| Determinant         Determinant         Determinant         Determinant           Determinant         Determinant         Determinant         <   |               |          | 3.4%            | R2 manusci     | ript Brucella Prof. Aym | an.doc        |                   |                                  |  |  |
|---|---------------|----------|-----------------|----------------|-------------------------|---------------|-------------------|----------------------------------|--|--|
| * All sources         22         O Internet sources         22           Ø         10         Oww.mplc.com/2075-1739/12/5647/htm         3           Ø         11         Oww.mplc.com/2075-1739/12/5647/htm         3           Ø         11         Oww.mplc.com/2075-1639/12/36/901         3           Ø         12         Oww.mplc.com/2075-1639/12/36/901         3           Ø         13         Oww.mplc.com/2075-1639/12/36/901         3           Ø         13         Oww.mplc.com/2075-1639/12/36/901         3           Ø         14         Oww.mplc.com/2075-1639/12/36/901         3           Ø         15         Oww.mplc.com/2075-1639/12/36/901         3           Ø         16         Oww.mplc.com/2075-1639/12/36/301-7327/2080000200011         3           Ø         16         Oww.mplc.com/2075-010-04-010-04-05-00         3           Ø         16         Oww.mplc.com/2075-010-04-05-00-00-00-00-0000000000000000  |               | 1 🖩 1    |                 |                | I                       |               |                   |                                  |  |  |
| v         (i)         Ownw.mipli.com/2075-1729/12/5047/htm           v         (i)         Tamathes         (ii)           v         (i)         Ownw.mipli.com/2075-4732/10/3/238/pdf           v         (i)         Ownw.mipli.com/2075-4732/10/3/238/pdf           v         (i)         Ownw.mipli.com/2079-4382/10/3/238/pdf           v         (i)         Ownw.mipli.com/2079-4382/10/3/238/pdf           v         (i)         Ownw.hindox/.com/journals/bm/2019/6762517/           v         (i)         Ownw.hindox/.com/journals/bm/12019/6762517/           v         (i)         Outnit.springer.com/article/10.1007/680705-015-04437-0           v         (ii)         Ownw.clin.nim.nit.gov/780-0009/Browser/wwtats.cgr/id=540272           v         (ii)         Ownw.clin.nim.nit.gov/780-0009/Browser/wwtats.cgr/id=5402201           (iii)         Imathes         (iii)         Imathes           v         (ii)         Ownw.clin.nim.nim.   |               |          |                 |                |                         |               |                   |                                  |  |  |
| v         [v]         [III]         • • • • • • • • • • • • • • • • • • •   |               |          | I               |                |                         |               |                   |                                  |  |  |
| v         11         [EB] 7         matches           v         12         0         www.sicince.tifreet.com/opics/medicine-and-dentistry/brucella           v         13         0         www.hindawi.com/opics/medicine-and-dentistry/brucella           v         13         0         www.hindawi.com/opics/medicine-and-dentistry/brucella           v         13         0         www.hindawi.com/opics/medicine-and-dentistry/brucella           v         14         0         pubmed.ncbi.m.nih.gov/16207953/           v         14         0         pubmed.ncbi.m.nih.gov/16207953/           v         15         0         bink.springer.com/article/10.1007/s00705-019-04437-0           v         16         0         www.science.infection.php?script=sci_artist.spid=50301-732:2008000200011           v         17         0         www.science.infection.on/ppi/script=sci_artist.spid=50301-732:2008000200011           v         17         0         www.science.infection.on/ppi/script=sci_artist.spid=50301-732:2008000200011           v         17         0         www.science.infection.on/ppi/script=sci_artist.spid=50301-732:2008000200011           v         171         0         www.science.infection.on/ppi/script=sci_artist.spid=50301-732:2008000200011           v         171         0         www.scienc   | <b>⊘</b> [0]  |          |                 |                |                         |               |                   |                                  |  |  |
| V         I/2         IEEE 7         T matches           V         13         Edm 4 matches         Imatches           V         14         Pubmed.ncbi.nlm.hg.gov/16207953/           I/2         25% 3 matches         Imatches           V         16         Ourseled.cl/sciele0.php?script=sci_arttext&pid=50301-732X2008000200011           I/2         16         Ourseled.cl/sciele0.php?script=sci_arttext&pid=50301-732X2008000200011           I/2         16         Ourseled.cl/sciele0.php?script=sci_arttext&pid=50301-732X2008000200011           I/2         T matches         Imatches           V         17         Ourseled.cl/sciele0.php?script=sci_arttext&pid=50301-732X2008000200011           I/2         T matches         Imatches           V         18         Odcplayer.es/5135559-Genotipos-de-alistados-de-campo-de-brucella-abortus-de-distintas-regiones-geograficas-de-chile.html           I/2         Ourse Sciencedirect.com/science/article/absipil/S0014489414001234         Imatches           V         19         Ourse Science/article/absipil/S0014489414001234           I/2         T matches         Imatches           V         10         Ourse Science/article/absipil/S0014489414001234           I/10         Ourse Science/article/absipil/S014489414001234         Imatches           V <td><b>₽</b> [1]</td> <td></td> <th>-6382/10/3/23</th> <th>3/pdf</th> <td></td> <td></td> <td></td> <td></td>  | <b>₽</b> [1]  |          | -6382/10/3/23   | 3/pdf          |                         |               |                   |                                  |  |  |
| v         13         EARS         4 matches           v         14         2 pubmed.ncbi.lnm.hl.gov/16207953/<br>EZR: 3 matches           v         15         0 link.springer.com/article/10.1007/s00705-019-04437-0<br>EZR: 2 matches           v         16         0 www.seled.clbi.elp.p?script=sci_arttext&pid=50301-732X2008000200011<br>EZR: 2 matches           v         17         0 www.seled.clbi.nlm.hl.gov/Taxonomy/Browser/wwwtax.cgi?id=546272<br>EZR: 2 matches           v         18         0 docplayer.ss/135559-Genotipos-de-aislados-de-campo-de-brucella-abortus-de-distintas-regiones-geograficas-de-chile.html<br>EXR: 2 matches           v         19         0 www.seled.clbi.amahospitals.com/<br>EXR: 2 matches           v         19         0 www.selencedirect.com/selence/article/abs/pil/S0014489414001234           v         10         0 ruw.badralsmaahospitals.com/<br>EXR: 1 matches           v         110         0 www.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saltou_and_Nei           v         111         0 www.researchgate.net/publication/19934093_A_note_on-the_Neighbor-Joining_Algorithm_of_Saltou_and_Nei           v         111         0 www.researchgate.net/publication/19934093_A_note_on-testing-The-initial-ramp-up-and-down-in-temperature-is-to_fig1_33109715           v         113         0 pubmed.ncbi.lnm.nih.gov/2788459/<br>EXR: 1 matches           v         114         0 www.  | <b>⊘</b> [2]  |          |                 |                |                         |               |                   |                                  |  |  |
