Supplementary Material

1 Supplementary Methods

1.1 Milk ring test (MRT)

Earlier studies have reported increased false positive reads for MRT following refrigeration already after 24 hours (1, 2), hence measures were taken to test milk samples collected the same day. Distributors in Kinna sold milk of cattle, goat and camel origin for consumers to purchase. The MRT is made and recommended for use on cattle milk only, but it has previously been shown that a modified milk ring test (mMRT) can be made to work for camel milk (3). Goat milk has reportedly been difficult to test by MRT and at least two studies testing the mMRT on goat milk have indicated limited functionality (4, 5).

Prior to testing, milk and MRT reagent were brought to RT. The MRT containing hematoxylin-stained *B. abortus* antigen (Animal Health and Veterinary Laboratories Agency, New Ham, Addlestone, Surrey, KT15 3N3, United Kingdom) was manually shaken before 30 µl was added to the milk followed by 10 gentle inversions for homogenous distribution. For cattle, one ml of milk was added to a narrow two ml microtube. For mMRT, goat and camel samples were added together with MRT-negative cow milk in a 3:1 ratio (camel: cattle) for a total volume of one ml. The microtubes were placed in a 37°C water bath for one hour for cattle according to OIE protocols (World Health Organization for Animal Health) and 1.5 hours for camel and goat. Results were interpreted accordingly. If positive, IgA, IgM and IgG antibodies are expected to locate in the upper cream section of the milk, binding the antigen to visualize a blue ring (Supplementary Figure 1A) (6, 7). Negative samples would retain a homogenous blue color due to unbound antigen. Results were graded according to the standard MRT system: - (blue milk, white cream), 1+ (same blue for milk and cream), 2+ (cream of darker blue than milk), 3+ (cream of strong dark blue, milk of blue hue) and 4+ (cream of strong dark blue, white milk) (2).

1.2 Rose Bengal test (RBT)

A total of 25 µl animal serum was added onto a clean white ceramic tile together with an equal proportion of RBT reagent (ID-Vet, Rapid Slide Agglutination Test Rose Bengal, RSA-RB 023). The reagent consisted of a heat- and phenol-inactivated suspension of *B. abortus* biovar 1 Weybridge strain No99 with Rose Bengal staining and was expected to detect IgG and IgM antibodies. Serum and reagent were mixed on the tile using a sterile toothpick, and the tile was rocked for four consecutive minutes. Obvious agglutination was denoted as a positive result (Supplementary Figure 1B) (8, 9).

1.3 Indirect enzyme-linked immunosorbent assay (iELISA)

IgG iELISA (ID-Vet, ID Screen® Brucellosis Serum Indirect Multi-species, BRUS-MS-10P) against *Brucella abortus*, *melitensis* and *suis* was performed. According to manufacturer's instructions, an experiment was considered valid if the mean value of two positive controls had an OD greater than 0.350 and if the ratio of the mean values between positive and negative controls was greater than 3. A result was considered positive if the ratio between sample and positive control was equal to or greater than 120%, and doubtful if between 110-120%. Plates were read at OD 450 nm in a microplate reader

(BioTek®, The Synergy™ HT Multi-detection microplate reader). Tests were run in technical duplicates.

1.4 Extraction of DNA from FTA filter papers

Multiple extraction protocols were tested to assess the best extraction efficiency for our samples. Four methods were used: (1) the "punch-in" method with FTA classic washing, (2) the punch-in method followed by classic washing and boiling, (3) the punch-in method with only boiling and (4) punch-in with a commercial extraction kit. Apart from the different methods, alternating numbers of FTA punches per reaction were assessed with 3, 5 and 50-60 punches. The 3-5 punches were created using a micro-puncher (Whatman™ WB100007 2 mm Harris Micro-Punch) while 50-60 punches were created using a scissor. All testing setups were carried out on blood, serum and milk samples separately.

The punch-in method with classic washing was based on the FTA classic cards standard protocol (28-9843-54 AA) for conventional PCR. FTA cards were punched-in directly to the PCR tube followed by three washes with purification agent (QIAGEN® GBS WB120204 FTA™ Purification Reagent) and three washes with pH8 TE buffer (Ambion®). The reaction mastermix was added on top of washed, dried filter punches.

The punch-in method with washing and boiling was carried out as follows; washing was performed exactly as explained in the previous step, followed by the addition of 50 µl 0.1% diethylpyrocarbonate-treated water (ThermoFisher, Invitrogen™ UltraPure™ DEPC-Treated Water). The tubes were placed in a heatblock (Techne, Dri-Block® DB-3) at 96°C for 10 min. The water containing released DNA was transferred to fresh tubes. The method using only boiling was equally carried out, excluding the primary washing steps.

