

**A novel multiplex qPCR-HRM assay for the simultaneous detection of four abortive zoonotic agents in cattle, sheep, and goats**

Boitumelo Magret Modise<sup>1,2\*</sup>, Sununguko Wata Mpoloka<sup>2</sup>, Tirumala Bharani Kumar Settypalli<sup>3</sup>, Joseph Hyera<sup>4</sup>, Alda Natale<sup>5</sup>, Letizia Ceglie<sup>5</sup>, Nomakorinte Gcebe<sup>6</sup>, Chandapiwa Marobela-Raborokgwe<sup>1</sup>, Gerrit Johannes Viljoen<sup>3</sup>, Giovanni Cattoli<sup>3</sup>, Charles Euloge Lamien<sup>3</sup>.

<sup>1</sup> Botswana National Veterinary Laboratory, Private Bag 0035, Gaborone, Botswana.

<sup>2</sup> University of Botswana, Department of Biological Sciences, Private Bag 00704, Gaborone, Botswana.

<sup>3</sup> Animal Production and Health Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Wagramer Strasse 5, P.O. Box 100, A-1400 Vienna, Austria.

<sup>4</sup> Botswana Vaccine Institute, Private Bag 0031, Gaborone, Botswana.

<sup>5</sup> Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Viale dell'Università, 10 35020 Legnaro, Italy.

<sup>6</sup> Agricultural Research Council–Bacteriology and Zoonotic Diseases Diagnostic Laboratory, Onderstepoort Veterinary Research, Pretoria, South Africa

\*Corresponding author email: [boikensmod@gmail.com](mailto:boikensmod@gmail.com)

## Supplementary information

**Supplementary Table S1:** Samples tested by the multiplex qPCR-HRM assay for this study.

Identification	Country	District	DNA. source	Species	Tm	Pathogen	Status
Bot 1894/2014	Botswana	Kasane	SC	Bovine	82.8	Brucella	Blind
Bot 3354/2014	Botswana	Gaborone	Tissues	Caprine	80	Coxiella	Blind
					82.8	Brucella	
Bot 3065/2015	Botswana	Gaborone	Placenta	Ovine	80.2	Coxiella	Blind
					82.8	Brucella	
Bot 667/2015	Botswana	Nata	Tissues	Caprine	83.4	Brucella	Blind
Bot 2881/2016	Botswana	Pitsane	SC	Bovine	80.2	Coxiella	Blind
					83	Brucella	
Bot 1310/2017	Botswana	Nata	Tissues	Caprine	80.2	Coxiella	Blind
					82.6	Brucella	
Bot 1162/2017	Botswana	Mochudi	Tissues	Caprine	80.8	Coxiella	Blind
					83.4	Brucella	
Bot 1396/2017	Botswana	Gaborone	AF/contents	Ovine	80.8	Coxiella	Blind
Bot 1396/2017	Botswana	Gaborone	Tissues	Ovine	82.8	Brucella	Blind
Bot 2756/2017	Botswana	Ramotswa	SC	Caprine	80.4	Coxiella	Blind
					83	Brucella	
Bot 2756/2017	Botswana	Ramotswa	Tissues	Caprine	80.6	Coxiella	Blind
Bot 2928/2017	Botswana	Mahalapye	Tissues	Caprine	80.6	Coxiella	Blind
					83	Brucella	
Bot 2884/2017	Botswana	Ramotswa	Tissues	Caprine	80.6	Coxiella	Blind
					83.2	Brucella	
Bot 1309/2017	Botswana	Nata	Tissues	Caprine	80.2	Coxiella	Blind
Bot 3616/2017	Botswana	Ramotswa	Placenta	Bovine	80.4	Coxiella	Blind
					82.8	Brucella	
Bot 2930/2017	Botswana	Mahalapye	Tissues	Caprine	80.2	Coxiella	Blind
					82.8	Brucella	
Bot 4077/2017	Botswana	Molepolole	Tissues	Caprine	80.2	Coxiella	Blind
Bot 3907/2017	Botswana	Mahalapye	Tissues	Caprine	80.2	Coxiella	Blind
					82.6	Brucella	

