Veterinary Parasitology 261 (2018) 67–72

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Direct detection of Tritrichomonas foetus in cattle genital fluid trough loop mediated isothermal amplification of elongation factor 1 alpha 1

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ARTICLE INFO

Keywords:
Disease
Diagnosis
LAMP
Animal
Venereal
Protozoa

ABSTRACT

Testing for *Tritrichomonas foetus* and exclusion of infected animals is an effective way of improving the reproductive efficiency in a herd. Conventional PCR is inherently more specific than the culture method and quantitative PCR can significantly increase the detection limit. Loop Mediated Isothermal DNA Amplification (LAMP) is gaining interest because the method does not require expensive equipment, specificity and sensitivity can be as high as quantitative PCR. The object of this study was to develop a sensitive and friendly test for point-of-care detection of *T. foetus*. The LAMP test that targeted *T. foetus* elongation factor 1 alpha 1 sequences showed high specificity. Sensitivity was 100–1000 times higher than that reached through culture, polymerase chain reaction or with a previously developed LAMP for 5.8 ribosomal sequences. Moreover, *T. foetus* detection could be performed without DNA purification from infected cervical vaginal mucus (CVM) or smegma samples. The tf-ef1a1 LAMP method was tested for field detection with paper strips soaked in CVM from infected cows and the results were observed 90 min later. Direct detection of *T. foetus* in CVM with the tf-ef1a1 LAMP showed high sensitivity and specificity, and an overall diagnostic odds ratio of 56 (CI: 13.3–235.0). The tf-ef1a1 LAMP showed great potential for diagnosis and control of *T. foetus* in resource-challenged regions.

1. Introduction

Bovine trichomoniasis is a sexually transmitted disease of cattle caused by the flagellate protozoan *Tritrichomonas foetus*. The infection is asymptomatic in bulls and may be perceived in cows that experience pregnancy loss, abortion within 5–7 months of gestation or infertility (Parsonson et al., 1974; Clark et al., 1983, 1986). *T. foetus* is suspected in a herd with prolonged calving intervals and high open rate at pregnancy checking time. The damage predicted in a herd with 20% prevalence of *T. foetus* was shown to be around 14% reduction in an annual calf crop (Rae, 1989).

T. foetus has a worldwide distribution and the incidence of the disease has significantly decreased in regions where artificial insemination is widely practised (Yao, 2013). In countries with extensive farming systems, the systematic approach of trichomonosis control relies upon the identification of infected bulls followed by their removal from the herd (OIE, 2017; Ondrak, 2016).

The diagnosis of *T. foetus* is based on the culture of preputial samples and microscopical examination (Schonmann et al., 1994; Clark and Diamond, 2002). Culture must be followed for seven days and the overall sensitivity is about 70–80 % (Mutto et al., 2006; Cobo et al., 2007). A second sampling and testing procedure after 2–4 weeks increases sensitivity to 80–90% (Yao 2013). A third culture test is mandatory in Texas if a bull comes from a heard of unknown status or when retesting positive animals.

Contamination of preputial samples with fecal flora is a source of saprophytic protozoa such as *Tetratrichomonas* sp. and *Pentatrichomonas hominis* that grow in culture and cause false positive diagnosis (Hayes et al., 2003; Dufernez et al., 2007; Parker et al., 2003; Corbeil et al., 2008). Staining techniques or phase contrast illumination may help in differentiating *T. foetus* from contaminant flagellated protozoa, but such methods are prone to error by the observer (Taylor et al., 1994; Lun et al., 2000).

T. foetus can be detected through Polymerase Chain Reaction (PCR) amplification of ribosomal DNA sequences (rDNA) (Riley et al., 1995; Felleisen et al., 1998; Gookin et al., 2002). Primers TFR1 and TFR2 anneal to sequences found in members of the Trichomonadidae family (Felleisen et al., 1998). The reaction yields a product of variable size that might help to differentiate *T. foetus* from other trichomonads with the assistance of a post-amplification technique such as Restriction Fragment Length Polymorphism (Hayes et al., 2003). The primer pair TFR3-TFR4 allows specific amplification of the 5.8S gene and flanking sequences ITS1 and ITS2 from *T. foetus* (Felleisen et al., 1998). The PCR detection of *T. foetus* takes less than five hours from DNA isolation to the results analysis and it has a similar overall sensitivity to the culture technique (Mutto et al., 2006; Cobo et al., 2007).