The method using an extraction kit was based on a blood and tissue protocol (Qiagen®, DNeasy® Blood and Tissue Kit) with a few modifications. FTA paper was treated as tissue, and for 50-60 punches, twice the volume of ATL had to be used in the primary step to cover the material. Heating was performed at 52°C for 1.5 hours with intermediate vortexing every 30 min, papers remain intact during this procedure. Before adding ethanol, pieces of FTA-paper were manually removed to prevent obstruction of the spin column. During the second wash with AW2, the samples were spun at 17,000g which was the max capacity of the micro-centrifuge (ThermoFisher, Fisherbrand™ accuSpin™ Micro 17).

After assessing extraction protocols, blood and serum were extracted by method 3 using 5-6 punches per 10 µl of DEPC water. Milk was extracted using method 4. All samples with extracted DNA were stored in -20°C for up to one week prior to analysis.

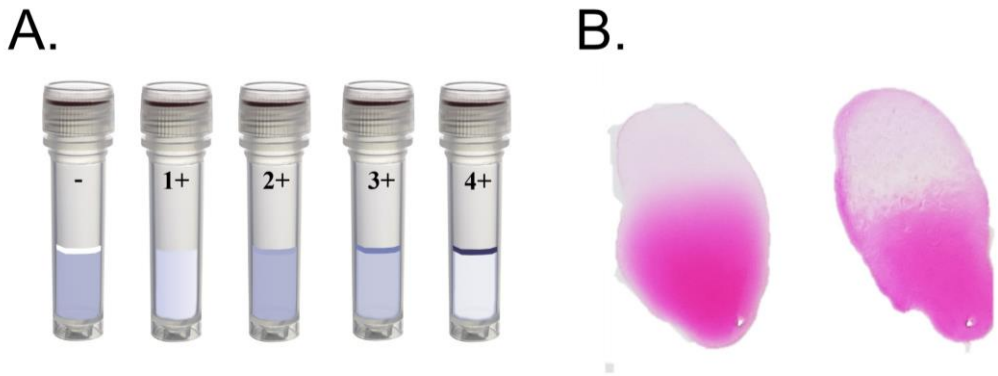
1.5 Quantitative polymerase chain reactions (qPCR)

Real-time qPCR was done to identify *Brucella* nucleic acid, using the genus-specific insertion element IS*711*, shared by all *Brucella* species (10), using a protocol previously described (11). The reaction was carried out with a Rotor-Gene 6000 qPCR machine (Corbett Research), the Rotor-Gene software 2.1.0.9 and a manual cycle threshold (CT) set to 0.055 (11). A mastermix was prepared with 2.5 U of DNA polymerase (Applied Biosystems, AmpliTaq Gold™**),** 1X of gene amplification buffer (AB, 10X