Bot 1315/2017	Botswana	Nata	Tissues	Caprine	80	Coxiella	Blind
					82.8	Brucella	
Bot 893/2017	Botswana	Gaborone	Tissues	Caprine	80.6	Coxiella	Blind
Bot 4122/2017	Botswana	Letlhakane	Swab A	Caprine	81	Coxiella	Blind
					83.6	Brucella	
Bot 4122/2017	Botswana	Letlhakane	Swab B	Caprine	81	Coxiella	Blind
Bot 2757/2017	Botswana	Ramotswa	Tissues	Caprine	80.2	Coxiella	Blind
Bot 807/2017	Botswana	Ramotswa	Tissues	Caprine	80.2	Coxiella	Blind
Bot 1327/2018	Botswana	Mochudi	Tissues	Caprine	80.8	Coxiella	Blind
Bot 4326/2019	Botswana	Lobatse	Tissues	Bovine	80.4	Coxiella	Blind
					83	Brucella	
Bot 1528/2019	Botswana	Lobatse	Cultured tissue -DNA	Bovine	80.6	Coxiella	Blind
					83	Brucella	
Bot 1530/2019	Botswana	Lobatse	Cultured tissue -DNA	Bovine	80.6	Coxiella	Blind
					83.2	Brucella	
Bot 4258/2019	Botswana	Lobatse	Cultured tissue -DNA	Bovine	80.6	Coxiella	Blind
Bot 1676/2019	Botswana	Ghanzi	SC	Caprine	80.2	Coxiella	Blind
					82.8	Brucella	
Bot 1530/2019	Botswana	Lobatse	Tissues	Bovine	80.8	Coxiella	Blind
					83.4	Brucella	
Bot 3303/2019	Botswana	Lobatse	Tissues	Bovine	80	Coxiella	Blind
					82.4	Brucella	
Bot 1359/2019	Botswana	Mochudi	Tissues	Caprine	79.8	Coxiella	Blind
					82.4	Brucella	
Bot 2859/2020	Botswana	Lobatse	Serum	Bovine	80	Coxiella	Blind
					82.8	Brucella	
Bot 3090/2020	Botswana	Gaborone	Placenta	Ovine	80.2	Coxiella	Blind
Bot 2330/2020	Botswana	Ghanzi	Tissues	Caprine	80.4	Coxiella	Blind
Bot 2395/2020	Botswana	Molepolole	SC	Caprine	80.6	Coxiella	Blind
					83	Brucella	
Bot 1109/2020	Botswana	Gaborone	FT	Ovine	80.8	Coxiella	Blind
					83.2	Brucella	
Bot 1792/2020	Botswana	Mochudi	SC	Caprine	83	Brucella	Blind
Bot 1340/2020	Botswana	Ramotswa	Placenta	Ovine	80.4	Coxiella	Blind
					83	Brucella	

