



Improved DNA extraction technique from blood clot for diagnosis of *Trypanosoma evansi* in camels

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Abstract

The detection of *Trypanosoma evansi* genetic material in clinical samples is considered an important diagnostic tool. When blood clots can be a valuable source of genetic information. In our paper, Forty blood samples were collected from camels at Cairo abattoir in plane tubes. Blood clots were dispersed by scissor and treated by lysis buffer and proteinase-K, then DNA was extracted according to protocol of Gene JET Whole Blood Genomic DNA Purification Mini Kit. The quality and integrity of the extracted DNA were corroborated by agarose gel electrophoresis and conventional PCR. Results confirmed that DNA purified from clotted blood can be used in further amplifications. Moreover 16 out of 40 samples (40%) were positive in PCR with bands at the expected molecular weight (164pb). The objective of the present study was to improve and develop a more sensitive method to recover *T. evansi* DNA from clot samples.

Key words: *Trypanosoma evansi*, Blood clot, DNA, PCR.

Introduction

Trypanosoma evansi is enzootic in camels in Egypt, and bovine hosts act as efficient reservoir for camel infection (Hilali et al., 2004; Elhaig et al., 2013). Although *T. evansi* infects a wide diversity of mammalian hosts but camels, horses and dogs remain the most perilous hosts for this parasite (Fernandez et al. 2009; Desquesnes et al. 2013; Rjeibi et al. 2015). Economical and medical impacts of *T. evansi* has donated by its capability to spread mutely via healthy carriers. Although *T. evansi* is inapparent in most cases, the parasite affects livestock productivity causing mortality, reduced animal production and reproduction performance and low carcass quality (Reid 2002; Desquesnes et al. 2013). Nucleic acid-based assays are considered to be the most effective tools for the detection of *T. evansi* in several animals and vectors (Sukhumsirichart et al. 2000). Moreover, polymerase chain reaction (PCR) based assays give chance for parasite identification at levels underneath the detection limit of other parasitological techniques. Several factors should be kept to adjust PCR sensitivity, amount and quality of extracted DNA is one of the most precarious factors (Gonzales et al. 2006). So, extraction methods of DNA with high-quantity, besides high-quality, is a critical stage for further molecular assays (Mardan-Nik et al, 2019). In the past, clotted blood was normally discarded even though it is considered a great source for DNA, so that some methods had optimized DNA extraction from blood clot (Zakaria et al. 2013). Blood clot should be physically smashed and dissolved prior DNA extraction, to improve the quality and quantity of DNA yield (Wong et al. 2007; Xu et al. 2010). Some techniques used mesh with centrifuge after separation in serum-separator tubes (Xu et al. 2010). Other techniques used scalpels for dispersing clotted blood (Siafakas et al.

1995). The aim of the present study is to develop a simple, safe, and efficient technique for dissolving of the blood clot before DNA extraction, furthermore using DNA yield in subsequent conventional PCR, to ensure the efficacy of our technique.

Material and Methods

A total number of 40 blood samples were collected from camels at Cairo abattoir in a plane tubes. Samples were clotted and transferred to Parasitology laboratory at Parasitology Department, Faculty of Veterinary Medicine, Alexandria University.

Blood Clot Treatment Before DNA Extraction:

Each blood clot was added into a petri dish then; it was dispersed using a sterilized scissor into small parts. Dispersed blood clots were returned back to blood collecting tube with addition of 1.5 ml of Blood lysis buffer and 20µl of proteinase K. Then they were left overnight at room temperature. In the next day, clots were completely dissolved and became ready for DNA extraction.

DNA Extraction

DNA was extracted following the protocol of Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermo scientific California). Briefly, Twenty µl of proteinase K solution were added to 200µl of dissolved blood, mix by vortexing, then 400µl of lysis solution were added, mixed thoroughly by vortexing to obtain a uniform suspension. The sample was then incubated at 56°C for 10 minutes while using shaking water bath. 200µl of ethanol (96-100%) were added and mixed by pipetting. The prepared mixture was transfered to the spin column, centrifuged for 1 minute at 6000 xg (about 8000rpm). The collection tube containing the flow-through solution was discarded. The column was placed into a new 2ml collection tube. 500µl of wash buffer1 (with ethanol added) were added, centrifuged for 1 min at 8000 xg (about 10000 rpm). The

flow-through was discarded and the column was placed back into the collection tube. 500µl of wash buffer 2 (with ethanol added) was added to the column, centrifuged for 3 min at maximum speed ($\geq 20000xg$, $\geq 14000rpm$). The collection tube containing the flow-through solution was discarded. The column was transferred to a sterile 1.5 ml micro centrifuge tube. 200µl of elution buffer were added to the centre of the column membrane to elute genomic DNA. The tubes were incubated for 2 min at room temperature and centrifuged for 1 min at 8000xg (about 10000 rpm). Extracted genomic DNA was stored at $-20^{\circ}C$ till used.