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https://doi.org/10.1016/j.vetpar.2018.08.011
Received 22 June 2018; Received in revised form 6 August 2018; Accepted 24 August 2018

Real-time or quantitative PCR (qPCR) combines the high specificity of the end-point PCR with a high sensitivity (McMillen and Lew, 2006). The high sensitivity of q-PCR procedures developed for *T. foetus* detection makes it possible to test a bull in a single sampling procedure and also to pool samples for screening which may help to reduce the cost of detection in expected negative herds (Kennedy et al., 2008; Guerra et al., 2013). However qPCR requires expensive amplification equipment, costly methods for DNA purification and highly trained operators that small laboratories are unable to afford.

Loop-mediated isothermal amplification (LAMP) is a technique developed for specific amplification of known DNA sequences (Notomi et al., 2000). The procedure employs a strand displacement DNA polymerase and four to six primers that recognize six to eight regions on the target DNA respectively, thereby providing a high level of specificity (Notomi et al., 2000; Nagamine et al., 2002; Imai et al., 2007). The LAMP test produces large amounts of DNA under isothermal conditions (60–65 °C) and it can be performed in a simple water bath or thermal block. In addition, a high amount of DNA can be generated in a LAMP reaction which makes it possible to evaluate the results by the naked eye (Iwamoto et al., 2003; Parida et al., 2005; Dukes et al., 2006; Hill et al., 2008). Unlike PCR tests, the LAMP has shown high tolerance to biological fluids, implying that DNA purification might be dispensable (Francois et al., 2011).

Rapid and easy-to-use procedures based in LAMP technique have been designed and are successfully used to detect microorganisms from different biological samples with high sensitivity and specificity in high or low complexity labs (Mori and Notomi, 2009). The benefits of this technique are reflected in public policies suggesting LAMP as an effective way to detect widely distributed common pathogens (Mansour et al., 2015; WHO, 2016).

A LAMP test based on the amplification of the *T. foetus* 5,8 ribosomal DNA sequence (tf-r5.8) was previously designed for *T. foetus* diagnosis (Oyhenart et al., 2013). The specificity of the tf-r5.8 LAMP is similar that of PCR and it shows a slight increment in sensitivity over culture and PCR. The tf-r5.8 LAMP can be performed without specific equipment in less than two hours. Furthermore the tf-r5.8 LAMP performed well with crude contaminated preputial samples, meaning that it can be used at point-of-care with no sample treatment.

The advantages of the qPCR test have shifted the focus on a highly sensitive test for *T. foetus* diagnosis. However, this test cannot be a common practice in resource-challenged regions. The aim of this study was to develop a new highly sensitive LAMP assay to detect *T. foetus*. The sequence of the first homologue encoding for the *T. foetus* elongation factor 1 alpha (EF1 A1) presents itself as an appropriate region for DNA amplification along with well known differences with homologous sequences in related organisms. The work focuses on determining the feasibility of using the tf-ef1a1 LAMP for *T. foetus* detection in untreated biological fluids.

2. Materials and methods

2.1. Culture and identification of Tritrichomonas foetus isolates

Isolates were obtained from diagnostic test cultures from bull smegma that are regularly sent to the laboratory for PCR test discrimination of *T. foetus*. Samples had 0.5–1.0 mL of bull sample fluid inoculated in three mL of liver infusion broth media containing 1 g/L streptomycin, 1,000,000 IU/L ampicillin and 10% v/v heat-inactivated horse serum. After microscopical observation of trophozoites, the isolates were tested through specific PCR amplification using primers TFR1-TFR2 and TFR3-TFR4 as described in Section 2.5. DNA amplicons from every isolate were bi-directionally sequenced using primers TFR1 and TFR2 as described in Section 2.8.