Bot 2833/2020	Botswana	Mochudi	FT	Caprine	80	Coxiella	Blind
Bot 2833/2020	Botswana	Mochudi	SC	Caprine	79.8	Coxiella	Blind
					82.4	Brucella	
Bot 2138/2020	Botswana	Mahalapye	Tissues	Caprine	79.8	Coxiella	Blind
Bot 1909/2020	Botswana	Lobatse	SC	Ovine	80	Coxiella	Blind
Bot 1571/2020	Botswana	Lobatse	Tissues A	Bovine	80	Coxiella	Blind
					82.6	Brucella	
Bot 1571/2020	Botswana	Lobatse	Serum	Bovine	82.6	Brucella	Blind
Bot 1571/2020	Botswana	Lobatse	Tissues B	Bovine	80.6	Coxiella	Blind
Bot 2933/2020	Botswana	Gaborone	Tissues	Caprine	80.2	Coxiella	Blind
					83	Brucella	
Bot 2132/2020	Botswana	Mochudi	Tissues	Caprine	80	Coxiella	Blind
					83	Brucella	
Bot 2112/2020	Botswana	Maun	Tissues	Caprine	80.4	Coxiella	Blind
Bot 3175/2020	Botswana	Shakawe	Tissues	Caprine	80.4	Coxiella	Blind
Bot 2046/2020	Botswana	Ghanzi	FT	Caprine	80.4	Coxiella	Blind
					83.2	Brucella	
Bot 2256/2020	Botswana	Ramotswa	Tissues	Caprine	80.2	Coxiella	Blind
Bot 2262/2020	Botswana	Mochudi	SC	Caprine	80.4	Coxiella	Blind
					82.6	Brucella	
Bot 2262/2020	Botswana	Mochudi	Tissues	Caprine	80	Coxiella	Blind
Bot 1920/2020	Botswana	Maun	SC	Bovine	80	Coxiella	Blind
					82.6	Brucella	
Bot 3677/2020	Botswana	Lobatse	Serum	Bovine	80	Coxiella	Blind
					82.6	Brucella	
Bot 2428/2020	Botswana	Molepolole	SC	Caprine	80	Coxiella	Blind
Bot 1883/2020	Botswana	Molepolole	SC	Caprine	79.9	Coxiella	Blind
Bot 2140/2020	Botswana	Mahalapye	Tissues	Caprine	80	Coxiella	Blind
Bot 686/2020	Botswana	Lobatse	Tissues	Bovine	83	Brucella	Blind
Bot 3098/2020	Botswana	Lobatse	Milk	Bovine	80.4	Coxiella	Blind
Bot 2535/2020	Botswana	Gaborone	Milk	Bovine	83.2	Brucella	Blind
Bot 3350/2020	Botswana	Lobatse	Milk	Bovine	82.6	Brucella	Blind
Bot 2799/2020	Botswana	Ghanzi	Milk A	Bovine	80.6	Coxiella	Blind
					82.8	Brucella	
Bot 2799/2020	Botswana	Ghanzi	Milk B	Bovine	80.8	Coxiella	Blind
Bot 1560/2020	Botswana	Lobatse	Milk	Bovine	83.2	Brucella	Blind
Bot 1509/2020	Botswana	Gaborone	Milk	Bovine	82.8	Brucella	Blind
Bot 2099/2020	Botswana	Lobatse	Milk	Bovine	80.4	Coxiella	Blind
Bot 1610/2020	Botswana	Lobatse	Milk	Bovine	80.2	Coxiella	Blind

Bot 2669/2020	Botswana	Lobatse	Milk	Bovine	80.4	Coxiella	Blind
Bot 2665/2020	Botswana	Lobatse	Milk	Bovine	80.4	Coxiella	Blind
Bot 1973/2020	Botswana	Gaborone	Milk	Bovine	80.8	Coxiella	Blind
					83	Brucella	
Bot 1611/2020	Botswana	Lobatse	Milk	Bovine	80.8	Coxiella	Blind
					83.2	Brucella	
Bot 2101/2020	Botswana	Serowe	Milk	Bovine	80.4	Coxiella	Blind
					82.8	Brucella	
Bot 1632/2020	Botswana	Gaborone	Milk	Bovine	80.2	Coxiella	Blind
Bot 1728/2020	Botswana	Gaborone	Milk	Bovine	80.4	Coxiella	Blind
Bot 2533/2020	Botswana	Gaborone	Milk	Bovine	80	Coxiella	Blind
Bot 808/2021	Botswana	Ramotswa	Swab	Caprine	80	Coxiella	Blind
					82.8	Brucella	
Bot 808/2021	Botswana	Ramotswa	WB	Caprine	80.6	Coxiella	Blind
Bot 808/2021	Botswana	Ramotswa	SC	Caprine	80	Coxiella	Blind
Bot 3345/2020	Botswana	Tsabong	Tissues	Caprine	80.2	Coxiella	Blind
Bot 1405/2021	Botswana	Ramotswa	SC	Caprine	80.6	Coxiella	Blind
Bot 523/2021	Botswana	Mahalapye	FT	Caprine	80.6	Coxiella	Blind
Bot 1368/2021	Botswana	Molepolole	SC	Caprine	80.8	Coxiella	Blind
					83.2	Brucella	
Bot 1048/2021	Botswana	Nata	SC	Caprine	80.6	Coxiella	Blind
Bot 1048/2021	Botswana	Nata	FT	Caprine	80.8	Coxiella	Blind
					83.2	Brucella	
Bot 952/2021	Botswana	Gaborone	SC	Caprine	80.8	Coxiella	Blind
					83.4	Brucella	
Bot 952/2021	Botswana	Gaborone	Tissues	Caprine	80.4	Coxiella	Blind
Bot 1175/2021	Botswana	Nata	SC	Caprine	80.2	Coxiella	Blind
					82.6	Brucella	
Bot 654/2021	Botswana	Nata	Serum	Bovine	82.8	Brucella	Blind
Bot 1064/2021	Botswana	Lobatse	FT	Bovine	83.4	Brucella	Blind
Bot 33/2021	Botswana	Lobatse	FT	Bovine	82.8	Brucella	Blind
ATCC 15313 GLi 11/2007	Austria	NK	NK	Rabbit	77.6	Listeria	known
ATCC 19114 GLi 18/2008	Austria	NK	Brain	Ruminant	77.6	Listeria	known
NCTC 10890 GLi 20/2008	Austria	NK	Faeces	Human	77.6	Listeria	known
GLI 22/2008	Austria	NK	NK	Poultry	77.6	Listeria	known
2776-2018	Italy	NK	Cheese	NA	77.8	Listeria	known

