw pork at the butchereries was obtained from the butchers and was used to compute the sampling interval. Three cooks were sampled from each butchery as they came to buy raw pork and take it home for cooking. Twenty-eight cooks were sampled from abattoirs in each of the three districts.

### Sample size

The sample size for the prevalence was calculated using Kish Leslie formula (1965) aiming for power of 80% and precision of 95%. There was 5% sample size adjustment for the cluster design. This gave a sample size of 385. For the associated risk factors, the sample size was calculated from a text book of designing clinical research by Steven Cummings (2013) for two proportions which yielded a sample size of 269 participants. However, due to voluntary participation, we managed to sample 302 raw pork handlers.

### Questionnaire data collection

A semi-structured questionnaire for the risk factors associated with *E. rhusiopathiae* infection was designed based on literature obtained from Google Scholar, PubMed, text books (Including; Wood 1981, Reboli and Farrar 1989. Key words used included *Erysipelothrix rhusiopathiae*, abattoir workers, diagnosis, treatment, meat handlers, Africa, Asia, Europe and America.

The questionnaire was written in English and translated before pretesting into Lusoga, the local language spoken in the area. The questionnaires were administered to the participants by trained research assistants who were fluent in Lusoga. We cross checked all questionnaires to ensure that no data were missing (demographic information, socio-economic factors and individual predisposition to identify factors associated with infection). Data from all butchers and abattoir workers were collected at their places of work and data from cooks were collected at the butcheries and abattoirs where they bought the raw pork. Other customers told the research team to follow them home for safety and privacy issues.

### Ethical clearance including informed consent

Permission to conduct this study was sought from the Clinical Epidemiology Unit at Makerere University, the Uganda National Council for Science and Technology and the Kamuli District Commissioner. Ethical approval was obtained from the School of Medicine, Research and Ethics Committee (SOMREC-013-2016) and the International Livestock Research Institute, Institutional Research Ethics committee (ILRI-IREC2014-07). The participants were informed about the study and their written consent was sought prior to commencement of the study. Feedback from the study was given to the respondents regarding their status about the infection. Participants who were found infected were referred to the area nurse who provided treatment that was paid for by ILRI. Additionally, butchers and abattoir workers were provided with personal protective equipment that included soap, hand washing facilities, gumboots, gloves and disinfectant.

### Collection of blood samples from study participants

All butchers and abattoir workers were sampled by the researcher as they worked. However, cooks (house wives and pork joint owners) who fulfilled the selection criteria were sampled consecutively as they came to buy pork from the butcheries and the abattoirs. Other cooks were sampled at their homes. Individual blood samples were taken aseptically from the cephalic vein into 5 ml EDTA-containing vacutainer tubes after tying a tourniquet on the upper arm and swabbing the area with 70% alcohol. The collected blood was kept on ice in a cool box and immediately transported to Kamuli District Regional Referral Hospital where it was kept in a deep freezer at -20°C before being transported to the microbiology laboratory, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, where it was kept frozen before analysis.

### Bacteriological culture, identification and confirmation

Bacteriological culture was performed according to standard procedures (Reboli and Farrar, 1989). Each EDTA blood sample was separately streaked on sterile trypticase soy agar (Laboratorios Conda S.A., Madrid, Spain) [14]. and modified blood agar containing 0.4% sodium azide (Laboratorios Conda S.A., Madrid, Spain) [15] and incubated for 24 - 48 hours at 37°C. The plates were examined after incubation for suspect *E. rhusiopathiae* colonies which were pinpoint (approximately 0.1 mm), convex, circular, translucent, smooth mildly-haemolytic colonies [16]. The colonies with morphological characteristics of *E. rhusiopathiae* were sub cultured on *Erysipelothrix*-selective media as described by Wang *et al.* (2010) [5] and subsequently incubated for 24 hours at 37°C. Bacterial colonies that grew on the selective media were identified by their morphology: rod shaped, small (0.3 - 0.6µm), circular, and transparent, with a smooth glisten-

ing surface and edge, and positive by Gram staining and were biochemically tested. *E. rhusiopathiae* isolates are facultative anaerobes, catalase negative, oxidase negative, aesculin negative, gelatin negative and generate hydrogen sulphide [17].