T. foetus B1 strain used in this study is a clone obtained from an isolate from a cow with pyometra (Cobo et al., 2002; Gracia Martinez et al., 2018). Trophozoites were cultured in liver infusion broth and were subcultured every two to three days.

2.2. Animal fluids

Bovine smegma was extracted from five Aberdeen Angus bulls that were aged between three to four years old. The fluid was obtained with a 20-cm long catheter attached to a 10 mL syringe. Ten millilitres of sterile physiological solution (150 mM NaCl) pre-warmed to 30–37 °C was flushed into the preputial cavity, then a gentle massage for five minutes ensured an even wash of the cavity and the liquid was aspirated and placed into sterile test tubes. A 1 mL aliquot was directly inoculated in liver infusion medium placed at 37 °C and inspected daily for seven days for the presence of trophozoites. A 1 mL sample was centrifuged at 13,000 xg for 15 min and the precipitate was resuspended in 100 µL of sterile water and frozen for DNA extraction. Other samples were centrifuged at 7,500xg for five minutes and the supernatant was stored at −20 °C until used in sensitivity tests (Section 2.10). The absence of *T. foetus* in smegma samples was checked by culture, PCR and LAMP.

Cervical vaginal mucus (CVM) samples were collected as previously described (Gracia Martinez et al., 2018). Briefly, young calves were mock treated or received an experimental vaccine containing fixed *T. foetus* cells. Vaccinated and unvaccinated cows were experimentally infected and the rate of clearance of the infection was observed for a period of a 120 days. Samples were obtained using a sterile Cassou pipette. Approximately 0.5 mL were immediately suspended in 3 mL of liver infusion broth media and the remnant was distributed in 5 mL sterile tubes and frozen as soon as possible at −20 °C until PCR or LAMP tests were performed.

2.3. Template DNA preparation

Culture samples were centrifuged for ten minutes at 7500 × g and the pellet was washed twice with a Tris-buffered saline solution (150 mM NaCl in 50 mM, Tris − HCl, pH 7.4). Genomic DNA extraction was performed by a phenol/chloroform method after proteinase K treatment (Sambrook et al., 1989). Ethanol insoluble pellets were suspended in 400 µl of milli-Q water and the concentration was estimated through the absorbance at 260 nm.

2.4. Primer sequences

The sequence of the first homologue encoding for the elongation factor 1 alpha (ef1a1) was chosen for DNA amplification. The reference sequence (GenBank accession number HM217356.1), two other known *T. foetus* homologous sequences for elongation factor 1 alpha (ef1a2 and ef1a3) and similar sequences described in related organisms are shown aligned in supplementary file 1.

LAMP forward and backward inner primers (FIP, BIP), outer primers (F3, B3) and loop forward and backward primers (LF, LB) were designed using the Primer Explorer v3 software (http:/primerexplorer.jp/lamp, Eiken Chemical Co Ltd). Primer sequences for *T. foetus* elongation factor 1 alpha 1 (tf-ef1a1) LAMP were: F3: 5′-TCGCTCTGGAAGTTCG AATC-3′, B3: 5′-TGACGGCGATGATGACTTG-3′, FIP: 5′-CGGCAGCATC AGCTTGTGATGTttAATCATCGATGCTCCAGGAC-3′, BIP: 5′-CAACACG TGGTGGTTTCGAAGCttCGAGGGTGAAAGCAAGAAGA-3′, LF: 5′−CCT GTGATCATGTTCTTGATGAAGT-3′ and LB: 5′-TGGTATCGCTGAACAG GGC-3′.

Primer sequences for *T. foetus* 5.8S ribosomal (tf-r5.8) LAMP were already described (Oyhenart et al., 2013): F3: 5′-CTTGGCTTCTTACAC GATGA-3′; B3: 5′-TCCTATATGAGACAGAACCCTT-3′; FIP: 5′-GTGCAT TCAAAGATCGAACTTGTCGTTTAGAACGTTGCATAATGCGATA-3′ and BIP: 5′-AGCTTGCTAGAACACGCATATATGTTTTTGTTTTTCGCTCTTTT GCTTAA-3′.

