ELSEVIER

Veterinary Parasitology 89 (2000) 129–132

veterinary parasitology

www.elsevier.com/locate/vetpar

Short communication

Comparative in vitro isolation of *Trypanosoma theileri* from cattle in Belgium

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Received 2 August 1999; received in revised form 12 November 1999; accepted 25 November 1999

Abstract

Ten blood samples randomly collected from cows on a farm nearby Antwerp, Belgium, were inoculated into KIVI culture medium (Kit for In Vitro Isolation of trypanosomes) and RPMI 10%+feeder medium. Within 3 weeks of incubation all KIVI cultures and four RPMI 10%+feeder revealed presence of *Trypanosoma theileri*. Some practical implications regarding the use of KIVI for isolation of pathogenic African trypanosomes from cattle and other Bovidae are discussed.

Keywords: *Trypanosoma theileri*; In vitro culture; Diagnosis; Cattle-Protozoa

1. Introduction

In cattle, the infection of *Trypanosoma* (*Megatrypanum*) *theileri* may persist for many years. Parasitaemia is often so low that it is only detectable by cultivation of blood samples (Dirie et al., 1990). Cultivation of the trypanosomes at 37°C has been reported in non defined or partially defined monophasic liquid media containing erythrocytes or erythrocyte products, in embryonal chicken eggs and in various types of cell cultures (Wells, 1971; McHolland-Raymond et al., 1978). The latter authors reported that *T. theileri* can be cultured at room temperature in several culture media such as veal infusion or blood agar.

The KIVI culture system (Kit for In Vitro Isolation of trypanosomes, Aerts et al., 1992) has been designed for the isolation of pathogenic African trypanosomes, particularly

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0304-4017/00/$ – see front matter
PII: S0304-4017(00)00191-6

T. brucei gambiense. Cell division starts after transformation into procyclic forms. The tool has been proven useful for detecting *T. brucei* and *T. congolense* in man or animals with low parasitaemia (Truc et al., 1992, 1994, 1997; Komoin-Oka et al., 1994; McNamara et al., 1995; Truc, 1996). Rosewell Park Memorial Institute (RPMI) based culture systems have been described for culturing bloodstream forms of *T. b. brucei* (Hirumi et al., 1980).

In Western Europe, *T. theileri* is the only trypanosome species occurring in cattle. In an attempt to isolate a stock of *T. theileri*, 10 blood samples from Belgian cattle were inoculated into both RPMI 10%+feeder and KIVI culture medium.

2. Materials and methods

2.1. Blood samples

Sampling was done at the end of June 1996 on a small farm nearby Antwerp, Belgium, by bleeding from the tail vein. Blood (10 ml) was taken from each of 10 different 1–3 year old Holstein cows chosen at random. As prescribed for KIVI, the anticomplementary anticoagulant Liquoid® (Roche) was added to a final concentration of 5% (v/v).

2.2. KIVI

KIVI (Aerts et al., 1992) is a ready-for-use kit allowing direct inoculation of blood into vials with culture medium.

2.3. RPMI 10%+feeder

RPMI medium 1640 containing 25 mM 4-(2-hydroxyethyl)-1 piperazine ethanesulfonic acid (HEPES), 200 mM L-glutamine, 50 mg/ml gentamycin and 10% foetal calf serum was supplemented with a feeder layer of murine splenic cells 24 h before inoculation. Reiter et al. (1987) previously reported growth of *T. theileri* on similar culture systems.

2.4. Inoculation and follow-up of the cultures

Part of each blood sample (5 ml) was put aseptically into a KIVI vial. KIVI vials were kept in the dark at 24°C. The remaining 5 ml of blood was centrifuged at $1000 \times g$ for 10 min, the plasma layer removed and the buffy coat transferred to a cell culture flask (25 cm^2, Nunclon^{TM} Delta Flasks) with 4 ml RPMI 10%+feeder. RPMI cultures were incubated and exposed to 5% CO_2 gassing at 37°C. Cultures were checked on Days 6, 9, 13 and 21. KIVI manipulations took place in a laminar flow workstation. Samples were taken from the vial with an autoclaved and flamed sterilized pipette. A drop was put on a microscope slide, covered with a cover slide and examined under the microscope at 10×25 magnification. RPMI vessels were monitored directly under a reversed microscope at 10×10 magnification. Cell culture media were not replaced during the period of the experiment (21 days).

Table 1
Presence of *Trypanosoma theileri* in KIVI and RPMI cultures in function of the day post inoculation

Sample	Days post inoculation KIVI					Days post inoculation RPMI				
	D0	D6	D9	D13	D21	D0	D6	D9	D13	D21
1	–[a]	+[b]	+	+	+	–	+	–	–	–
2	–	–	+	+	+	–	–	–	–	–
3	–	–	+	+	+	–	–	–	–	–
4	–	–	–	–	+	–	–	–	–	–
5	–	+	+	+	+	–	–	–	–	–
6	–	+	+	+	+	–	+	–	–	–
7	–	+	+	+	+	–	+	+	+	–
8	–	–	+	+	+	–	–	–	–	–
9	–	+	+	+	+	–	–	–	–	–
10	–	+	+	+	+	–	+	+	+	–

[a] No trypanosomes found.
[b] At least one trypanosome found.

3. Results

Within 21 days after inoculation, all 10 KIVI vials showed presence of *T. theileri*, six vials were already found positive on Day 6 (Table 1). In four out of ten RPMI vessels, some trypanosomes were temporarily seen between Days 6 and 13 but no longer on Day 21. Although their number was not counted it appeared that trypanosomes were far more numerous in KIVI than in RPMI.