57448 C9-2018	Italy	NK	Foetus	Bovine	80.6	Coxiella	known
57448 C11-2018	Italy	NK	Foetus	Bovine	80.8	Coxiella	known
Milk Bolzano-reference material	Italy	Bozen	Milk	Caprine	80.8	Coxiella	known
Leptospira _ Icterohaemorrhagiae _ Bianchi	Italy	Pavia	Strain in EMJH medium	Man	75.8	Leptospira	known
Leptospira _ Canicola _ Alarik	Netherlands	NK	Strain in EMJH medium	Dog	75.8	Leptospira	known
Leptospira _ Grippothyphosa _ Moskva V	Russia	Rostov	Strain in EMJH medium	Man	75.8	Leptospira	known
Leptospira _ Copenhageni _ Wijmberg	Netherlands	NK	Strain in EMJH medium	Man	75.8	Leptospira	known
Leptospira _ Pomona _ Pomona	Australia	North Queensland	Strain in EMJH medium	Man	75.8	Leptospira	known
Leptospira _ Bratislava _ Riccio 2	Italy	Pisa	Strain in EMJH medium	Hedgehog	75.8	Leptospira	known
Leptospira _ Sejroe _ M84	Denmark	NK	Strain in EMJH medium	House mouse ( <i>Mus musculus spicilegus</i> )	75.6	Leptospira	known
Leptospira _ Tarassovi _ Mitis Johnson	Australia	NK	Strain in EMJH medium	Man	75.8	Leptospira	known
Leptospira serovar Ballum strain Mus 127	Denmark	NK	Strain in EMJH medium	Mouse ( <i>Mus musculus</i> )	75.8	Leptospira	known
Leptospira serovar Saxkoebing	Denmark	NK	Strain in EMJH medium	Yellow necked field	75.6	Leptospira	known

strain Mus 24				mouse ( <i>Apodemus flavicollis</i> )			
Leptospira serovar Hardjo strain Harjoprajitno	Indonesia	Sumatra	Strain in EMJH medium	Man	75.8	Leptospira	known
Brucella B1 625 -2019	Botswana	Lobatse	SC-DNA	Bovine	83.2	Brucella	known
Brucella B2 646 - 2019	Botswana	Lobatse	Culture-DNA	Bovine	83.8	Brucella	known
Brucella B3 050 - 2019	Botswana	Lobatse	Lung culture -DNA	Bovine	83	Brucella	known
Brucella B4 652 -2019	Botswana	Lobatse	SC-DNA	Bovine	82.8	Brucella	known
Bot 2332/2014	Botswana	Lobatse	SC	Caprine	NA	Negative	Blind
Bot 1024/2016	Botswana	Ramotswa	Tissues	Caprine	NA	Negative	Blind
Bot 2929/2017	Botswana	Mahalapye	Tissues	Caprine	NA	Negative	Blind
Bot 2947/2017	Botswana	Gaborone	Tissues	Caprine	NA	Negative	Blind
Bot 2992/2017	Botswana	Gaborone	Tissues	Caprine	NA	Negative	Blind
Bot 3223/2017	Botswana	Gaborone	Tissues	Caprine	NA	Negative	Blind
Bot 3224/2017	Botswana	Kanye	Tissues	Caprine	NA	Negative	Blind
Bot 3177/2017	Botswana	Ramotswa	Tissues	Caprine	NA	Negative	Blind
Bot 3531/2017	Botswana	Ramotswa	Placenta	Caprine	NA	Negative	Blind
Bot 1100/2019	Botswana	Kasane	Tissues	Ovine	NA	Negative	Blind
Bot 3040/2019	Botswana	Molepolole	SC/Tissues	Caprine	NA	Negative	Blind
Bot 2825/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 1633/2020	Botswana	Gaborone	Milk	Bovine	NA	Negative	Blind
Bot 1760/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 2654/2020	Botswana	Palapye	Milk	Bovine	NA	Negative	Blind
Bot 2822/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 1608/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 1606/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 1763/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 1630/2020	Botswana	Gaborone	Milk	Bovine	NA	Negative	Blind
Bot 1644/2020	Botswana	Ramotswa	FT	Caprine	NA	Negative	Blind
Bot 1469/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind

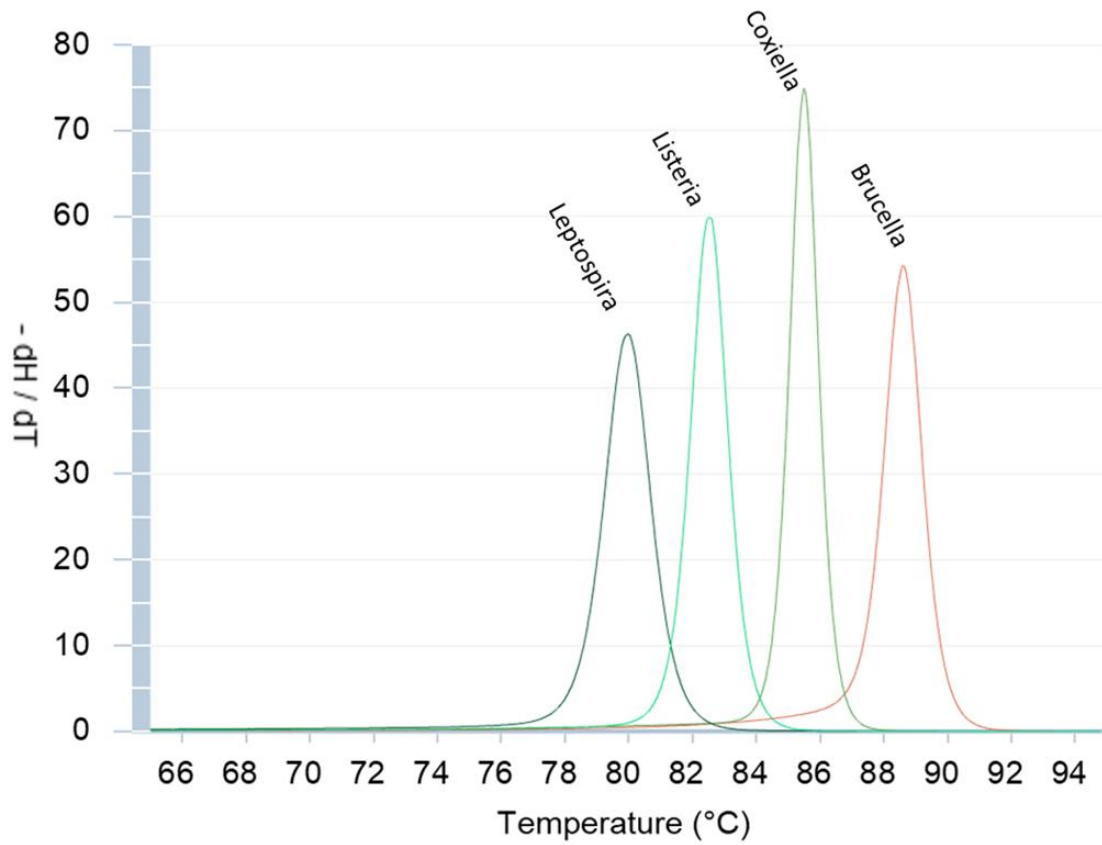
Bot 2094/2020	Botswana	Gaborone	Milk	Bovine	NA	Negative	Blind
Bot 2097/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 1986/2020	Botswana	Gaborone	Milk	Bovine	NA	Negative	Blind
Bot 1766/2020	Botswana	Gaborone	Milk	Bovine	NA	Negative	Blind
Bot 3183/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 3351/2020	Botswana	Lobatse	Milk	Bovine	NA	Negative	Blind
Bot 1570/2020	Botswana	Gaborone	Milk	Bovine	NA	Negative	Blind
Bot 2175/2020	Botswana	Mochudi	SC/FT	Caprine	NA	Negative	Blind
Bot 1792/2020	Botswana	Mochudi	Tissues	Caprine	NA	Negative	Blind
Bot 3671/2020	Botswana	Lobatse	Serum	Bovine	NA	Negative	Blind
Bot 3676/2020	Botswana	Lobatse	Serum	Bovine	NA	Negative	Blind
Bot 1802/2020	Botswana	Ramotswa	Tissues	Caprine	NA	Negative	Blind
Bot 2116/2020	Botswana	Maun	Tissues	Caprine	NA	Negative	Blind
Bot 2419/2020	Botswana	Nata	FT	Caprine	NA	Negative	Blind
Bot 2428/2020	Botswana	Molepolole	FT	Caprine	NA	Negative	Blind
Bot 902/2021	Botswana	Molepolole	FT	Caprine	NA	Negative	Blind
Bot 0439/2021	Botswana	Palapye	WB	Caprine	NA	Negative	Blind
Bot 0735/2021	Botswana	Shakawe	Tissues	Caprine	NA	Negative	Blind
Bot 0711/2021	Botswana	Lobatse	Serum	Bovine	NA	Negative	Blind
Bot 0677/2021	Botswana	Kanye	Serum	Bovine	NA	Negative	Blind
<i>Brucella abortus</i> 1	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 2	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 3	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 7	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 8	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 9	South Africa	NK	Bacterial culture	Bovine	83.2	Brucella	known
<i>Brucella abortus</i> 10	South Africa	NK	Bacterial culture	Bovine	83.2	Brucella	known
<i>Brucella abortus</i> 11	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 12	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 13	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 14	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 15	South Africa	NK	Bacterial culture	Buffalo	83.0	Brucella	known
<i>Brucella abortus</i> 16	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 17	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known