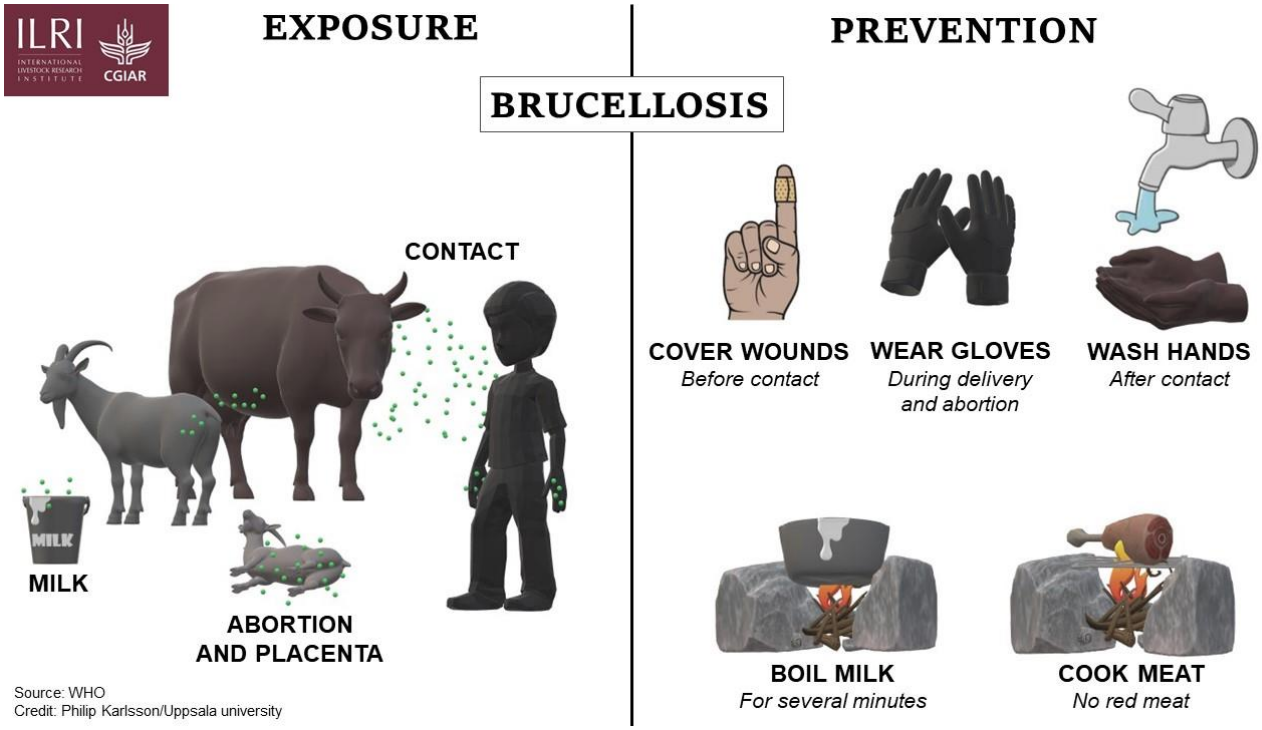
GeneAmp buffer II), 6 mM of $MgCl_2$ (AB), 800 µM of dNTP (AB, Warrington, UK, dNTP Mix), 300 nM of forward primer (5'-GGC CTA CCG CTG CGA AT-3') (Invitrogen), 300 nM of reverse primer (5'-TTG CGG ACA GTC ACC ATA ATG-3') (Invitrogen), 250 nM of probe (5'-AAG CCA ACA CCC GC-3') (AB, 6-FAM MGBNFQ HPLC), nuclease free water (Ambion®) and 2 µl of template. The final volume was 50 µl, and the qPCR protocol included a hot-start (95˚C, 10 min) followed by 50 cycles of denaturation (95˚C, 15 sec) and annealing (60˚C, 60 sec). The qPCR results were categorized based on CT-values, with **30≥CT<35** being positive, **35≥CT<40** being weak positive and **40≥CT≤50** being doubtful. The categorical grading was settled with values from the previously reported study in mind, and the results of our positive control (11). Samples with no detectable CT, or samples without a clear sigmoidal shape, were classified as negative. Samples were run in triplicates, and if any of the three fulfilled the criteria for positive, the sample was registered as such. Every run was carried out with negative (water) and positive controls (*Brucella suis* Biotype 1NA, INGENAS).

2 Supplementary Figures and Tables

2.1 Supplementary Figures



Supplementary figure 1. Brucellosis infographic. Agglutination tests for antibodies against *Brucella* spp. **A.** Milk Ring test (MRT) for testing raw cattle milk. Figure showing the grading scale with illustration. **B.** Rose Bengal test (RBT) for testing cattle serum. Figure showing negative (left) and positive (right) results obtained from field footage.



Supplementary figure 2. Brucellosis infographic. Infographics provided to patients tested positive for brucellosis, farmers having MRT-positive milk and for participants of the community meeting. Illustrations of exposure through contact, abortions, placenta and milk, and prevention through covering of wounds, wearing gloves, washing hands, boiling milk and properly cooking meat.

2.2 Supplementary tables

Supplementary table 1. Kinna health centre reports

	January			February			March		
	Tested	Positive	Ratio	Tested	Positive	Ratio	Tested	Positive	Ratio
2018	13	2	0.15	23	2	0.09	11	2	0.18
2019	36	5	0.14	50	5	0.10	25	2	0.08
2020	62	15	0.24	39	10	0.26	8	3	0.38

Supplementary table 2. Patient data

Patient	Livestock	Unpasteurized milk	FBAT-positive	qPCR-positive
1	No	Yes	Yes	Yes
2	No	Yes	No	Yes
3	No	Yes	Yes	Yes
4	No	Yes	No	No
5	No	Yes	Yes	Yes
6	No	Yes	Yes	Yes
7	No	Yes	No	Yes
8	No	Yes	NA	No
9	Yes	Yes	Yes	No
10	No	Yes	Yes	No
11	Yes	Yes	Yes	No
12	No	Yes	No	No