*E. rhusiopathiae* infections resemble streptococcal infections. *Streptococcus pyogenes* (Group A Streptococcus) isolated in the laboratory were stained with Gram and biochemical tests were performed on the same isolates. This was done to differentiate streptococcal infections from *E. rhusiopathiae* infections. Isolated streptococci appeared as cocci in clusters with short chains whereas *E. rhusiopathiae* isolates were slender and rod shaped. Further, streptococci isolates were catalase negative and beta hemolytic.

**Statistical analysis**

Data were double-entered into Epidata, (<http://www.epidata.dk/credit.htm>) and validated. The data were then transferred to STATA version 12.0 (StataCorp LLC) for analysis to determine the overall prevalence and factors associated with *E. rhusiopathiae* infection among raw pork handlers. Continuous variables were summarized using median and interquartile range. Categorical variables were summarized using frequencies, proportions, and percentages. Bivariate logistic regression analysis was performed and all variables with a P-value of ≤ 0.2 were subjected to multivariate analysis. Interaction between the variables was assessed using the maximum log-likelihood ratio (Chunk test). Confounding was assessed using a difference of ≥ 10% between the crude and adjusted odds ratio for the variables.

**Results**

The median age of the study participants was 39 years with a range of 18 - 47 years. Most participants were from Namwendwa sub-county 155/302, (51%) and those of Anglican religion dominated 157/302, 52%. Most of the participants were married 219/302, (73%) and had completed elementary education 158/302, (52%). Cooks dominated the study population, 205/302, (68%) (Table 1 and Figure 1).

Variable	Frequency ( N=302)	Percent
<b>Sub county</b>		
Namwendwa	154	51
Kitayunjwa	99	32.8
Bugulumbya	49	16.2
<b>Sex</b>		
Males	155	51.3
Females	147	48.7
<b>Religion</b>		
Catholic	113	37.4
Anglican	157	52
Others*	32	10.6
<b>Education level</b>		
Never	78	25.8
Primary	158	52.3
Secondary	54	17.9
Tertiary	12	4
<b>Raw pork handler</b>		
Butcher	59	19.5
Abattoir worker	38	12.6
Cooks	205	67.9
<b>Marital status</b>		
Single	47	15.6
Married	219	72.5
Divorced	22	7.3
Widowed	14	4.6

**Table 1:** Socio-demographic characteristics of the 302 study participants in Kamuli District Eastern Uganda, 2016.

Most participants reported less than 10 years of exposure to raw pork, 261/302 (86%) with a weekly frequency of exposure, 113/302, (37%). Roughly half of the participants reported not engaging in other pig related activities like pig keeping or trade but solely slaughtering and meat processing (158/302; 52%). (Table 2). The overall prevalence of *E. rhusiopathiae* infection was 9.9%, (95% CI:7.4 - 13) after adjusting for clusters. The prevalence was highest among participants from Kitayunjwa sub-county 10/99, (11%), followed by Namwendwa, 15/154, (10%) and Bugulumbya, 4/49, (8.1%). Males were more affected by *E. rhusiopathiae* infection, 21/155, (14%) compared to females, 9/147, (6.1%). Participants who were single were most affected, 5/47, (11%), followed by the “married” 21/219, (9.6%), divorced, 2/22, (9.1%) and widowed, 2/14, (14%) (Table 3).

Variable	Frequency (N=302)	Percent
Alcohol consumption	165	54.6
Yes		
No	137	45.4
<b>Participant’s duration on exposure to raw pork</b>		
Below 10 years	261	86.4
Above 10 years	41	13.6
<b>Engagement in other pig related activities</b>		
Yes	144	47.7
No	158	52.3
<b>Frequency of handling raw pork</b>		
Daily	99	32.8
Weekly	113	37.4
Others**	90	29.8

**Table 2:** Individual Characteristics of Raw Pork Handlers in Kamuli District Eastern Uganda, 2016.

\*\* Monthly and Yearly Handling of Raw Pork.