PCR primers for recognition of *T. foetus* 5.8S ribosomal and flanking sequences were previously reported (Felleisen et al., 1998; Hayes et al.,

2003): TFR1: 5′-TGCTTCAGTTCAGCGGGTCTTCC-3′; TFR2: 5′-CGGTAGGTGAACCTGCCGTTGG-3′; TFR3: 5′-CGGGTCTTCCTATATGAGACAGAACC-3′ and TFR4:5′−CCTGCCGTTGGATCAGTTTCGTTAA-3′. LAMP and PCR primers were synthesized by Operon (Huntsville, USA).

2.5. Polymerase chain reaction

The primer pair TFR1-TFR2 was used for amplification of DNA from the trichomonad group and the primer pair TFR3-TFR4 for specific amplification of *T. foetus* DNA. PCR reactions were performed in 25 µl containing 10 mM Tris−HCl, pH 8.3, 50 mM KCl, 2.0 mM MgCl2, 0.5 µM each primer (TFR1 and TFR2, or TFR3 and TFR4), 150 µM each dNTP and 2 U of Taq DNA polymerase (PB—L, Argentina). Fifty nanograms of genomic DNA or 2.5 µl of serially diluted samples were used as the template. Amplification comprised 36 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s and extension at 72 °C for 90 s, with a final extension step of seven minutes at 72 °C.

2.6. Loop-mediated isothermal DNA amplification

Desalted primers concentration varied between 0.4–2.4 µM, dNTPs 100–400 µM, betaine 0–1.6 M (N,*N*,*N*-trimethylglycine), and 0–4 mM $MgSO_4$. Closed tube reactions included calcein (25 µM) and $MnCl_2$ (0.25–1.2 mM). LAMP reactions were optimized in a Multigene gradient thermal cycler (Labnet) at 58–70 °C for 0–120 minutes and *T. foetus* detection in fluid samples were performed in a heat block set at 62 °C.

2.7. Detection of amplified DNA products

PCR and LAMP products were resolved in 1–2% agarose gels stained with ethidium bromide and observed under UV light. At the end of PCR amplification 6 µL of loading buffer (50% Glycerol, 11 mM EDTA, 0.017% SDS, 0.015% Bromophenol Blue in 3.3 mM Tris−HCl pH 8.0) added and 12 µL of sample resolved in 2% agarose gels at 10 V/cm for 30 min and stained with ethidium bromide (0.5 µg/ml). PCR products were also resolved in 5% polyacrylamide gels (39:1 methylene-bis-acrylamide in Tris-borate EDTA) (Sambrook et al., 1989). For agarose gel electrophoresis of LAMP products, 4 µL of loading buffer were added to 20 µL of reaction and 2 µL analyzed on agarose gels as indicated. In LAMP reactions containing calcein, DNA amplification was assessed by direct visualization under white, ultraviolet and blue (470 nm) light. An orange to light yellow colour change could be observed under natural light and a green fluorescent signal under ultraviolet or blue illumination was indicative of positive reaction. Alternatively LAMP DNA amplification was ascertained through direct inspection of tubes after addition of SYBR Green I (Sigma Aldrich). Fluorescent signals were obtained after mixing 5 µL of LAMP reaction product with 200 µl of 1/10000 dilution of SYBR Green I.

2.8. DNA sequencing

Gel bands from PCR reactions were sliced and DNA was purified using Wizard SV Gel and PCR clean-up system (Promega). Pure DNA was sequenced with Big Dye Terminator system (Applied Biosystems) in an ABI/Hitachi Genetic Analyzer 3130. The sequences were aligned with target sequences using the Basic Local Alignment Search Tool (Altschul et al., 1990).

2.9. LAMP specificity

A total of 63 parasite strains were tested in order to determine the specificity of the LAMP assay. Positive LAMP reactions were run overnight through 2% agarose gels and the lowest band was sliced for DNA purification and sequencing with F3 or B3 primers.