Evaluation of Extracted DNA

Extracted DNA was evaluated by Agarose gel electrophoresis and conventional PCR.

Agarose Gel Electrophoresis

In order to assess DNA degradation and the molecular weight of the DNA, gel electrophoresis was performed by loading 5µL of extracted DNA on 1.2% agarose gels prepared in 0.5X TBE buffer and using a 100 bp marker ladder (Thermo scientific).

Conventional PCR

PCR was carried out for amplification of 164 bp by using minichromosomesatellite DNA, subgenus trypanozoon specific primers, TBR 1/2 (TBR-1 Forward 5'-GAATATTAACAATGCGCAG-3; TBR-2Reverse5' CCATTTATTAGCTTTGTTGC-3'). The primer was used at a concentration of 10 pmol/µl. The PCR protocol was according to Herrera et al (2005) with some modifications. Briefly, the PCR amplification was performed in a total reaction volume of 25 µl containing: 12.5 µl Dream Taq Green master mix (Thermo Scientific), 1.5 µl DNA template, 1µl of each primer and was completed with nuclease free water. The samples were thermo cycled in 3Prime thermal cycler (TECHNE, UK) with initial denaturation at $95^{\circ}C$ for 3 min. then 35 cycles of denaturation at $95^{\circ}C$ for 30 seconds,

annealing at $50^{\circ}C$ for 30 seconds and extension at $72^{\circ}C$ for 30 seconds with a final extension at $72^{\circ}C$ for 7 min.

Results

Agarose gel electrophoresis showed intact bands for DNA (Fig. 1). Results of conventional PCR confirmed that DNA purified from clotted blood can be used in further amplifications. 16 out of 40 samples (40%) were positive with bands at the expected molecular weight (164bp) (Fig. 2).

Fig (1): Gel containing intact DNA bands

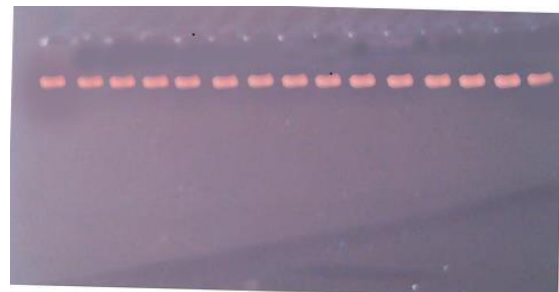
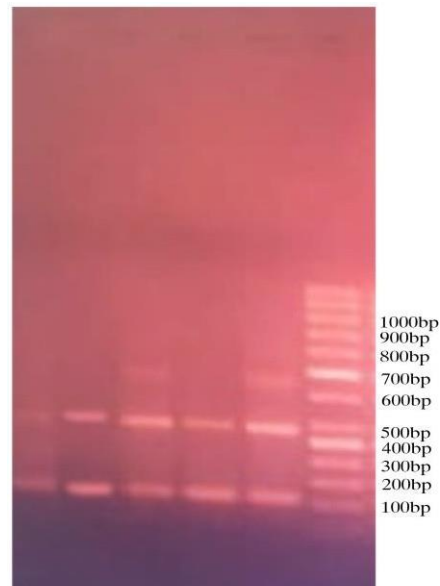


Fig (2): Ethidium bromide stained gel of PCR for *T.evansi* at 164bp showing repetitive nature of this gene



Discussion

Molecular diagnostic techniques are considered critical tools for the detection of *T.evansi* infection globally due to their accuracy and reliability. (Desquesnes & Davila, 2002; Sengupta et al. 2010). In the present study, we have developed and improved a rapid, easy technique for DNA extraction from clot samples. The technique is based on the initial disruption of clot using a scissor, lysis buffer and proteinase K, followed by the internationally standardized DNA extraction protocol for the molecular diagnosis of *T.evansi* in camels. DNA yield was highly purified with enough amounts to be used in molecular techniques. Moreover, the extracted DNA was used for detection of *T evansi* using conventional, which revealed infection rate of 40%. Our results agreed with that of De Abreu et al. (2018) who used the extracted DNA from clotted blood in diagnosis of *Plasmodium* spp. in human and non-human primates from the Brazilian Atlantic Forest. Moreover, Mayta et al. (2019) added that DNA extraction of clot samples showed better sensitivity than either whole blood or buffy coat in diagnosis of *T cruzi*. In addition to, Lundblom et al (2011) confirmed that high speed shaking of frozen blood clot is an efficient method to extract DNA for detection of *P. falciparum* parasites and the human thalassaemia gene. Frozen archived blood clots can be used as a source of DNA in cases where serum is routinely collected for clinical tests. (Bank et al, 2013). Zakaria et al (2013) developed an alternative method to extract DNA and RNA from clotted blood using sonication with QIAamp spin column for use in various genomic and molecular investigations. Moreover, Adkin et al (2002) obtained high quality DNA from 15 individual