| V       14       22% 3 matches         V       15       Q link-springer.com/article/10.1007/s00705-019-04437-0         Q2% 2       matches         V       16       Q www.scieto.clice.php?script=sei_arttext&pid=S0301-732X2008000200011         Q       If       Q www.scieto.clink.mih.gov/Taxonomy/Browser/wwwtax.cgi7id=546272         Q       If       Q www.scieto.clice.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.com/science.dlret.gov         Q       If       Q uwww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         Q       If       Q uwww.researchgate.net/figure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_ligi_33109715         Q       If       Q www.ncebi.nlm.nih.gov/Zaxonomy/Browser/wwwtax.cgi7/d=64114  | <b>⊘</b> [3]  |          | urnals/bmri/2   | 019/6762517/   |                         |               |                   |                                  |  |  |
| N       19       Exhip 2 matches         V       16       Quives sciels of Siciels ophp?script=sci_arttext&pid=50301-732X2008000200011         V       17       Quives sciels of Siciels ophp?script=sci_arttext&pid=50301-732X2008000200011         V       17       Quives sciels of Siciels ophp?script=sci_arttext&pid=50301-732X2008000200011         V       18       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=546272         V       18       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=546272         V       19       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=546272         V       19       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=546272         V       19       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=546272         V       10       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=546272         V       11       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=54140         V       113       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=641140         V       114       Quives science/Instanting ov/Taxonomy/Browser/wwwtax.cgi?id=641140         V       Quives science/Instanting ov/Taxonomy/Browser/wwtax.cgi?id=641140         V       Quives science/Instanting ov/Taxonomy/Browser/wwtax.cgi?id=641140         V       Quives science/I  | <b>☑</b> [4]  |          | h.gov/162079    | 53/            |                         |               |                   |                                  |  |  |
| V       [0]       Exts: 2 matches         V       [7]       Queww.ncbi.nlm.nih.gov/Taxonomy/Browser/www.tax.cgi?id=546272         [2]       [2]       [2]       matches         V       [8]       Quecplayer.es/5135559-Genotipos-de-aislados-de-campo-de-brucella-abortus-de-distintas-regiones-geograficas-de-chile.html         V       [8]       Quecplayer.es/5135559-Genotipos-de-aislados-de-campo-de-brucella-abortus-de-distintas-regiones-geograficas-de-chile.html         V       [9]       Queww.sciencedirect.com/science/article/abs/pii/S0014489414001234         V       [10]       Queww.sciencedirect.com/science/article/abs/pii/S0014489414001234         V       [10]       Queww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         [0]       Quewww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         [11]       Quewww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         [12]       Quelxhuwair.badralsamaahospitals.com/         [13]       Quebmed.ncbi.nlm.nih.gov/2888250/         [24]       Quebmed.ncbi.nlm.nih.gov/27888459/         [25]       Quewww.researchgate.net/ligure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_flig1_331099715         [21]       Quebmed.ncbi.nlm.nih.gov/27057678/         [25] <td><b>⊘</b> [5]</td> <td></td> <th>ticle/10.1007/s</th> <th>00705-019-04</th> <td>1437-0</td> <td></td> <td></td> <td></td>  | <b>⊘</b> [5]  |          | ticle/10.1007/s | 00705-019-04   | 1437-0                  |               |                   |                                  |  |  |
| V       [1]       E2% 2 matches         V       [8]       Odocplayer.es/5135559-Genotipos-de-aislados-de-campo-de-brucella-abortus-de-distintas-regiones-geograficas-de-chile.html         V       [9]       Oww.sciencedirect.com/science/article/abs/pii/S0014489414001234         V       [9]       Oww.sciencedirect.com/science/article/abs/pii/S0014489414001234         V       [9]       Oww.sciencedirect.com/science/article/abs/pii/S0014489414001234         V       [10]       Orruw.bddratsamaahospitals.com/         [011]       Oww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saltou_and_Nei         V       [11]       Oww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saltou_and_Nei         V       [11]       Oww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saltou_and_Nei         V       [11]       Outch.neth.searchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saltou_and_Nei         V       [12]       Oalkhuwair.badralsamaahospitals.com/       Saltou_and_Nei         V       [12]       Oalkhuwair.badralsamaahospitals.com/       Saltou_and_Nei         V       [14]       Opubmed.ncbi.nlm.nih.gov/8882501/       Saltou_and_Nei         V       [15]       Outch.nlm.nih.gov/27888459/       Saltou_and_Nei         V       [   | <b>☑</b> [6]  |          | .php?script=s   | ci_arttext&pic | l=S0301-732×20080002    | 200011        |                   |                                  |  |  |
| V       [9]       §155 2 matches         V       [9]       @vww.sciencedirect.com/science/article/abs/pii/S0014489414001234         V       [10]       @ruwi.badralsamaahospitals.com/         V       [11]       @vww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         V       [11]       @ www.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         V       [11]       @ al-khuwair.badralsamaahospitals.com/         V       [12]       @ al-khuwair.badralsamaahospitals.com/         V       [13]       @ pubmed.ncbi.nlm.nih.gov/8882501/         V       [14]       @ pubmed.ncbi.nlm.nih.gov/27688459/         V       [14]       @ pubmed.ncbi.nlm.nih.gov/27888459/         V       [15]       @ www.researchgate.net/figure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_fig1_331099715         V       [16]       @ www.ncbi.nlm.nih.gov/27057678/         @ itims 1 matches       V         V       [17]       @ pubmed.ncbi.nlm.nih.gov/27057678/         @ itims 1 matches       V         V       [18]       @ link.springer.com/article/10.1007/s102666-016-1335-1         @ www.ncbi.nlm.nih.gov/30977901/       @ pubmed.ncbi.nlm.nih.gov/30977901/  | <b>⊘</b> [7]  |          | ov/Taxonomy/    | Browser/www    | tax.cgi?id=546272       |               |                   |                                  |  |  |
| [9]       E1%       2 matches         [7]       [10]       © ruwi.badralsamaahospitals.com/         [5]       [11]       @ uww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         [7]       [11]       @ uww.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         [7]       [12]       @ al-khuwair.badralsamaahospitals.com/         [7]       [13]       @ ubmed.ncbi.nlm.nih.gov/8882501/         [7]       [14]       @ ubmed.ncbi.nlm.nih.gov/8882501/         [8]       [14]       @ ubmed.ncbi.nlm.nih.gov/27888459/         [8]       [9]       [14]       @ ubmed.ncbi.nlm.nih.gov/27888459/         [9]       [15]       @ www.researchgate.net/ligure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_fig1_331099715         [9]       [16]       @ www.ncbi.nlm.nih.gov/71xxonomy/Browser/wwwtax.cgi?id=641140         [9]       [17]       @ pubmed.ncbi.nlm.nih.gov/27057678/         [9]       [18]       @ link.springer.com/article/10.1007/s12275-016-6410-3         [9]       @ link.springer.com/article/10.1007/s12275-016-6410-3         [9]       @ link.springer.com/article/10.1007/s12275-016-6410-3         [9]       @ link.springer.com/article/10.1007/s12275-016-6410-3         [9] <t< td=""><td><b>⊘</b> [8]</td><td></td><th>i59-Genotipos</th><th>-de-aislados-</th><td>de-campo-de-brucella-a</td><td>abortus-de-di</td><td>istintas-regiones</td><td>-geograficas-de-chile.html</td></t<>   | <b>⊘</b> [8]  |          | i59-Genotipos   | -de-aislados-  | de-campo-de-brucella-a  | abortus-de-di | istintas-regiones | -geograficas-de-chile.html       |  |  |