2.10. LAMP sensitivity

The LAMP sensitivity was determined assaying serial dilutions of intact or lysed cells. *T. foetus* cells in fresh cultures in the exponential growth phase were washed five times in a saline buffered solution (150 mM NaCl, 50 mM Tris HCl, pH 7.4), counted and serially diluted (1/10) in the same buffer. Samples from serial dilution tubes were directly used for seeding culture medium and for artificial contamination of bovine smegma or CVM. For the culture detection of trophozoites 2.0 µl of each dilution were added to 3,0 mL of medium in culture tubes and daily examined for seven days. For artificial contamination or smegma or CVM, 10 µl of each dilution were added to 1,0 mL of fluid. Contaminated fluids were then vortexed for two minutes. Serial dilutions in saline or bovine fluids were tested directly through PCR or LAMP, or heated at 80 °C for ten minutes and stored at −20 °C until use. A volume of 2.0 µl of each dilution was used as the template in LAMP and PCR amplification assays. Results are representative of at least three independent assays.

2.11. LAMP test in field samples

The sensitivity of LAMP was directly assessed in cervical fluid contained in 5 mL plastic tubes. The tip of an FTA paper strip was introduced into the neck of the tube and retained with the plug in an inverted position for ten minutes as shown in Fig. 3. The embedded paper was removed from the tube and the extremity (< 2 mm) was cut by closing the cap of a 0.2 mL thin-wall tube containing 20 µL of tf-ef1a1 reaction. The piece of paper inside the 0.2 mL thin-wall tube was then pushed to the bottom of the tube with a sterile tip and the mix was incubated for 60 min at 62 °C. After the indicated time, *T. foetus* DNA amplification was ascertained by migration in agarose gels as indicated in Section 2.7.

3. Results

3.1. Tritrichomonas foetus elongation factor 1 alpha 1 (EF1 A1) loop mediated isthermal amplification

LAMP reactions targeting the *T. foetus* elongation factor 1 alpha gene 1 (tf-ef1a1) showed amplification between 58 °C and 66 °C (Fig. 1A). Control reactions with *T. foetus* 5.8S ribosomal (tf-r5.8) DNA primers showed products of amplification between 58–64 °C with the strongest signal at 62 °C. The temperature for tf-ef1a1 LAMP reactions and for control tf-r5.8 LAMP was therefore set to 62 °C in order to make simultaneous comparisons.

LAMP control reactions with no DNA performed in absence of betaine showed faint calcein fluorescence signal. In order to increase the contrast between positive and negative samples, betaine concentration was varied between 0–1.6 M. The betaine concentration that showed the highest signal to noise ratio was 0.8 M. Finally, 8.0 mM Mg^{++} concentration showed non-specific amplification while a specific signal and efficient Bst DNA polymerase amplification was observed at ≥2.0 mM Mg_2Cl (Fig. 1B).

The time required for the amplification of 10 ng of DNA of T. fetus with ef-1α LAMP was 30 min while reactions of tf-r5.8 LAMP showed amplification in 90 min (Fig. 1C). Meanwhile, calcein containing tubes excited with UV or blue LED light showed fluorescent signals after 45–60 minutes in tf-ef1a1 LAMP reactions and 100–120 minutes were necessary for tf-r5.8 LAMP.

The optimized conditions for LAMP reaction contained 1.6 µM of each FIP and BIP primers, 0.8 µM of each LF and LB primers, 0.2 µM of each F3 and B3 outer primers, 200 µM each dNTP, 0.8 M betaine (Sigma), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1%v/v Triton X-100 and 10U Bst DNA polymerase in 20 mM Tris−HCl, pH 8.8. The reaction time was set to 60 or 90 min at 62 °C and the enzyme was inactivated by increasing the temperature to 80 °C for ten minutes at the