Variable	Number (n=302)	ER infection positive	Prevalence (%)	95% CI
<b>Overall Prevalence</b>	302	30	9.9	7.4-13
<b>Sub county</b>				
Namwendwa	154	15	9.7	5.02-14.46
Kitayunjwa	99	11	11.1	4.86-17.36
Bugulumbya	49	4	8.1	0.39-15.94
<b>Sex</b>				
Males	155	21	13.5	8.12-18.98
Females	147	9	6.1	2.21-10.03
<b>Religion</b>				
Catholic	113	13	11.5	3.02-17.44
Anglican	157	14	8.9	2.28-13.40
Others*	32	3	9.4	0.93-19.68
<b>Education level</b>				
Never	78	10	12.8	3.81-20.32
Primary	158	18	11.4	2.54-16.38
Secondary	54	2	3.7	2.59-8.81
Tertiary	12	0	0	
<b>Type of raw pork handler</b>				
Butcher	59	9	15.3	5.90-24.54
Abattoir worker	38	14	36.8	21.1-52.44
Cooks	205	7	3.4	0.90-5.92
<b>Marital status</b>				
Single	47	5	10.6	1.69-19.58
Married	219	21	9.6	5.66-21.44
Divorced	22	2	9.1	3.25-21.44
Widowed	14	2	14.3	4.81-33.38

**Table 3:** Prevalence of *Erysipelothrix Rhusiopathiae* Infection Among Raw Pork Handlers Within the Socio Demographic Characteristics in Kamuli District Eastern Uganda, 2016.

\*Other Religions Studied Include Born-Again Christians, Pentecostals and Muslims.

**Bivariate analysis of factors associated with *E. rhusiopathiae* infection among raw pork handlers**

Working in the abattoir was associated with *E. rhusiopathiae* infection (OR = 17, 95% CI: 6.1 - 45). Similarly, working at a butchery was associated with *E. rhusiopathiae* infection, (OR = 5.1, CI: 1.8 - 14). Males were more likely to be infected than females (OR = 2.4, 95% CI: 1.0 - 5.4) (Table 4).

Variable	OR	95% CI	Pvalue
<b>Type of raw pork handler</b>			
Cooks	1		
Butcher	8.37	1.79-39.10	0.007
Abattoir worker	26.13	5.29-129.10	<0.0001
<b>Alcohol</b>			
No	1		
Yes	4.02	1.07-15.03	0.038
<b>Confounder</b>			
<b>Sex</b>			
<b>Females</b>	1		
Males	3.85	0.91-16.23	0.067

**Table 4:** Results of multivariate analysis for *E.rhusiopathiae* infection among raw pork handlers in Kamuli district Eastern Uganda, 2016.

**Multivariate analysis for the factors associated with *E. rhusiopathiae* among raw pork handlers**

Type of raw pork handler was associated with *E. rhusiopathiae* infection. Abattoir workers were 26 times more likely to develop the infection compared to cooks (OR = 26, 95% CI: 5.3 - 129). The butchers were 8.4 times more likely to develop the infection compared to the cooks, (OR = 8.4, 95% CI: 1.8 - 39). Participants who consumed locally brewed alcohol were four times more likely to develop *E. rhusiopathiae* infection compared to those who reported no alcohol consumption (4.0, 95% CI: 1.1 - 15). Sex of the participants was retained in the model because it was confounding the association between type of raw pork handler and *E. rhusiopathiae* infection.

**Discussion**

The overall prevalence of *E. rhusiopathiae* infection among raw pork handlers in Kamuli district was 9.9%. In Uganda and the East African region, this is the first paper to report infection with *E. rhusiopathiae* among raw pork handlers. Therefore, there were no reference studies from this region to compare our results with and most of the papers referred to are from Europe.

Our findings have affirmed our suspicion that *E. rhusiopathiae* infection is present in the human population in Kamuli district, Uganda [11]. This indicates the need to employ a One Health approach to contain the situation, given the devastating consequences associated with the infection in humans. Human sampling for the current findings was done at butcheries where raw pork that tested positive at a prevalence of 45% was obtained. The present findings show a lower prevalence (9.9%) among occupationally exposed individuals compared to those of Brhel and Bartnicka (2003) studying occupational infectious diseases in Czech Republic who reported a prevalence of 29% of *E. rhusiopathiae* infection (erysipeloid) among agriculturalists, forestry workers and game park managers [18]. This could be explained by our study population which enrolled three kinds of raw pork handlers with cooks dominating which resulted in an underestimation of the overall prevalence. Subgroup analysis found that the prevalence of *E. rhusiopathiae* infection amongst abattoir workers

was 36.8%. This was more than two times higher than that reported in abattoir workers in Sweden [19]. This is possibly because this the most at risk occupational group due to the frequency of handling raw pork.