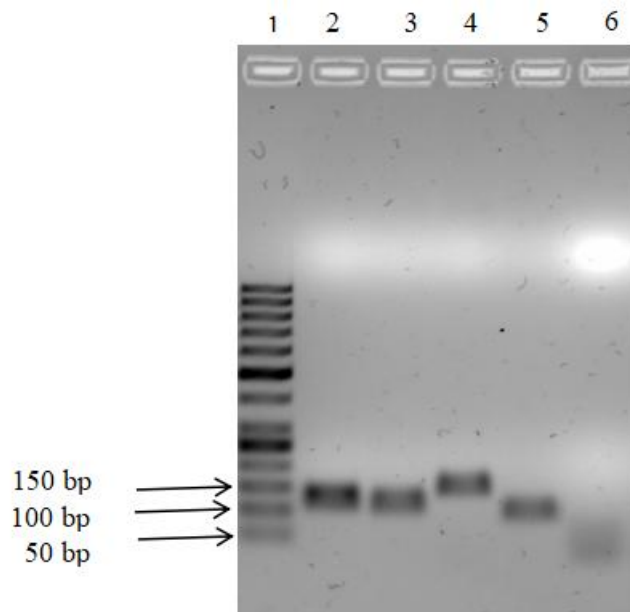
<i>Brucella abortus</i> 18	South Africa	NK	Bacterial culture	Bovine	83.0	Brucella	known
<i>Brucella abortus</i> 19	South Africa	NK	Bacterial culture	Bovine	83.2	Brucella	known
<i>Brucella abortus</i> 20	South Africa	NK	Bacterial culture	Bovine	83.2	Brucella	known
<i>Brucella melitensis</i> 2	South Africa	NK	Bacterial culture	Goat	83.2	Brucella	known
<i>Brucella melitensis</i> 3	South Africa	NK	Bacterial culture	Goat	83.2	Brucella	known
<i>Brucella ovis</i> 1	South Africa	NK	Bacterial culture	Sheep	83.2	Brucella	known
<i>Brucella ovis</i> 2	South Africa	NK	Bacterial culture	Sheep	83.2	Brucella	known
<i>Brucella ovis</i> 3	South Africa	NK	Bacterial culture	Sheep	83.2	Brucella	known
<i>Brucella suis</i> 2	South Africa	NK	Bacterial culture	Pig	83.2	Brucella	known
<i>Brucella suis</i> 3	South Africa	NK	Bacterial culture	Pig	83.2	Brucella	known
<i>Brucella abortus</i> bv9, NCTC 10507	UK	NK	Bacterial culture	NK	83.2	Brucella	known
<i>Brucella melitensis</i> 16m, NCTC 10094	UK	NK	Bacterial culture	NK	83.2	Brucella	known
<i>Brucella ovis</i> 63/290, NCTC 10512	UK	NK	Bacterial culture	NK	83.2	Brucella	known
<i>Brucella suis</i> bv3 686, NCTC 10511	UK	NK	Bacterial culture	NK	83.0	Brucella	known
<i>Coxiella burnetii</i>	South Africa	NK	Tissue	Cattle	80.8	Coxiella	known
<i>Coxiella burnetii</i> 2	South Africa	NK	Tissue	Cattle	80.8	Coxiella	known
<i>Coxiella burnetii</i> 3	South Africa	NK	Tissue	Cattle	80.8	Coxiella	known
<i>Coxiella burnetii</i> 4	South Africa	NK	Tissue	Cattle	80.8	Coxiella	known
<i>Coxiella burnetii</i> 5	South Africa	NK	Tissue	Cattle	80.8	Coxiella	known
<i>Coxiella burnetii</i> 6	South Africa	NK	Tissue	Cattle	80.8	Coxiella	known
<i>Coxiella burnetii</i> 7	South Africa	NK	Tissue	Cattle	80.8	Coxiella	known
<i>Coxiella burnetii</i> 8	South Africa	NK	Vaginal swab	Sheep	80.8	Coxiella	known
<i>Coxiella burnetii</i> 10	South Africa	NK	Vaginal swab	Sheep	80.8	Coxiella	known
<i>Coxiella burnetii</i> 11	South Africa	NK	Vaginal swab	Sheep	80.8	Coxiella	known
<i>Coxiella burnetii</i> 12	South Africa	NK	Vaginal swab	Sheep	80.8	Coxiella	known
<i>Coxiella burnetii</i> 13	South Africa	NK	Tissue	Goat	80.6	Coxiella	known
<i>Coxiella burnetii</i>	South	NK	Tissue	Goat	80.8	Coxiella	known

