

THE MAGNITUDE AND RISK FACTORS OF BRUCELLOSIS IN URBAN AND PERI-URBAN CATTLE IN NORTHERN AND EASTERN UGANDA

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BACKGROUND

- Agriculture = backbone of developing economies
- Livestock supports 70 % of the world's poor
- Livestock contribute 5.2% of Uganda's GDP
- Most of the livestock products in Uganda are produced by small holder farmers.

Problem statement

- Civil strife in Northern and Eastern Uganda concentrated the small holder farmers in and around towns in these areas
- Genesis of urban and peri-urban farming in these areas
- Need for food was further enhanced by the ensuing rural-urban migration

Problem statement Ctd...

- Urban and peri-urban livestock farming is the major source of unprocessed foodstuffs in these towns
- High disease burden including zoonotic ones
- Brucellosis



Bovine brucellosis

- Caused by biovars of *Brucella abortus*, *B. melitensis* and *B. suis*
- Causes abortion, mastitis, placentitis, orchitis and hygromas in cattle
- Causes relapsing fever and abortion in humans
- Its zoonotic nature and resulting infertility in cattle impact on people's livelihoods and health.

Objectives

- Determine the sero-prevalence of brucellosis in indigenous and imported cattle in urban and peri-urban areas of Gulu and Soroti towns in Uganda.
- Identify the associated risk factors for transmission of brucellosis in cattle in the study areas.

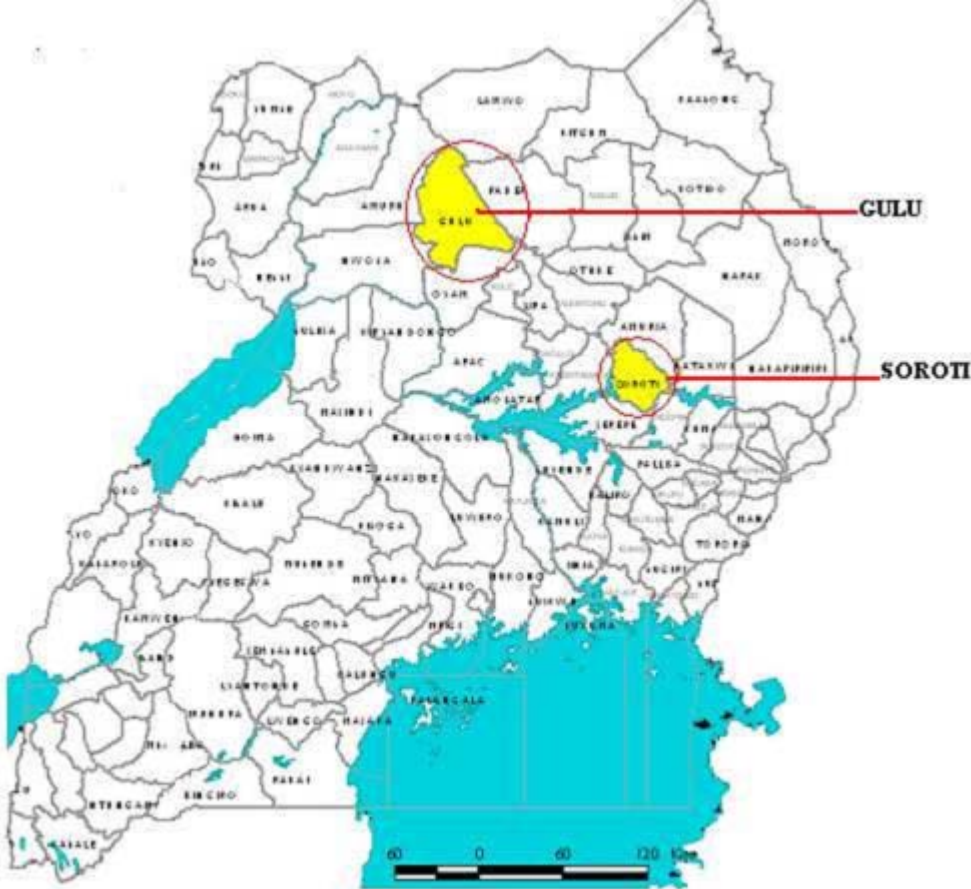
Objectives ctd ...

- Isolation, biotyping and molecular characterization of the *Brucella* infecting cattle in the study areas.
- Determine the susceptibility of *Brucella* isolates to commonly used anti-microbials in Uganda.
- Disseminate research information to the various stakeholders so as to initialize mitigation strategies against brucellosis in cattle.