| V [10]       E1%       1 matches         V [11]       O www.researchgate.net/publication/19934093_A_note_on_the_Neighbor-Joining_Algorithm_of_Saitou_and_Nei         V [11]       O al-khuwair.badralsamaahospitals.com/         E189       1 matches         V [12]       O al-khuwair.badralsamaahospitals.com/         E193       O pubmed.ncbi.nlm.nih.gov/8882501/         E193       O pubmed.ncbi.nlm.nih.gov/7888459/         V [14]       O pubmed.ncbi.nlm.nih.gov/27888459/         V [15]       O www.researchgate.net/ligure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_fig1_331099715         V [15]       O www.researchgate.net/ligure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_fig1_331099715         V [15]       O www.ncbi.nlm.nih.gov/7axonomy/Browser/wwwtax.cgi?id=641140         V [17]       O pubmed.ncbi.nlm.nih.gov/27057678/         V [17]       O pubmed.ncbi.nlm.nih.gov/27057678/         V [18]       O link.springer.com/article/10.1007/s12275-016-6410-3         Q Q Bilnk.springer.com/article/10.1007/s00606-016-1335-1         Q Q Dilnk.springer.com/article/10.1007/s00606-016-1335-1         Q Q Dilnk.springer.com/article/10.1007/s00606-016-1335-1         Q Q Dilnk.springer.com/article/10.1007/s00606-016-1335-1         Q Q Dilnk.springer.com/article/10.1007/s00606-016-1335-1         Q Dil  | <b>v</b> [9]  |          |                 |                |                         |               |                   |                                  |  |  |
| Image: | <b>7</b> [10] |          | spitals.com/    |                |                         |               |                   |                                  |  |  |
| ▼ [12]       ● 1 matches         ▼ [13]       ● pubmed.ncbi.nlm.nih.gov/8882501/         ● [14]       ● pubmed.ncbi.nlm.nih.gov/27888459/         ● [14]       ● pubmed.ncbi.nlm.nih.gov/27888459/         ● [14]       ● pubmed.ncbi.nlm.nih.gov/27888459/         ● [15]       ● www.researchgate.net/figure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_fig1_331099715         ● [15]       ● www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=641140         ● [16]       ● www.ncbi.nlm.nih.gov/27057678/         ● [17]       ● pubmed.ncbi.nlm.nih.gov/27057678/         ● [18]       ● 1 matches         ▼ [18]       ● link.springer.com/article/10.1007/s12275-016-6410-3         ● ∞0% 1 matches       ■         ▼ [19]       ● link.springer.com/article/10.1007/s00606-016-1335-1         ● ∞0% 1 matches       ■         ▼ [20]       ● patents.google.com/patent/CN113249495A/en         ● ∞1% 1 matches       ■         ▼ [21]       ● pubmed.ncbi.nlm.nih.gov/30977901/  | 🔽 [11]        |          | et/publication  | /19934093_A_   | _note_on_the_Neighbo    | or-Joining_Al | gorithm_of_Saite  | ou_and_Nei                       |  |  |
| ✓ [13]       0.1%       1 matches         ✓ [14]       O pubmed.ncbi.nlm.nih.gov/27888459/         Ø pubmed.ncbi.nlm.nih.gov/27888459/         Ø 0.0%       1 matches         ✓ [15]       O www.researchgate.net/figure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_fig1_331099715         Ø 115       0.1%       1 matches         ✓ [16]       O www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=641140         Ø 116       0.1%         Ø 117       O pubmed.ncbi.nlm.nih.gov/27057678/         Ø 117       0.1%         Ø 118       O link.springer.com/article/10.1007/s12275-016-6410-3         Ø 118       0.0%         Ø 118       0.1%  | <b>7</b> [12] |          | naahospitals.   | com/           |                         |               |                   |                                  |  |  |
| ✓       [14]       0.0% 1 matches         ✓       [15]          • www.researchgate.net/figure/The-thermal-profile-used-for-testing-The-initial-ramp-up-and-down-in-temperature-is-to_fig1_331099715         Ø       [15]          • www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=641140         Ø       [16]          • www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=641140         Ø       [16]          • www.ncbi.nlm.nih.gov/27057678/         Ø       [17]          • pubmed.ncbi.nlm.nih.gov/27057678/         Ø       [18]          • link.springer.com/article/10.1007/s12275-016-6410-3         Ø          • 0.0% 1 matches         ✓       [19]          • link.springer.com/article/10.1007/s00606-016-1335-1         Ø          • 0.0% 1 matches         ✓       [19]          • pink.springer.com/patent/CN113249495A/en         Ø          • [20]          • patents.google.com/patent/CN113249495A/en         Ø          • [17]          • pubmed.ncbi.nlm.nih.gov/30977901/   | 🔽 [13]        | · .      | h.gov/8882501   | 1              |                         |               |                   |                                  |  |  |
|   | <b>⊘</b> [14] | <u> </u> | h.gov/278884    | 59/            |                         |               |                   |                                  |  |  |
|   | 🖌 [15]        |          | et/figure/The-  | hermal-profil  | e-used-for-testing-The∙ | initial-ramp- | up-and-down-in-t  | temperature-is-to_fig1_331099715 |  |  |
| ▶       [17]       0.1% 1 matches         ▶       [18]       ● link.springer.com/article/10.1007/s12275-016-6410-3         0.0% 1 matches       0.0% 1 matches         ▶       [19]       ● link.springer.com/article/10.1007/s00606-016-1335-1         ●       0.0% 1 matches         ▶       [20]       ● patents.google.com/patent/CN113249495A/en         ●       0.1% 1 matches         ▶       [21]       ● pubmed.ncbi.nlm.nih.gov/30977901/   | 🔽 [16]        |          | ov/Taxonomy/    | Browser/www    | tax.cgi?id=641140       |               |                   |                                  |  |  |
| ▶       [18]       0.0% 1 matches         ▶       [19]       ● link.springer.com/article/10.1007/s00606-016-1335-1         ●       0.0% 1 matches         ▶       [20]       ● patents.google.com/patent/CN113249495A/en         ●       0.1% 1 matches         ▶       [21]       ● pubmed.ncbi.nlm.nih.gov/30977901/  | <b>⊘</b> [17] |          | h.gov/2705767   | /8/            |                         |               |                   |                                  |  |  |
|   | <b>₽</b> [18] |          | ticle/10.1007/s | 12275-016-64   | 10-3                    |               |                   |                                  |  |  |
| <ul> <li>✓ [20] 0.1% 1 matches</li> <li>✓ [21] Ø pubmed.ncbi.nlm.nih.gov/30977901/</li> </ul>   | <b>7</b> [19] |          | ticle/10.1007/s | 00606-016-13   | 35-1                    |               |                   |                                  |  |  |
|   | <b>₽</b> [20] |          | /patent/CN113   | 249495A/en     |                         |               |                   |                                  |  |  |
|   | <b>₽</b> [21] |          | h.gov/3097790   | )1/            |                         |               |                   |                                  |  |  |