14	Africa						
<i>Coxiella burnetii</i> 15	South Africa	NK	Tissue	Goat	80.6	Coxiella	known
<i>Coxiella burnetii</i> 17	South Africa	NK	Tissue	Goat	80.8	Coxiella	known
<i>Leptospira Canicola-Grippotyphosa-Hardjo-Icterohaemorrhagiae-Pomona</i> Bacterin 1	South Africa	NK	Leptoferm-5 vaccine (Zoetis)	NA	75.8	Leptospira	known
<i>Listeria monocytogenes</i> 1	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 3	South Africa	NK	Bacterial culture	NK	77.4	Listeria	known
<i>Listeria monocytogenes</i> 5	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 9	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 10	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 11	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 13	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 14	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 15	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 16	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 17	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 18	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 19	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known
<i>Listeria monocytogenes</i> 20	South Africa	NK	Bacterial culture	NK	77.6	Listeria	known

Abbreviations: AF = Abdominal fluid; FT = Foetal tissues; NK = Not known; SC = Stomach contents; WB = Whole blood



**Figure S1:** Umelt simulation graph showing melting curve, the negative derivative of the fluorescence (F) over temperature (T) ( $dF/dT$ ) against the temperature (T).



**Figure S2:** Gel picture showing the expected amplicon size of each of the primers.

Lane 1: 50-bp ladder; lane 2: *Brucella* spp. (101 bp) ; lane 3: *Listeria monocytogenes* (93 bp); lane 4: *Coxiella burnetii* (121); lane 5: *Leptospira* spp (77 bp); lane 6: Negative control without DNA.