Our study was done in a setting with a vulnerable population with high level of poverty in the household. Pig farming and trading are major businesses the population engages in as incoming generating activities. Men in the district operate butcheries and work in the abattoir but also eat pork at the butcheries whereas women buy raw pork from the butcheries and prepare the meat at home. *E. rhusiopathiae* infection is likely under reported and underdiagnosed in the region because of lack of diagnostic capacity (field- and laboratory-based) and limited awareness among health practitioners and people working in the pig industry.

It has been reported that 89% of *E. rhusiopathiae* infection in humans is present in people who handle animals and raw meat regularly; at work, highest at risk are veterinarians, housewives, butchers, abattoir workers and animal farm workers [20]. Therefore, as a means of improving occupational health, it is necessary to increase awareness of the disease/infection route to veterinarians and all health practitioners on diagnosis and treatment. The government extension services should formulate guidelines that should be followed by the abattoir workers like wearing personal protective equipment (gloves, gumboots) and hand washing after handling raw pork.

According to Romney, *et al* (2001), alcohol consumption leads to immunosuppression hence the body can easily be attacked by the bacteria [22]. A study conducted by Kichloo reported that alcohol dependency is an important risk factor for acquiring *E. rhusiopathiae* infection [3]. In our study, too, the type of raw pork handler and consumption of alcohol increased the likelihood of being infected with *E. rhusiopathiae*. Compared to the cooks, abattoir workers were 26 times and butchers eight times more likely to be infected. All respondents who reported consuming alcohol weekly were four times more likely to develop the infection compared to non-consumers of alcohol. Infection in people is occupationally related, occurring principally from contact with infected animals and their by-products. Frequent exposure to infected animals and their by-products increases the risk of *E. rhusiopathiae* infections in humans [16]. Alcohol consumption was reported to be at 55% in Kamuli District among the respondents; 67.7% of men drink alcohol compared to 40.8% of women.

Sex was reported as a confounder because men usually work in the butchers and abattoirs. However, in this study, four females worked in the butcheries (two of whom were found infected with *E. rhusiopathiae*) and two in the abattoir while the rest were cooks. Women are responsible for cutting meat into small pieces at the butcheries prior to cooking for customers. Nine of the 30 participants were infected with *E. rhusiopathiae*. These findings are in line with those of Brooke and Riley (1999) who reported that males were twice as likely to develop *E. rhusiopathiae* infection as compared to females, due to their frequent occupational exposure [1].

The current study found that Catholics and Anglicans were more affected which could be attributed to the fact that people who belong to the above groups are known to freely participate in pig keeping activities. In addition, they believe that keeping pigs generates a lot of income. In addition, there were three Muslims among the study participants indicating the diversity of the occupation and the role of pigs towards improving livelihoods of people in rural parts of the county.

Participants who had never gone to school were most affected. Majority of the people in rural Uganda have limited access to schools thus many engage in income-generating activities like pig keeping, slaughter and butchering to earn a living. Unmarried participants were most affected. Previous studies have not found any association between marital status and developing *E. rhusiopathiae* infection. However, in our study this result can be explained by the number of participants who were mostly youths and the roles they play in the pig industry.

### Limitations

The estimated sample size was not the sample size that was analyzed. We estimated a population size of 385, but obtained 302 since the remaining number of participants withdrew at the time of drawing blood from them. The consecutive sampling approach for cooks could have introduced some bias but this was collected by the census conducted for the butchers and abattoir workers. Since interviews for the abattoir workers and butchers were conducted in open space this would have resulted in reporting of false formation about their practices. Males were studied more than the females which could have presented reporting bias because the males are known to be occupationally associated with the infection.

### Conclusions and Recommendations

The current study shows that *E. rhusiopathiae* infections exist in humans in Uganda. This poses a high risk characterized by economic losses and highlights the need for urgent attention since the infection is a public health threat in developing countries.

*E. rhusiopathiae* infection had never been reported in humans in Uganda previously. The disease is preventable and relatively treatable when diagnosed in time. Further research should include serotyping of isolates from animals and humans and identification of control measures, especially vaccination, that could be implemented in Kamuli District.

### Conflict of Interest

Authors declare no conflict of interest.

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