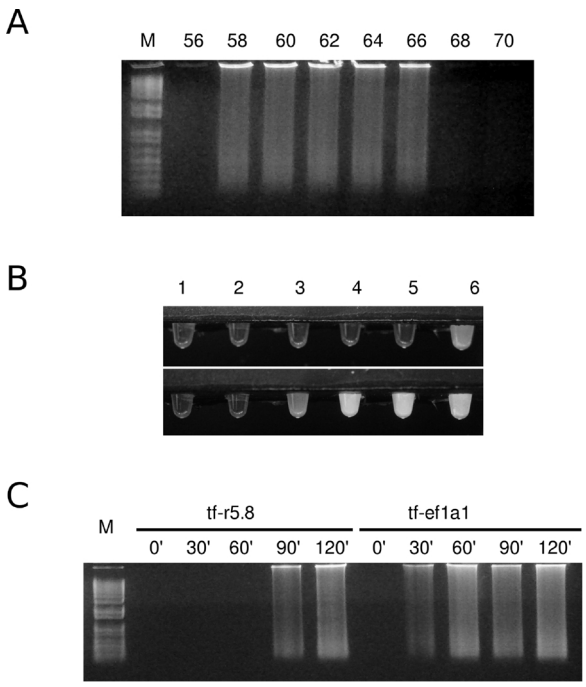

Fig. 1. Loop mediated isothermal amplification of *Tritrichomonas foetus* elongation factor 1 alpha 1. A) Gel detection after DNA amplification with tf-ef1a1 in a temperature range from 56 to 70 °C. B) Direct observation of calcein fluorescence in tf-ef1a1 LAMP reactions containing variable concentrations of magnesium chloride. Tubes 1 to 6 contained 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 M of MgCl2 respectively. Reactions in the tubes on the top lane were performed in absence of *T. foetus* DNA while bottom tubes contained 10 ng of *T. foetus* B1 DNA. C) Time course for detection of DNA amplification with 5.8 ribosomal primers (left) or elongation factor 1 alpha 1 primers (right). The time in minutes is shown on top of the figure. M: 50-bp ladder.

end of the reaction.

3.2. Specificity of the tf-ef1a1 LAMP

The tf-ef1a1 LAMP technique was used to amplify DNA from samples containing motile protozoans commonly mistaken by *T. foetus*. Results from ef-1α LAMP were in agreement with results from tf-r5.8 LAMP and TFR3-TFR4 PCR. A positive result was obtained when tested samples contained *T. foetus* (n = 56) while no amplification was observed from samples with *Pentatrichomonas hominis* (n = 16) or *Tetratrichomonas* spp. (n = 22).

3.3. Sensitivity of the tf-ef1a1 LAMP

The sensitivity of the LAMP assay was analyzed through detection of DNA amplification after incubation with variable amounts of purified DNA or with different numbers of cells in a saline solution. The concentration of DNA necessary to give a positive pattern in gel with the tf-ef1a1 LAMP was 100-fold higher than that necessary to show amplification products with tf-5.8 r LAMP or TFR3-TFR4 PCR. With serial dilutions of intact cells in saline solution or culture medium, the tf-ef1a1 LAMP also showed a better performance than tf-5.8 r LAMP and TFR3-TFR4 PCR. Positive reactions were obtained in two additional dilutions meaning that sensitivity was 100 times higher.

3.4. Direct detection of Tritrichomonas foetus in bovine genital fluids

Cervical vaginal mucus (CVM) and smegma with trophozoites were tested for direct amplification of DNA. The tf-ef1a1 LAMP test showed 100–1000 times more sensitive than tf-5.8 r LAMP, TFR3-TFR4 PCR and culture in both fluids (Fig. 2). The tf-5.8 r LAMP showed no difference over TFR3-TFR4 PCR in smegma (Fig. 2A). However, DNA from *T. foetus* cells suspended in CVM was detected at a high dilution in tf-ef1a1 LAMP, while the tf-5.8 r LAMP showed signal from 50,000 cells/mL and the PCR signal was absent 500000 cells/mL (Fig. 2B). The culture test gave positive results starting at 5000 cells/mL while the minimal quantity of cells that were detected with tf-ef1a1 LAMP was around 5 cells/mL.