12 pages, 4237 words

PlagLevel: 3.4% selected / 3.4% overall

31 matches from 22 sources, of which 22 are online sources.

#### Settings

Data policy: Compare with web sources, Check against my documents

Sensitivity: *High* 

Bibliography: Consider text

Citation detection: Reduce PlagLevel

Whitelist: --

1IS711 nucleotide sequencing of Brucella melitensis and Brucella abortus strains, and use of microchip-2based real-time PCR for rapid monitoring 3

4 Ayman Elbehiry<sup>1,2\*</sup>, Musaad Aldubaib<sup>3</sup>, Osamah Al Rugaie<sup>4</sup>, Eman Marzouk<sup>1</sup>, Ihab

5 Moussa<sup>5,6\*</sup>, Mohamed H. El-Husseiny<sup>7</sup>, Adil Abalkhail<sup>1</sup>, Khalid Abou-Gazia<sup>8</sup>, Ahmed

6

7<sup>1</sup> Department of Public Health, College of Public Health and Health Informatics, Qassim University, Al-8Bukairiyah, Saudi Arabia. Email: ar.elbehiry@qu.edu.sa

Allam<sup>9</sup>

9<sup>2</sup> Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, University10of Sadat City, Sadat City, Egypt.

11<sup>th</sup> College of Agriculture and Veterinary Medicine, Qassim University, Qassim, Saudi Arabia.

12<sup>12</sup> Department of Basic Medical Sciences, College of Medicine and Medical Sciences, Qassim University,

13Unaizah, P.O. Box 991, Qassim 51911, Saudi Arabia

14<sup>5</sup> Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, 15Riyadh 11451, Saudi Arabia.

16<sup>6</sup> Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

177 Animal Health Research Institute - Agriculture Research Center, Giza 12618, Egypt.

18<sup>8</sup> Brucella unit, Animal Reproductive Research Institute, Agriculture Research Central (ARC).

199 Animal Health Research Institute (AHRI), Agriculture Research Central (ARC).

20

21Corresponding author\*: Ayman Elbehiry

22

# 23

24Abstract

25In animal production systems around the world, brucellosis is a serious zoonotic disease that creates 26public health hazards and losses in economic terms. The aim of the study is to genotype and molecularly 27characterize Brucella melitensis (B. melitensis) and Brucella abortus (B. abortus) collected from 28different animal species and humans. A total of 50 isolates of Brucella species (16 B. melitensis and 34 29B. abortus) were isolated from 1081 animal and human samples using a culture technique, followed by 30biochemical identification using the Vitek 2 compact system and proteomic identification using mass 31spectrometry technology. Molecular genotyping was performed on all isolates using multiplex real-time

1

32PCR. Six isolates from each genotype of Brucella species were selected and genetically evaluated by 33IS711 insertion sequences. Microchips-based real-time PCR for Brucella species identification was 34performed on twelve genetically characterized isolates as a first attempt. Forty-four (88%) isolates of 35Brucella species were detected using multiplex real-time PCR. Based on IS711 nucleotide sequencing, 36twelve isolates were phylogenetically clustered into their specific clusters. The results of the 37comparative analysis of conventional real time and microchips-based real time indicated that the later is 38faster and qualitatively more sensitive than conventional real time; however, further studies are needed 39to ensure that it is capable of serving as a gold standard alternative for Brucella species monitoring. 40

41Keywords: Brucella species, phylogenetic analysis, microchip-based real-time PCR, IS711 sequencing 42

## 431. Introduction

44Brucellosis is one of the most common zoonotic disease with public health importance and industrial 45 farming systems around the world suffer substantial financial losses as a result of it. (Seleem et al., 462010; Janowicz et al., 2018). The disease remains endemic in the Middle East despite being well-47 controlled in western countries. (Kirk et al., 2015). Brucella is a gram negative intracellular bacterium 48that cause disease in domesticated animals such as cattle, sheep, goats and camels (Richomme et al., 492006; Saeed et al., 2019). All of the Brucella species identified from livestock, including Brucella 50 melitensis (B. melitensis), Brucella abortus (B. abortus), Brucella suis (B. suis), and Brucella canis (B. 51canis), are virulent to humans (Al Jindan, 2021).<sup>[2]</sup> Human-animal contact and environmental boundaries 52 are often points of transmission of Brucella strains that infect humans and animals (Assenga et al., 2015; 53Godfroid, 2017) since humans, livestock, and wildlife often share the same habitats. The humans' 54infection with brucellosis was frequently due to damaged skin during direct contact with infected 55parturition materials as in gynaecological examination or as in examining and flaying slaughtered 56animals. Infection could be also through the mucous membranes (mucosa) and airways. Moreover, 57 infection could be occurred during handling the infected animals' manure (Solecki, 1999; Galinska and 58Zagórski, 2013). While infections by ingestion of infected milk or dairy products are rare (Solecki, 591999). Middle East has an endemic case of brucellosis (Greco et al., 2018). B. abortus and B. melitensis 60have been isolated from animals and Humans (Sayour and Sayour, 2018; Sayour et al., 2020), while B. 61suis has only been isolated from animal (Khan et al., 2019; Khan et al., 2020). Generally, diagnosis of

62brucellosis is based on classical isolation and identification methods, serological tests or molecular 63techniques (Boussetta, 1999; Yagupsky et al., 2019).