Supplementary table 3. Milk vendor data

Hawker	Kinna location	Source	Farms pooled	Origin	Intervention	MRT
1	A_1	Cow	1	Duse	Boiling	-
2	A_2	Cow	1	NA	Boiling	-
3	A_3	Camel	3	NA	Boiling	-
4	A_4	Camel	1	Duse	No	-
5	$Walking_1$	Cow	2	Hidaya	No	++++
6	$Walking_2$	Cow	1	Machesa	No	+++
7	B_1	Camel	1	Duse	Boiling	-
8	B_2	Camel	1	Machesa	Boiling	-
9	B_3	Camel	1	Machesa	Boiling	-
10	B_4	Cow	1	Kukuu	Boiling	-
11	B_5	Cow	1	Duse	Boiling	-
12	$Motorbike_1$	Camel	1	Boji	No	-
13	$Motorbike_2$	Goat	1	Boji	No	-
14	$Motorbike_3$	Camel	1	Boji	No	+++
15	$Motorbike_4$	Camel	1	Boji	No	-
16	C_1	Camel	1	Duse	No	-
17	C_2	Goat	1	Duse	No	+
18	C_3	Cow	1	Rapsu	No	++++
19	C_4	Camel	1	Duse	No	-
20	C_5	Cow	1	Machesa	No	++++
21	C_6	Camel	1	Rakoila	No	-
22	C_7	Cow	1	Machesa	No	-

23	C_8	Cow	1	Machesa	No	-
24	C_9	Cow	1	Machesa	No	-
25	C_{10}	Cow	1	Machesa	No	-
26	C_{11}	Cow	1	Duse	No	-
27	C_{12}	Cow	1	Machesa	No	++++
28	C_{13}	Cow	NA	NA	NA	-
29	C_{14}	Camel	1	Machesa	No	-
30	C_{15}	Cow	NA	NA	NA	-
31	C_{16}	Camel	NA	NA	NA	-
32	$Household_1$	Cow	1	Moliti	No	-
33	$Motorbike_5$	Cow	1	Moliti	No	++++
34	D_1	Cow	1	Moliti	No	-
35	E_1	Cow	1	Bibi	No	-
36	$Household_2$	Camel	1	Moliti	No	-
37	F_1	Camel	1	Kukuu	Boiling	-
38	F_2	Camel	1	Moliti	No	-
39	F_3	Cow	1	Halibor	Boiling	-
40	F_4	Camel	1	Moliti	Boiling	-
41	$Walking_3$	Cow	1	Machesa	No	++
42	$Household_3$	Cow	1	Gubadhidha	No	-
43	G_2	Cow	1	Machesa	No	-
44	$Walking_4$	Cow	1	NA	NA	+
45	H_1	Cow	1	Machesa	Boiling	-
46	H_2	Cow	1	Machesa	No	++++
47	H_3	Cow	1	Machesa	No	+++
48	H_4	Cow	1	Machesa	No	+
49	H_5	Cow	1	Machesa	No	-
50	H_6	Cow	1	Machesa	No	-
51	H_7	Cow	1	Machesa	No	++++
52	H_8	Cow	1	Machesa	No	++++

Supplementary table 4. Farm animals data

Farmer	Herd	Location	MRT	RBT-positive	iELISA-positive	qPCR-positive
A	1_1	Machesa	-	No	No	Yes
A	1_2	Machesa	-	No	No	Yes
A	1_3	Machesa	-	No	No	Yes
A	1_4	Machesa	-	No	No	No
A	1_5	Machesa	-	No	No	Yes
A	2_1	Machesa	-	No	No	Yes
A	2_2	Machesa	-	No	No	Yes
A	2_3	Machesa	+++	NA	NA	No*
A	2_4	Machesa	-	No	No	Yes
A	2_5	Machesa	-	No	No	No
B	1_1	Machesa	-	No	No	Yes
B	1_2	Machesa	+	No	No	Yes
B	1_3	Machesa	-	No	No	No
B	1_4	Machesa	-	No	No	Yes
B	1_5	Machesa	-	No	No	Yes

B	2_1	Machesa	-	No	No	No
B	2_2	Machesa	++++	Yes	Yes	Yes
B	2_3	Machesa	-	No	No	No
B	2_4	Machesa	+	No	No	Yes
B	2_5	Machesa	-	No	No	No
B	3_1	Machesa	++++	Yes	Yes	No
B	3_2	Machesa	-	No	No	Yes
B	3_3	Machesa	-	No	No	No
B	3_4	Machesa	-	No	No	Yes
B	3_5	Machesa	-	No	No	No
B	4_1	Machesa	-	No	No	No
B	4_2	Machesa	-	No	Yes	Yes
C	1_1	Rapsu	+	Yes	Yes	Yes
C	1_2	Rapsu	++++	Yes	Yes	Yes
C	1_3	Rapsu	++++	Yes	Yes	Yes
C	1_4	Rapsu	+++	Yes	Yes	Yes
C	1_5	Rapsu	++++	No	No	No
C	2_1	Rapsu	+	No	No	No
C	2_2	Rapsu	-	No	No	Yes
C	2_3	Rapsu	-	No	No	Yes
C	2_4	Rapsu	-	Yes	Yes	No
C	2_5	Rapsu	-	Yes	Yes	No

* qPCR based on only milk

3 Supplementary References

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