clotted blood samples used for single nucleotide polymorphism genotyping.

Conclusion

In conclusion, blood clots can be a valuable source of genetic information, optimizing costs, handling time and reducing the amount of blood collected from each subject. Thereby, the creation of a blood clot bank could aid the resolution of several ecological, evolutionary and epidemiological issues.

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References:

- Adkins, K.K.; Strom, D.A.; Jacobson, T.E.; Seemann, C.R.; O'Brien, D.P. and Heath, E.M. (2002):** Utilizing Genomic DNA Purified From Clotted Blood Samples for Single Nucleotide Polymorphism Genotyping. Arch. Pathol. Lab. Med., 126: 266–270.
- Bank, S.; Nexø, B.A.; Vibeke Andersen, V.; Vogel, U. and Andersen, P.S (2013):** High-Quality and-Quantity DNA Extraction from Frozen Archival Blood Clots for Genotyping of Single-Nucleotide Polymorphisms. Genet. Test Mol. Biomarkers.17(6): 501-503.
- De Abreu, F.V.S; Gomes, L.R.; Mello, A.R.L.; Bianco-Junior, C.; de Pina-Costa, A.; Santos, E.D.; Teixeira, D.S.; Brasil, P.; Daniel-Ribeiro, C.T.; Lourenco-de-Oliveira, R. and Ferreira-da-Cruz, M.D. (2018):** Frozen blood clots can be used for the diagnosis of distinct *Plasmodium* species in man and non-human primates from the Brazilian Atlantic Forest. Malar .J., 17: 338.

Desquesnes, M. and Davila, A.M.R. (2002): Applications of PCR-based tools for detection and identification of animal trypanosomes: A review and perspectives', *Vet. Parasitol.*, 109(3–4): 213–231.

Desquesnes, M.; Holzmuller, P.; Lai, D.; Dargantes, A.; Lun, Z. and Jittaplapong, S. (2013): *Trypanosoma evansi* and Surra: A review and

perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomed. Res. Int.* ? :1–22.

Elhaig, M.; Youssef, A. and El-Gayar, A. (2013): Molecular and parasitological detection of *Trypanosoma evansi* in Camels in Ismailia, Egypt, *Vet. Parasitol.*, 198(1–2): 214–218.

Fernandez, D.; Gonzalez-Baradat, B.; Eleizalde, M.; Gonzalez-Marcano, E.; Perrone, T. and Mendoza, M. (2009): *Trypanosoma evansi*: A comparison of PCR and parasitological diagnostic tests in experimentally infected mice, *Exp. Parasitol.*, 121(1): 1–7.

Gonzales, J. L.; Loza, A. and Chacon, E. (2006): Sensitivity of different *Trypanosoma vivax* specific primers for the diagnosis of livestock trypanosomosis using different DNA extraction methods. *Vet. Parasitol.* 136: 119–126.

Herrera, H. M.; Norek, A.; Tatiana P. T.; Freitas; Rademaker, V.; Fernandes, O. and Jansen, A. M. (2005): Domestic and wild mammals infection by *Trypanosoma evansi* in a pristine area of the Brazilian Pantanal region. *Parasitol. Res.*, 96: 121–126.

Hilali, M., Abdel-Gawad, A., Nassar, A., Abdel-Wahab, A., Magnusb, E. & Büscher, P. (2004): Evaluation of the card agglutination test (CATT/*T. evansi*) for detection of *Trypanosoma evansi* infection in water buffaloes (*Bubalus bubalis*) in Egypt, *Vet. Parasitol.*, 121(1–2): 45–51.

Lundblom, K.; Macharia, A.; Lebbad, M.; Mohammed, A. and Färnert, A. (2011): High-speed shaking of frozen blood clots for extraction of human and malaria parasite DNA. *Malar. J.*, 10: 229.