Many different methods have been used worldwide to identify and characterize Brucella species and 65determine how they spread to other mammals, including human (Ntirandekura et al., 2020). Isolation is 66considered a gold slandered for diagnosis but has many disadvantages such as, need prolonged time and 67poses a high risk of infection to the veterinarians work with it. Moreover, to handle samples and live 68bacteria for ultimate identification and biotyping, level 3 biocontainment facilities and highly qualified 69technical employees are required. (Yu and Nielsen, 2010; Khan and Zahoor, 2018). Fast and precise 70diagnostic technologies are necessary in order to prevent disease transmission from animals to humans, 71reduce health risks, and minimize economic losses. The most effective diagnostic approach is the PCR 72test in order to detect Brucella strains (Yu and Nielsen, 2010; Khan and Zahoor, 2018). Microchip real-73time PCR is considered as a friendly alternative to traditional real-time PCR. It has been shown to 74deliver reliable, sensitive, and specific results in less time (Cojocaru et al., 2021).

Molecular and computational techniques are providing us with an improved understanding of how 76Brucella species differ in terms of evolutionary development, specificity, and pathogenicity in various 77hosts (Vidal et al., 2018). For the purpose of establishing relationships and grouping of Brucella species, 78phylogenetic analyses based on random repeats, genomic loci and 16S rRNA gene sequencing are useful 79(Menshawy et al., 2014; Shome et al., 2016; Khan et al., 2018). A high homology of DNA is found 80between Brucella species, with more than 90%. Based on the polymorphism of the IS711 insertion 81sequence in the Brucella genome, it is a prospective molecular method for distinguishing between 82species of Brucella and its biovars (Bricker et al., 1994; Mancilla et al., 2011). Selim et al. (2019) 83explained how identification of the common Brucella and characterization of its molecular 84characteristics makes it easier to determine the source of the infection and take the appropriate measures 85to control brucellosis. The current work intends to identify and molecular characterize the isolated 86samples from several governorates in Saudi Arabia and Egypt and throw the light on rapid technique for 87<mark>Brucella</mark> species diagnosis.

## 88 892. Material and Methods

902.1. Ethical statement

91A written authorization or ethical approval were not necessary for this study because neither humans nor 92animals actively participated. Neither human nor animal samples were used. Our only source of bacteria

3

93was routine medical testing or strain collection. As a result, none of the clinical strains were obtained 94from patients or animals for use in this study. Samples obtained from routine diagnostic procedures were 95used instead.

96

972.2. Samples collections, isolation and identification

98In Saudi Arabia's Al-Qassim province, samples of milk, vaginal swabs, and blood from 364 animals 99 with a high rate of brucellosis, and 70 human blood samples from individuals who suffered from 100hyperthermia after close contact with suspect animals were collected. Moreover, 617 different tissues 101(spleen and lymph nodes) of aborted fetuses or animal carcasses and milk were collected from Egyptian 102governorates. From cow and goat farms, we collected 15 ml of each milk and blood sample, as well as 103vaginal swabs. A tissue sample was collected aseptically, extraneous materials were removed, and tissue 104samples were sliced into small pieces and then macerated in sterile phosphate buffer saline (PBS), as 105described in the OIE manual (2018). The biosafety level two (BSL2) was applied to all microbiological 106samples deemed to have relatively high impacts. In brief, the samples were rotated at 6000 rpm for 10 107 minutes to concentrate the organism, after which the sediment was inoculated onto a specific, antibiotic-108containing medium (Brucella Selective Agar), after which the cultured plates were examined for 109Brucella species on the 4<sup>th</sup> day and then on a daily basis throughout the next 2–4 weeks at 37°C in the 110existence of 10% CO<sub>2</sub>. After several subcultures, the Brucella colonies appeared spherical, shiny, 111pinpointed, and honey-colored. The bacterial colonies were then identified biochemically using both the 112Vitek 2 Compact System (bioMérieux, France) and other similar approaches such as catalase activity, 113oxidase activity, CO2 requirements, urease, hydrogen sulfide production, lactose fermentation, and 114nitrate reduction. The MALDI Biotyper (Bruker Daltonics, Bremen, Germany) was used to identify 115Brucella species from their proteomic data.

### 116

117

1182.3. DNA extraction and molecular detection

1192.3.1. Conventional real time PCR

120The biochemical confirmed colonies were subjected to molecular detection by standard conventional 121real time PCR. At first the DNA was extracted from bacterial pellet using GeneJET Genomic DNA 122Purification Kit (thermofisher, cat# K0722) according to manufacturer's instruction. The extracted DNA 123was detected for Brucella species by uniplex real time PCR. Then genotyped for B. abortus and B. 124meletensis by multiplex realtime PCR. The primers and probes used are listed in table 1.<sup>[2]</sup>The kit used for

4

11

125standard real time PCR is Ambion<sup>TM</sup>, Path-ID<sup>TM</sup> (applied biosystem, cat# 4388644M). The master mix 126was prepared by adding 12.5  $\mu$ l of 2× qPCR Master Mix and 0.5  $\mu$ l of each primer (50 pmol) and 0.125 127 $\mu$ l of probe (30 pmol) and 6.375  $\mu$ l nuclease-free water to adjust the final volume 25  $\mu$ l, finally 5  $\mu$ l of 128the extracted DNA was added. The thermal profile starts with enzyme activation and DNA denaturation 129at 95C° for 10 min. The amplification cycles were done at 95C° for 15 sec. and 57C° for 30 sec., finaly 13072C° for 30 sec.<sup>[2]</sup>(40 cycles) for Brucella species and B. melitensis and B. abortus genotyping. The 131conventional real time PCR was conducted in Stratagene MX30005P thermal cycler machine (Aligent 132Technologies Inc, Santa Clara, CA, USA).