4. Discussion

This study suggests that KIVI is an excellent tool for isolation of *T. theileri* from cattle in Belgium with a much higher sensitivity than the RPMI 10%+feeder cultures.

Although no culture medium was replaced in both systems to keep the manipulations as basic as possible, the trypanosomes stayed alive in the KIVI during the experiment while in the RPMI 10%+feeder medium they did not survive for more than 7 days suggesting that medium replacement is required to optimalise the RPMI 10%+feeder culture system.

If required, large amounts of culture forms can easely be grown in KIVI, allowing the preparation of *T. theileri* antigen, DNA or RNA for specificity evaluation of serological and molecular diagnosis tests for pathogenic trypanosomes.

On the other hand, the easy growth of *T. theileri* might render KIVI less efficacious for the isolation of pathogenic African trypanosomes. Mataa (1997) used the KIVI for parasitological follow-up of an experimental *T. b. brucei* infection in six bovines in Zambia. It was observed that some of the inoculated vials showed *T. theileri* instead of *T. b. brucei*. Also one of the two control animals showed *T. theileri* in KIVI. The fact that a mixture of *T. b. brucei* and *T. theileri* was not observed indicates the possibility that *T. theileri* overgrows *T. b. brucei*. Therefore for differential diagnosis in regions where other trypanosomes than

T. theileri occur, KIVI should be complemented by other parasitological, serological or molecular techniques.

Acknowledgements

This work received financial support from the International Livestock Research Institute (ILRI) in Nairobi, Kenya. The authors also like to thank Yves Claes and Rita Beudeker for practical assistance.

References

Aerts, D., Truc, P., Penchenier, L., Claes, Y., Le Ray, D., 1992. A kit for in vitro isolation of trypanosomes in the field: first trial with sleeping sickness patients in the Congo Republic Trans. R. Soc. Trop. Med. Hyg. 86, 394–395.

Dirie, M.F., Bornstein, S., Wallbanks, K.R., Stiles, J.K., Molyneux, D.H., 1990. Zymogram and life-history studies on trypanosomes of the subgenus *Megatrypanum* Parasitol. Res. 76, 669–674.

Hirumi, H., Hirumi, K., Doyle, J.J., Cross, G.A.M., 1980. In vitro cloning of animal-infective bloodstream forms of *Trypanosoma brucei* Parasitology 80, 371–382.

Komoin-Oka, C., Truc, P., Bengaly, Z., Formenty, P., Duvallet, G., Lauginie, F., Raath, J.P., N'Depo, A.E., Leforban, Y., 1994. Etude de la prévalence des infections à trypanosomes chez différentes espèces d' animaux sauvages du parc national de la Comoé en Côte d' Ivoire : résultats préliminaires sur la comparaison de trois méthodes de diagnostic Rev. Elev. Méd. Vét. Pays Trop. 47, 189–194.

Mataa, L., 1997. Experimental infection of *Bos indicus* with *Trypanosoma brucei brucei*: diagnostic aspects. M.Sc. Dissertation, Antwerp, Prince Leopold Institute of Tropical Medicine, Department of Veterinary Medicine, pp. 90.

McHolland-Raymond, L.E., Kingston, N., Trueblood, M.S., 1978. Continuous cultivation of *Trypanosoma theileri* at 37°C in bovine cell culture J. Protozool. 25, 388–394.

McNamara, J.J., Bailey, J.W., Smith, D.H., Wakhooli, S., Godfrey, D.G., 1995. Isolation of *Trypanosoma brucei gambiense* from northern Uganda: evaluation of the kit for in vitro isolation (KIVI) in an epidemic focus Trans. R. Soc. Trop. Med. Hyg. 89, 388–389.

Reiter, I., Büttner, M., Seitz, A., 1987. *Trypanosoma theileri* Laveran, 1902, in naturally and experimentally infected cattle: parasite isolation, serological and cellular reactions and BERENIL® sensitivity J. Vet. Med. 34, 380–390.

Truc, P., 1996. A miniature kit for the in vitro isolation of *Trypanosoma brucei gambiense*: a preliminary field assessment on sleeping sickness patients in Côte d'Ivoire Trans. R. Soc. Trop. Med. Hyg. 90, 246–247.

Truc, P., Aerts, D., McNamara, J.J., Claes, Y., Allingham, R., Le Ray, D., Godfrey, D.G., 1992. Direct isolation in vitro of *Trypanosoma brucei* from man and other animals, and its potential value for the diagnosis of gambian trypanosomiasis Trans. R. Soc. Trop. Med. Hyg. 86, 627–629.

Truc, P., Bailey, J.W., Doua, F., Laveissière, C., Godfrey, D.G., 1994. A comparison of parasitological methods for the diagnosis of gambian trypanosomiasis in an area of low endemicity in Côte d'Ivoire Trans. R. Soc. Trop. Med. Hyg. 88, 419–421.

Truc, P., Formenty, P., Duvallet, G., Komoin-Oka, C., Diallo, P.B., Lauginie, F., 1997. Indentification of trypanosomes isolated by KIVI from wild mammals in Côte d'Ivoire: diagnostic, taxonomic and epidemiological considerations Acta Tropica 67, 187–196.

Wells, E.A., 1971. Studies on *Trypanosoma theileri*-like trypanosomes of cattle I. Culture and storage of isolations Br. Vet. J. 127, 466–475.