Because of the highest sensitivity demonstrated by the tf-ef1a1

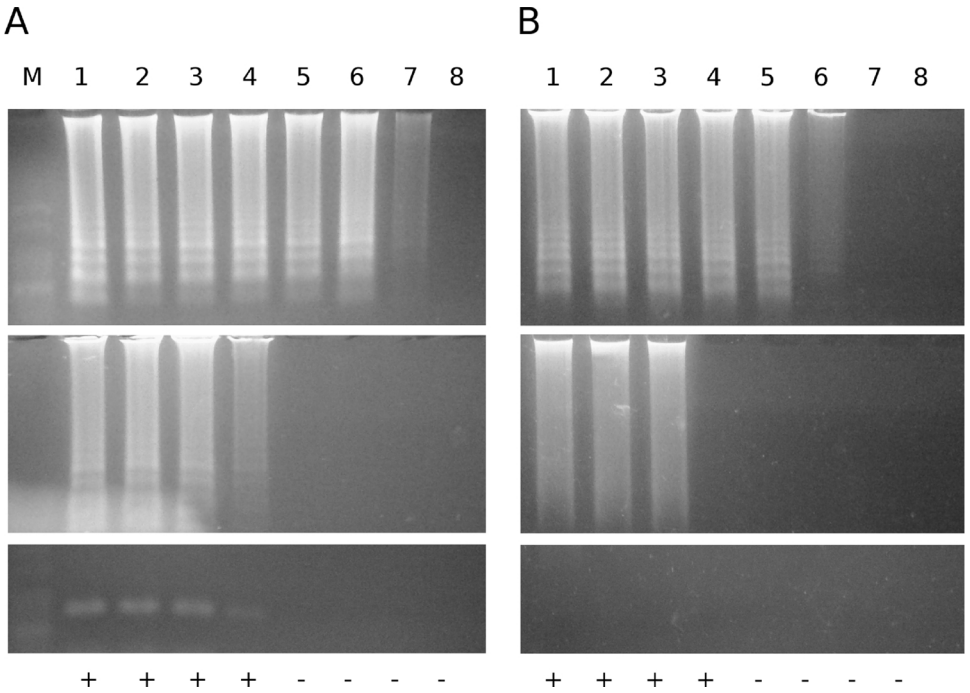

Fig. 2. Sensitivity of the tf-ef1a1 LAMP. Washed trophozoites serially diluted in smegma (left) or cervical-vaginal mucus (right). LAMP with elongation factor 1 alpha 1 primers (top), 5.8 ribosomal primers (middle) or PCR with primers TFR3 and TFR3 (bottom). Cuture results for the same dilutions are shown below (+ = positive, - = negative). From 1–8 dilutions are 500000, 50,000, 5000, 500, 50, 5, 0.5 and 0.05 trophozoites/mL.

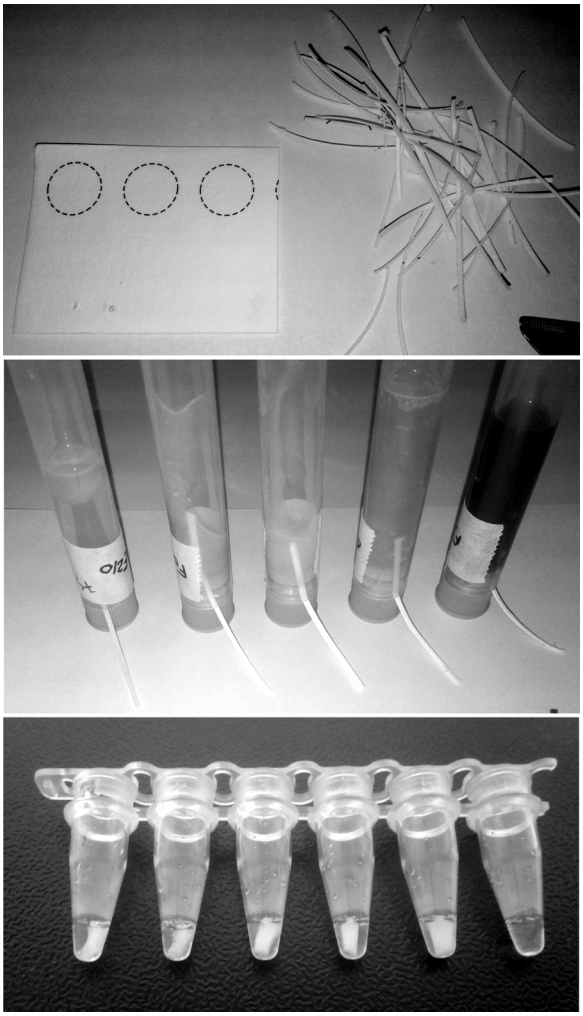

Fig. 3. Direct detection of *T. foetus* DNA with the tf-ef1a1 LAMP. FTA paper strips (top) were introduced into plastic tubes containing cervical vaginal samples and held in place with the pressure cap. The tubes were kept in a inverted position (middle) for ten minutes. The tip of the paper was then put into a tube containing the LAMP reaction for *T. foetus* elongation factor 1 alpha detection (bottom).