#### 133

#### 1342.3.2. Microchip real time PCR

135The developed & optimized microchips with lyophilized reagents ready to use by Lumex Instruments 136for real-time PCR analyzer AriaDNA <sup>TM</sup> (lumex, Mission, Canada) were used to detect Brucella species. 137Two 25.4x25.4x0.5 mm<sup>3</sup> glass slides consist the microchip; the bottom slide considers the PCR reaction 138chamber while the top slid has a thin heater. In the reaction chamber (bottom slid) there are two different 139size holes; the inlet (2 mm) and outlet (1 mm).<sup>[1]</sup>A total of  $1.2^{[9]}$ µl of DNA (six isolates for each genotype) 140were loaded individually into the reaction chamber through the 2 mm hole. The thermal profile was 141adjusted as follow with fast ramp rate; 80C° for 10 sec. then 94C° for 180 sec. for activation and initial 142denaturation followed by amplification cycles at 94C° for 1 sec for denaturation and 60C° for 30 secs for 143annealing and polymerization for 45 cycles.

#### 144

1452.4. Molecular characterization of the insertion sequence (IS711) by DNA Nucleotide sequencing

146The IS 711 of 12 samples of both **B**. abortus and **B**. melitensis (6 for each genotype) were partially 147amplified by conventional PCR using Phusion<sup>®</sup> High-Fidelity PCR Master Mix with HF Buffer (Thermo 148Fisher Scientific, USA) according to the manufacturer's instructions, using specific primers (Table 1). 149<sup>[15]</sup>he thermal profile as follow;<sup>[3]</sup>he initial denaturation 98C° for 30 secs then the cycling stage began with 150denaturation at 98C° for 10 secs, annealing at 65C° (both genotype) for 30 secs then extention at 72C° 151for 30 secs (35 cycles) and the final extension was at 72C° for 5 min. By using a QIAquick<sup>®</sup> gel 152extraction kit (Qiagen, Gmbh, Hilden, Germany), the positive amplicons were purified. Bigdye<sup>®</sup> 153Terminator V3<sup>[3]</sup> cycle sequencing kit was used to conduct the sequence reactions (PerkinElmer, Foster 154City, CA).<sup>[1]</sup> The sequencing reactions were purified using a DyeEx<sup>®</sup> kit (Qiagen, Gmbh, Hilden,

155Germany) before they were mounted in the Applied Biosystems 3500 xl genetic analyzer machine (Life 156Technologies, Carlsbad, CA, USA).

157

1582.5. Alignment and Phylogenetic Analysis

159The nucleotide sequence was aligned using Bioedit 7.2 software (Hall, BioEdit). Mega 7.0.26 software 160was used to construct a nucleotide phylogenetic tree of the sequenced isolates using the neighbor-joining 161method with 1000 bootstrap (Kumar et al., 2016). The analysis of the sequenced isolates was carried out 162in comparison with different genotypes and biovars retrieved from the Gene Bank, their accession No. 163included within the taxa of the phylogenetic tree.

164

165

167

1663. Results

1683.1. Molecular Detection of the isolated colonies

169A total of 50 Brucella species (16 B. melitensis and 34 B. abortus) were detected using culture and 170biochemical methods in this study. In Saudi Arabia's Al-Qassim province, 25 Brucella species isolates 171(11 B. melitensis and 14 B. abortus). Moreover, 25 Brucella species isolates (5 B. melitensis and 20 B. 172abortus) in Egyptian governorates. The molecular detection of the biochemical identified isolates (50 173isolates) by standard real time PCR revealed that the 44 isolates are positive for Brucella species and 174genotyped as 29 isolates of B. abortus and 15 isolates of B. melitensis (Fig. 1). The genetically 175confirmed isolates by gene sequencing were subjected to detection by microchip real time PCR 176and the comparison between Ct values are shown in table 2.

177

1783.2. Phylogenetic and sequence analysis

179The purified PCR amplicons of the selected positive isolates of 498 bp in case of B. 180abortus (6 isolates) and 733bp in case of B. melitensis (6 isolates) were sequenced 181for insertion Sequence (IS711). The accession Number of the sequenced B. 182melitensis isolates are from ON402790 to ON402795 and The Accession Number of 183the sequenced B. abortus isolates are from ON402796 to ON402801. The all 184partially sequenced isolates of B. abortus are 100% identity with each other, also 185the selected isolates of B. melitensis are 100% identity. The phylogenetic tree 186clustered all the partially sequenced isolates of B. abortus with the same genotype

<sup>187</sup>and all the partially sequenced isolates of B. melitensis with their genotype as <sup>188</sup>shown in figs. 2 and 3. In both trees for B. abortus and B. melitensis the root of <sup>189</sup>tree is B. suis by 5str. CVI73 with accession No. CP054953.1 in gene bank.

### 1904. Discussion

191Brucellosis is a public-health hazard zoonotic disease that causes significant economic losses owing to 192mortality, morbidity, infertility, abortion, medical care costs as a direct consequence or revenue loss and 193vaccination as an indirect effect (Donev, 2010; Khan and Zahoor, 2018). The brucellosis has been an 194endemic disease in middle east for several years (Wareth et al., 2014). In this study, 50 samples were 195isolated from suspected 1051 clinical samples collected from human and different species (cattle, 196buffaloes, sheep, and goats). These positive isolates were molecular detected by both systems; standard 197conventional real time PCR and microchip real time PCR. Forty-four isolates only are positive by real 198time PCR by both systems for general Brucella species detection representing 88% of the biochemical 199characterized isolates. This percent emphasis the specificity of the PCR system used in the current study 200more over, other studies ensured that sensitivity of real time PCR is more than other tests including 201bacterial culture and isolation from clinical samples (Ilhan et al., 2008; Yu and Nielsen, 2010).

As far as our knowledge goes, this is the first study to include microchip real time PCR as a test for 203Brucella species. Microchip real time PCR was positive for all isolates that had been genetically 204characterized by conventional real time PCR, indicating its accuracy. The microchip real time PCR 205offers a less expensive and faster equivalent to the most reliable and sensitive test available today 206(Cojocaru et al., 2021). It engrosses about 30 minutes versus the standard real time PCR that takes 207about 80 minutes and at the same time the Microchip based real time PCR keeps the same gold standard 208in sensitivity qualitatively as it was measured in the current study, the comparison between the cut 209threshold (Ct) of the standard real time PCR and Microchip based real time PCR are listed in table 3 for 210the sequenced isolates only for proper genetic typing of the isolates under ct comparison between both 211real time PCR systems. While the quantitative sensitivity of the Microchip based real time PCR tested 212by other studies (Gill et al., 2018;<sup>211</sup> fong et al., 2019).