Significance of the study

- Findings will be a starting point towards improvement of people's livelihoods and public health

Study area



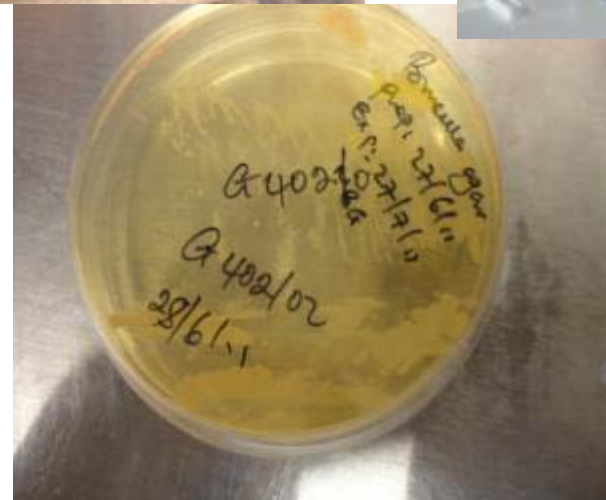
Production system & Climate

- Dry climatic (21.5-34°C)
- Bimodal annual, rainfall of 700-800mm



Study design

- Collected samples
- Lab. analyses
 - RB plate test
 - i-ELISA
 - c-ELISA
 - Culture and isolation
 - Drug susceptibility
 - Molecular typing



Drug susceptibility testing

- Was carried out using the MIC broth micro-dilution panel method.
- The panel consisted of commonly used antimicrobials in Uganda e.g. Gentamycin, Streptomycin, Tetracycline, Rifampicin, Penicillin, Ampicillin, Trimethoprim and Ciprofloxacin.

DNA extraction and molecular typing

- Genomic DNA was extracted from colonies using the Norgen DNA extraction kit
- Isolates confirmed as *Brucella* using real time PCR (Probert *et al.*, 2004).
- A Multiplex PCR (Bruce-ladder) protocol was used (Lopez Goni *et al.*, 2008) for speciation
- Biovar and genotype identification was done using the MLVA-16 assay (Le Fleche *et al.*, 2006).

DATA ANALYSIS

- The data collected was captured in Excel & transferred into SPSS version 17 for windows
- Univariable and multivariable analyses (Logistic regression) were used to establish relationship between disease outcome and risk factors

RESULTS

- Sero-positivity was significantly ($p < 0.001$) higher in Soroti than Gulu.

Number of included herds and animals and *Brucella* sero-prevalence

District	Gulu	Soroti	Overall
Herds sampled	116	50	166
Serum samples	500	507	1007
Herd sero-prevalence	19%	46%	27%
Animal sero-prevalence	6%	9%	7.5%

Multivariable analyses of herd and individual animal risk factors for *Brucella* seropositivity in cattle in Gulu

Variable	Category	p-value	OR	95% C.I for OR	
				Lower	Upper
Herd level					
Herd size		0.03			
	1 - 5 cattle (ref)	-	1.0	-	-
	6 - 10 cattle	0.998	< 0.0001	< 0.0001	< 0.0001
	11 - 20 cattle	0.22	2.6	0.6	12
	> 20 cattle	0.002	7.8	2	29
Individual animal level					
Age		0.002			
	1 - 2 years (ref)	-	1.0	-	-
	>2 - 5 years	0.12	0.4	0.1	1.3
	>5 - 7 years	0.28	1.9	0.6	6.6
	> 7 years	0.09	3.0	0.9	11
Region of origin of the cow		< 0.0001			
	Western Uganda (ref)	-	1.0	-	-
	Central Uganda	0.3	0.5	0.1	1.8
	Northern Uganda	< 0.0001	0.1	0.05	0.4

Multivariable analyses of herd and individual animal risk factors for *Brucella* seropositivity in cattle in Soroti

Variable	Category	p-value	OR	95% C.I for OR	
				Lower	Upper
Herd level					
Introduction of new cattle in last 2 years	No (ref)	-	1.0	-	-
	Yes	0.01	6.8	1.6	29.5
Keeping pigs	Yes (ref)	-	1.0	-	-
	No	0.02	4.9	1.2	19