Table 1
Detection of Tritrichomonas foetus in cervical vaginal samples. Comparison of culture and direct tf-ef1a1 LAMP results.

		Culture		
		Positive	Negative	Total
tf-ef1a1 LAMP	Positive	28	7	35
	Negative	3	42	45
Total		31	49	

LAMP and the apparent absence of inhibition of DNA amplification in bovine genital fluids the test was used for direct amplification of *T. foetus* DNA from field samples. Paper strips were embedded in CVM extracted from infected and non-infected cows and were directly used for amplification under isothermal conditions (Fig. 3). As detailed in Table 1, from a total of 80 samples tested, 35 were positive tf-ef1a1 LAMP including 28 with a positive culture. Meanwhile, DNA amplification was negative in 45 samples that included 42 by culture. According to these values tf-ef1a1 LAMP had 80% (28/35) positive predictive value and 93,3% (42/45) negative predictive value. Calculated sensitivity was 0.90 (CI: 0.75-0.96) and specificity was 0.85 (CI: 0.73-0.92). The diagnostic odds ratio was estimated to be 56.0 (CI: 13.341–235.0) meaning that the test is discriminating correctly.

4. Discussion

The control of bovine trichomoniasis is based on the identification of *T. foetus*. The method most commonly used consists of the cultivation and observation of the microorganism. This is a relatively simple technique although of low sensitivity, low specificity, and in addition requires one week for the delivery of results (Cobo et al., 2007). Repeated sampling and analysis with a minimum interval of 1 week allows the sensitivity to increase to ~ 90%. However the occurrence of false positives is still high.

The PCR approach based on the amplification of *T. foetus* ribosomal DNA sequences provides a rapid diagnostic tool and specificity is higher than culture tests (Felleisen et al., 1998). The sensitivity of standard PCR *T. foetus* detection is comparable to the culture method (Cobo et al., 2007). The sensitivity of PCR for *T. foetus* diagnosis can be highly increased through q-PCR (McMillen and Lew, 2006; Dufernez et al., 2007). However q-PCR requires highly trained operators and expensive equipment and reagents.

A LAMP test based on the amplification of the *T. foetus* 5.8S ribosomal DNA sequence was previously designed (Oyhenart et al., 2013). It showed a slight increase in sensitivity over the ongoing culture method and PCR test. Moreover, the tf-5.8 r LAMP test made it possible to directly detect *T. foetus* DNA in smegma in a laboratory with low complexity equipment.

Recent sequencing projects have provided more information into expressed and genomic sequences and have made possible to develop new diagnostic tools. A single copy of the elongation factor alpha 1 gene is predicted per *T. foetus* genome (Malik et al., 2011; Noda et al., 2012). The ef1a1 was chosen for DNA amplification through LAMP because the GC content was between the limits for primer prediction and because several nucleotide changes were present in the alignment with homologous genes in related organisms.

The tf-ef1a1 LAMP assay sensitivity was superior to that reached by the tf-5.8 r LAMP or with the PCR test in every condition tested. Moreover, the tf-ef1a1 LAMP was able to amplify *T. foetus* DNA present in infected CVM. The experience was carried out by simply embedding a fragment or FTA paper in cervical fluid and DNA amplification proceeded after inclusion of this paper in the LAMP mix. Because of the minimal sample preparation, the high sensitivity achieved and the low need for equipment the tf-ef1a1 LAMP presents itself as a useful application for an instrument-free readout.

Acknowledgments

This study was supportedby CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) and the Faculty of Exact and Natural Sciences of the National University of La Pampa. Special thanks to Marie Oudot.

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