Mardan-Nik, M.; Soflaei, S.S.; Biabangard-Zak, A.; Asghari, M.; Saljoughian, S.; Tajbakhsh, A.; Meshkat, Z.; Ferns, G.A.; Pasdar, A. and Ghayour-Mobarhan, M. (2019): A method for improving the efficiency of DNA extraction from clotted blood samples. *J. Clin. Lab. Anal.*, ? :1-7

Mayta, H.; Romero, Y.K.,; Pando, A.; Verastegui, M.; Tinajeros, F.;Bozo, R., Henderson-Frost, J.; Colanzi, R.; Flores, J.; Lerner, R.; Bern, C. Gilman, R.H. (2019): Improved DNA extraction technique from clot for the diagnosis of Chagas disease. *PLoS Negl. Trop. Dis.*, 13(1): 1-11.

Reid, S.A., (2002) *Trypanosoma evansi* control and containment in Australasia. *Trends Parasitol.*, 18(5): 219–224.

Rjeibi, M.R.; Ben Hamida, T.; Dalgatova, Z.; Mahjoub, T.; Rejeb, A. and Dridi, W. (2015): First report of surra (*Trypanosoma evansi* infection) in a Tunisian dog. *Parasite*, 22(3): 1-4.

Sengupta, P.P. Balumahendirana, M.; Suryanaryanab, W.; Raghaven-dra, A.G.; Shomea, B.R. and Gajendragada, M.R. (2010): PCR-based diagnosis of surra-targeting VSG gene: Experimental studies in small laboratory rodents and buffalo. *Vet. Parasitol.*, 171(2): 22–31.

Siafakas, N.; Burnett, L. and Bennetts, B. (1995): Non enzymatic extraction of DNA from blood collected into serum separator tubes. *Clin. Chem.*, 41: 1045- 1046.

Sukhumsirichart, W.; Khuchareonaworn, S.; Sarataphan, N.; Viseshakul, N.; and Chansiri, K. (2000): Application of PCR-based assay for diagnosis of *Trypanosoma evansi* in different animals and vectors. *J. Trop. Med. Parasitol.*, 23: 1–6.

Wong, S.; Kuei, J.J., and Prasad, N. (2007): A simple method for DNA isolation from clotted blood extricated rapidly from serum separator tubes. *Clin. Chem.*, 53: 522- 524.

Xu, R.; Ye, P.; Luo, L.; Wu, H.; Dong, J. and Deng, X. (2010): A simple and efficient method for DNA purification from samples of highly clotted blood. *Mol. Biotechnol.*, 46: 258- 264.

Zakaria, Z.; Umi, S.H.; Mokhtar, S.S.; Mokhtar, U.; Zaiharina, M.Z.; Aziz, A.T.A. and Hoh, B.P. (2013): An alternate method for DNA and RNA

extraction from clotted blood. Genet. Mol.
Res. (GMR). 12: 302-311.

المخلص العربى

طريقة مطورة استخالص الحمض الزوى من الدم المتجلط لتشخيص التريپانوسوما ايفانسى فى الجمال

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يُنْبَر وجود المادة الوراثية للتريپانوسوما ايفانسى فى العينة وسيلة جيدة للتشخيص. بينما يعبر الدم المتجلط مصدر جيد للمادة الوراثية. نفى هذه الدراسة تم تاجمىع 40 عينة من دم الجمال اثناء عملية الدبح بمجزر القاهرة فى انابيب خالية من مضادات التجلط للكشف عن وجود طفيل التريپانوسوما ايفانسى. تم اخذ الدم المتجلط وتغطيه بالمقصر ومعامله بالليسيز باثر والبروتينازك وتركه طول الليل , ثم عمل استخالص للحمض الزوى من العينة بانواع التعليمات فى الكيت الخاصة باستخالص الحمض الزوى من الدم. ثم تم توييم الحمض الزوى المسخالص من الدم المتجلط عن طريق تمريره على جل فى جهاز اللكتروفوريسز وزنج عنه وجود حمض زوى جيد ف كل العينات. ثم عمل تفاعل البلمرة المتسلسل والذى اسفر عن وجود المادة الوراثية للتريپانوسوما ايفانسى فى 16 عينة من اجمالى 40 عينة بنسبة 40% عزد الوزن الجزيئى الخاص بالتريپانوسوما ايفانسى (164 بيز بيير). الهدف من هذه الدراسة هو تطوير طريقة جديدة بسيطة وحساسة لمتخراج الحمض الزوى للتريپانوسوما ايفانسى من الدم المتجلط.