However quantitative sensitivity, specificity and limit of detection criteria are required to ensure the 214use of the microchip real time PCR in Brucella species monitoring and genotyping as a gold standard 215alternate in Brucella diagnosis. Regarding to the molecular characterization of the current circulating 216Brucella species, 6 isolates were selected from genotyped B. abortus and another 6 isolates were 217selected from genotyped B. melitensis by real time PCR for molecular characterization by partial

7

20

218sequencing of the insertion sequence IS711. The Insertion sequence (IS711) is a short DNA sequences 219transpose within and between genomes causing genomic rearrangements. It inserts randomly and takes 220genomic locations. It can have used for distinguishing between different isolates and its typing (Halling 221et al., 1993; Mancilla et al., 2011).

The selected partially sequenced 6 isolates of B. abortus isolates are identical and the 6 isolates 223sequenced of B. melitensis are identical too, referring the conservancy of IS711 between sequenced 224isolates. Despite Brucella being a relatively homogenous and ultramonomorphic genus, there were no 225differences between isolates obtained from various animals living in various governorates. (Khan et al., 2262021). Phylogenetically, the 6 isolates of B. abortus isolates were clustered within the B. abortus clade 227and the other 6 isolates of B. melitensis were clustered within its clade as shown in figs 2 & 3. The 228presence of different biovars for the same Brucella type in its clade of the phylogenetic tree indicating 229the limitation of the IS711 sequence to differentiate between subspecies or biovars (Whatmore, 2009). In 230conclusion, the current study spots the light to the urgency of implementation of rapid accurate tests to 231monitor and genotyping of the Brucella species due to its hazard impact on public health and animal 232production and reproduction.

233

234Declaration of competing interests

235There are no conflicts of interest or personal ties that could have influenced the research presented in 236this paper.

237

238

## 239Acknowledgments

240The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of 241Education and Qassim University, Saudi Arabia for funding this research work through the project 242number (QU-IF-1-4-2).

243

## 244Funding

245The Deputyship for Research & Innovation, Ministry of Education and Qassim University, Saudi 246Arabia (the project number: QU-IF-1-4-2).

8

247

#### 248References

23

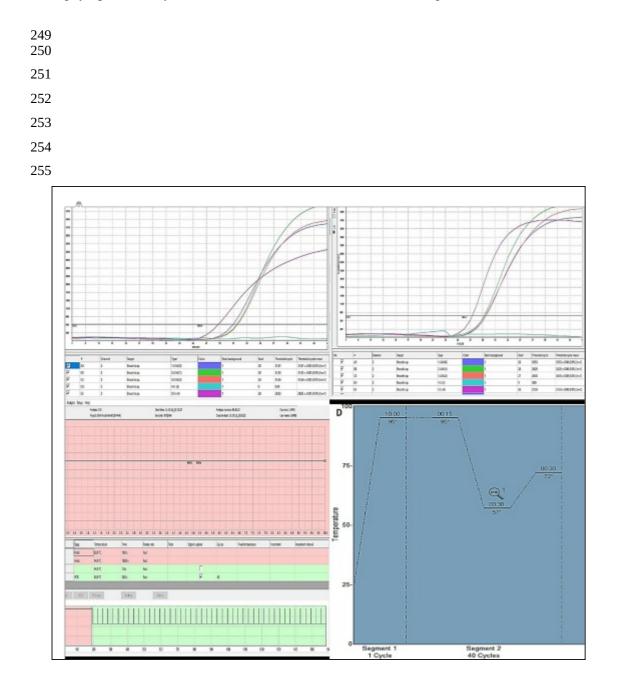
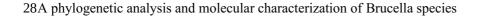


Fig. 1. Shows the amplification plots of microchip based real time PCR for Brucella species. (a) Amplification plot for a group of selected B. abortus isolates. (b) Amplification plot for a group of the selected B. melitensis isolates. (c) The thermal profile used in this study for microchip based real time PCR for Brucella species. (d) Thermal profile used in conventional Real time PCR



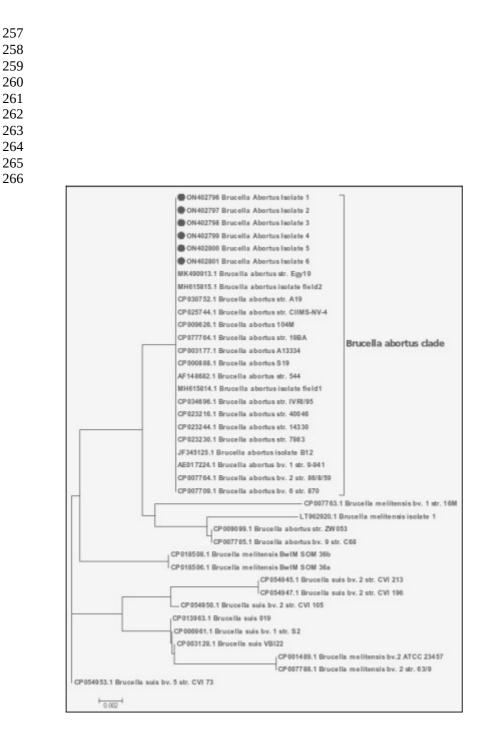


Fig. 2. Phylogenetic tree of the selected isolates (B. abortus) in the study and indicated by filled circle.