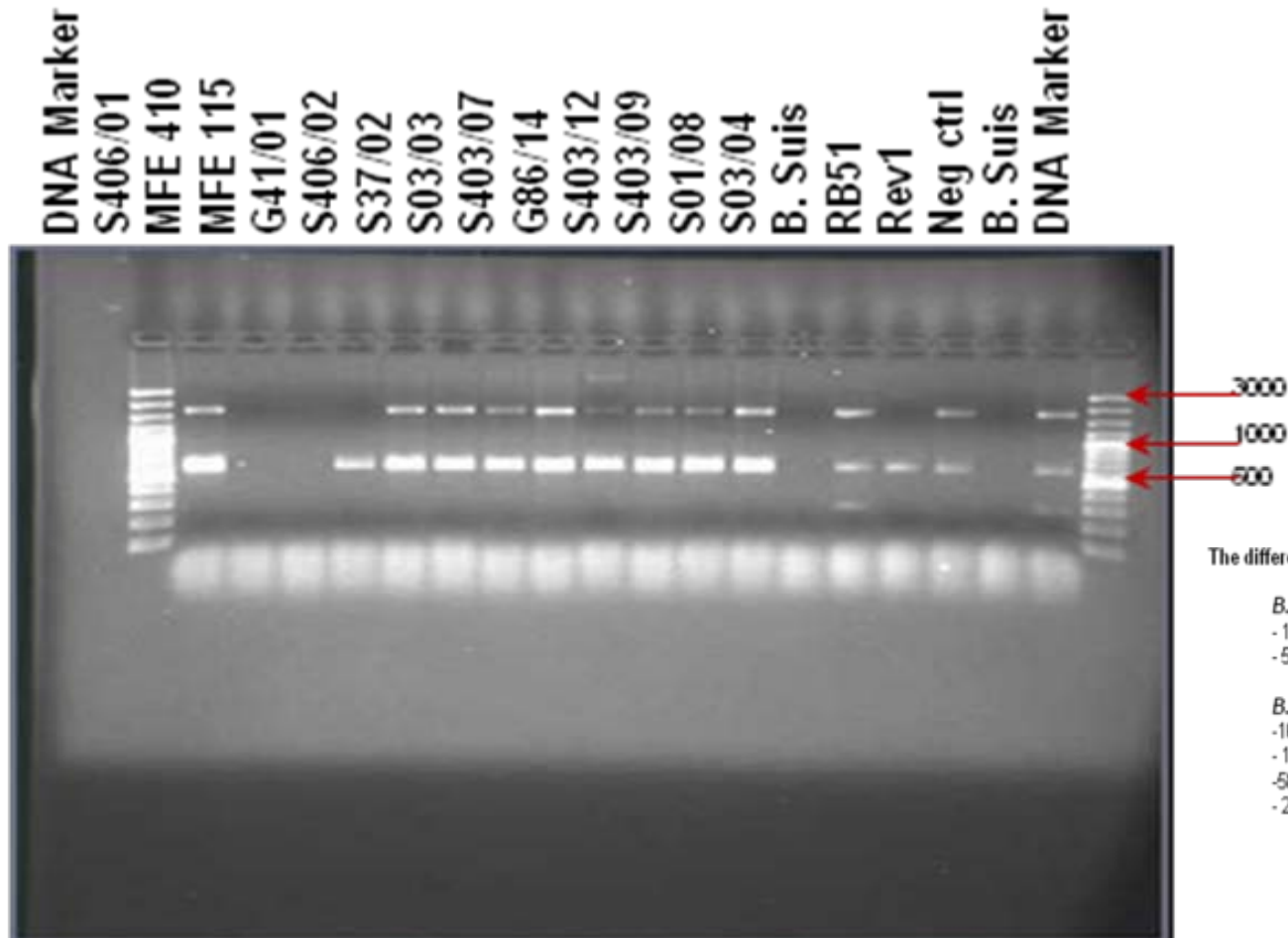
Isolates

- 11 isolates obtained from 207 milk samples (5.3%)
- All the isolates were from sero-positive cattle (11/17)
- All isolates were bio-typed and confirmed with monospecific sera as *B. abortus*

Drug susceptibility of isolates

MIC (µg/ml) for <i>B. abortus</i> isolates			
Antimicrobial	Isolate range (µg/ml)	CLSI recommended susceptibility break point	No. of isolates with MIC above the CLSI values
Ampicillin	64-128	0.25-8	11
Ciprofloxacin	0.5-1	0.12-1	0
Gentamicin	>16	≤4	11
Streptomycin	>256	≤8	11
Tetracycline	2-4	≤1	11
Florfenicol	16-32	-----	-----
Sulfamethoxazole	>1024	≤0.056-0.1	11
Trimethoprim	8-16	0.05	11
Chloramphenicol	>64	0.25-4	11
Cefotaxime	>2	0.5-2	11
Ceftazidime	>16	-----	-----
Penicillin	>16	0.25-8	11
Linezolid	>16	-----	-----
Rifampicin	2-4	0.06-4	0

Bruce-ladder agarose gel picture



The different species should give following pattern of bands:

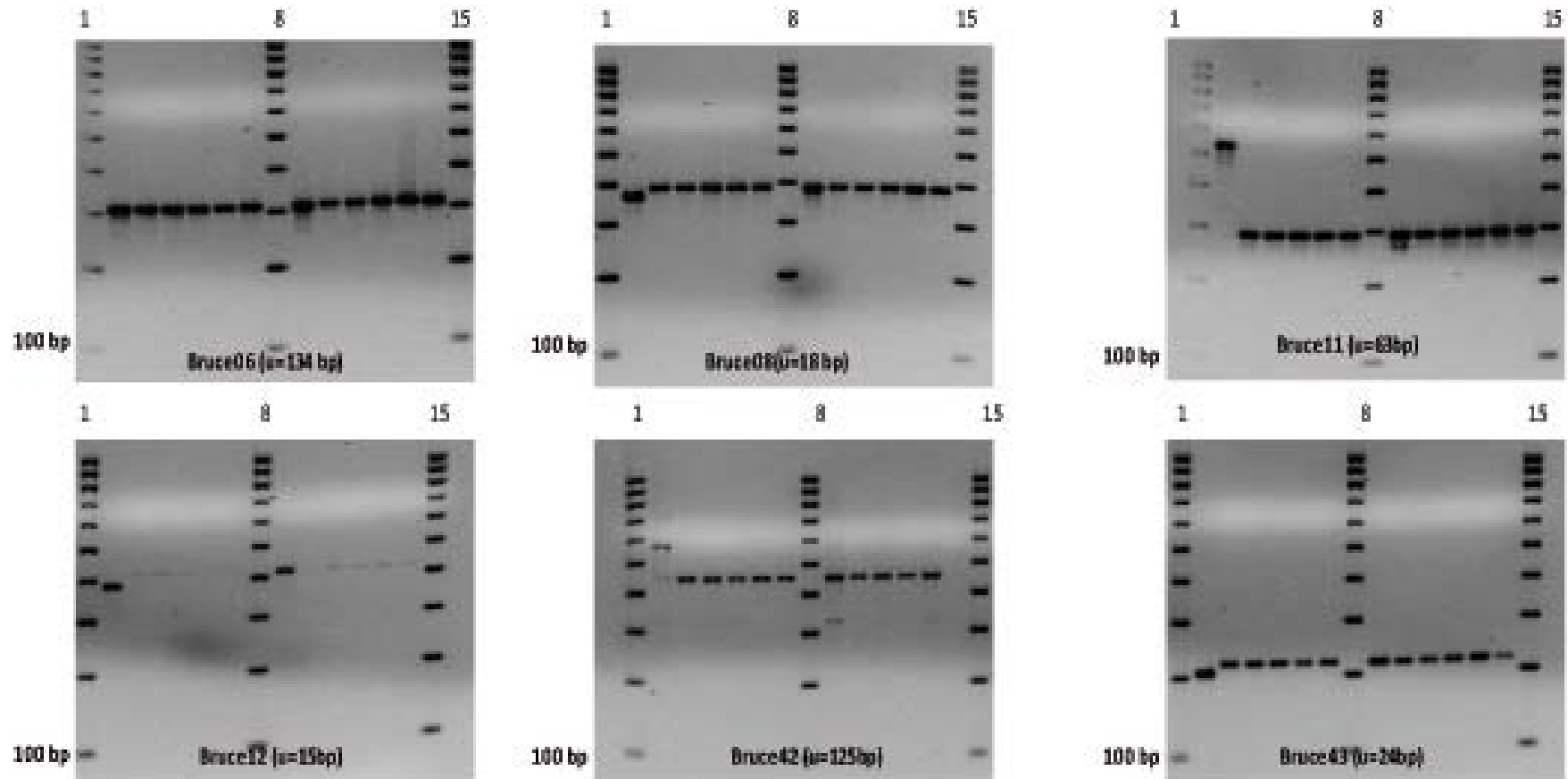
B. Abortus
- 1682 bp
- 587 bp

B. Ovis
- 1071 bp
- 587 bp

B. suis
- 1682 bp
- 1071 bp
- 587 bp
- 272 bp

B. melitensis
- 1682 bp
- 1071 bp
- 587 bp

**Gel picture showing MLVA-16 assay; locus 1-6; extreme left,
right and intermediate lanes = ladder, lane 2 (left) = B. Suis
and 11 samples**



Biovar isolated

- Conclusion awaits sequence alignment
- But a quick blast search revealed that all the 11 isolates bare a close relationship to *Brucella melitensis biovar abortus 2308*. This is a wild strain of the rough vaccine strain RB51.

Policy Guidance

- Urban and peri-urban farmers are advised to keep smaller manageable herds of high producing cattle
- Cattle should be bought from brucella free herds, or screened before introduction
- Rifampicin remains the drug of choice in treating brucellosis in humans

THANK YOU