| Γ             | I● ON402790 Brucella Melitensis Isolate 1  | 1                                   |                   |
|---------------|--|-------------------------------------|-------------------|
|               | ● ON402791 Brucella Melitensis Isolate 2   |                                     |                   |
|               | ON402792 Brucella Melitensis Isolate 3   |                                     |                   |
| 31A phylog    | enetic analysis and molecular cheracterization of Brucella Melitensi relate 4  | pecies                              |                   |
|               | ON402795 Brucella Melitensis Isolate 6   |                                     |                   |
|               | MK913897 Brucella melitensis str 5794  |                                     |                   |
| evolution     | hary distances were computed using soft Evaluation in the Compo  | site Likelihood me                  | ethod (Tamura et  |
| al. 2004)     | All positions containing gaps att 4909171 Strift and the art of the elim   |                                     |                   |
|               | d in MEGA7 (Kumar et al., 201) HK490046, Brucella melitensis str. EGY20 at the   |                                     | re clustered with |
| B. abortu     | is isolates and other genotypes strains the strain  | NCBI. The acces                     | sion No. of the   |
| sequence      | s were illustrated within the taxa CP058597.1 Brucella melitensis str. CVI 7   |                                     |                   |
|               |  |                                     |                   |
| 267           | CP007760.≹ Brucella melitensis bv. 3 str. Ether<br>CP007788.1 Brucella melitensis bv. 2 str. 63/9  |                                     |                   |
|               | LT963349 Brucella melitensis isolate 1   |                                     |                   |
|               | CP016983.1 Brucella melitensis str. 2008724259   |                                     |                   |
|               | CP007762.1 Brucella melitensis bv. 1 str. 16M  |                                     |                   |
| Fig. 3. Pl    | hylogenetic tree of the selected is the select | he study and indic                  | ated by           |
| filled cire   | cle. The evolutionary history was http://tetallsinitgsithes NEighb   | or-Joining method                   | (Saitou           |
| and Nei,      | 1987). The evolutional states are computed using the state of the stat | he Maximum Cor                      | nposite           |
|               | od method (Tamure and Brupella suppresses, 1 Brupella suppresser, Chip13<br>CP95999, 1 Brupella suppresser, Chip13 positions containing gr   |                                     |                   |
|               | d. Evolutionary analysis average of the contract of the sub of the contract of |                                     |                   |
| shows th      | at the all six isolates with Brucella abortus by 2 st. 86/9/59 B. melitensis iso   | lates and other get                 | notypes           |
| strains th    | at retrieved from NCBI. The accossion Naboutui solate B16  | es were illustrated                 | within            |
| the taxa.     | AE017223.1 Brucella abortus bv 1 str. 9-941<br>CP030751.1 Brucella abortus str. A19  |                                     |                   |
|               | CP007738.1 Brucella abortus str. BFY   |                                     |                   |
| 268           | CP033079.1 Brucella abortus str. BJ1   |                                     |                   |
| 2691 able 1   | CP044338.1 Brucella abortus str. clpP  |                                     |                   |
|               | CP003176.1 Brucella abortus A13334   | C D 11                              | 1 .1              |
| 270The prin   | ters and crossed sources and crossed sources and crossed sources and crossed and the second sources and the second | of Brucella spec                    | es and the        |
| 271differenti | al mentiplex real time PCR for R abortus and B. melitensis   | <mark>s. Th</mark> e table also ill | ustrates the      |
|               |  |                                     |                   |

| 272primers | used in | conventional PC | CR and | sequencing. |
|------------|---------|-----------------|--------|-------------|
|            |         |                 |        |             |

| S | Genotype      |       | Primer Sequence                       | PCR type                                      | Reference             |
|---|---------------|-------|---------------------------------------|---|-----------------------|
| 1 | Brucella      | F     |                                       |   |                       |
|   | species       | R     | GGGTAAAGCGTCGCCAGAAG                  | Rel time                                      |                       |
|   |               | Probe | FAM-AAATCTTCCACCTTGCCCTTGCCATCA-Tamra | PCR   |                       |
|   |               |       |                                       |   |                       |
| 2 | B. melitensis | F     | AACAAGCGGCACCCCTAAAA                  | Multiplex<br>Real time<br>PCR<br>(Genotyping) | (Dal et al.,<br>2019) |
|   | R<br>Pro      | R     | CATGCGCTATGATCTGGTTACG                |   |                       |
|   |               | Probe | FAM-CAGGAGTGTTTCGGCTCAGAATAATCCACA-   |   |                       |
|   |               |       | Tamra                                 |   |                       |
| 3 | B. abortus    | F     | GCGGCTTTTCTATCACGGTATTC               |   |                       |
|   |               | R     | CATGCGCTATGATCTGGTTACG                |   |                       |
|   |               | Probe | HEXCGCTCATGCTCGCCAGACTTCAATG-Tamra    |   |                       |
| 4 | B. melitensis | Bm    | AAATCGCGTCCTTGCTGGTCTGA               | Conventional                                  |                       |
|   |               | IS711 | TGCCGATCACTTAAGGGCCTTCAT              | PCR and                                       | (Che et al.,          |
| 5 | B. abortus    | Ва    | GACGAACGGAATTTTTCCAATCCC              | sequencing                                    | 2019)                 |
|   |               | IS711 | TGCCGATCACTTAAGGGCCTTCAT              |   |                       |

## 274Table 2

275Comparison between real time PCR and Microchips real time according to the Cycle 276Threshold (Ct). The comparison was done to the genetically confirmed 12 isolates 277by sequence.

| Isolates No.  | Brucella species<br>Real time PCR<br>Ct | <mark>B. abortus</mark><br>Real time PCR Ct | <mark>B. melitensis</mark><br>Real time PCR Ct | Microchip<br>Real time<br>PCR Ct. | Genotype      | Accession<br>Number in<br>Gene Bank |
|---------------|---|---|--|-----------------------------------|---------------|-------------------------------------|
| Isolate No. 1 | 31.87                                   | Negative                                    | 32.91  | 29.53                             | B. melitensis | ON402790                            |
| Isolate No. 2 | 30.93                                   | Negative                                    | 31.11  | 29.25                             | B. melitensis | ON402791                            |
| Isolate No. 3 | 30.62                                   | Negative                                    | 30.98  | 29.35                             | B. melitensis | ON402792                            |
| Isolate No. 4 | 32.43                                   | Negative                                    | 33.99  | 33.02                             | B. melitensis | ON402793                            |
| Isolate No. 5 | 21                                      | Negative                                    | 21.51  | 20.16                             | B. melitensis | ON402794                            |
| Isolate No. 6 | 26.14                                   | Negative                                    | 25.00  | 23.34                             | B. melitensis | ON402795                            |
| Isolate No. 1 | 34.86                                   | 32.37                                       | Negative                                       | 31.01                             | B. abortus    | ON402796                            |
| Isolate No. 2 | 33.13                                   | 31.17                                       | Negative                                       | 31.53                             | B. abortus    | ON402797                            |
| Isolate No. 3 | 33.23                                   | 32.94                                       | Negative                                       | 31.66                             | B. abortus    | ON402798                            |
| Isolate No. 4 | 19.65                                   | 15.56                                       | Negative                                       | 15.32                             | B. abortus    | ON402799                            |
| Isolate No. 5 | 17.59                                   | 14.67                                       | Negative                                       | 14.22                             | B. abortus    | ON402800                            |
| Isolate No. 6 | 24.75                                   | 18.53                                       | Negative                                       | 20.43                             | B. abortus    | ON402801                            |