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***Full Article***

# Susceptibility of various cell lines to *Neospora caninum* tachyzoites cultivation

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**ABSTRACT**

*Neospora caninum* is a coccidian protozoan parasite which is a major cause of bovine abortions and neonatal mortality in cattle, sheep, goat and horse. Occasionally, cultured cells are used for isolation and multiplication of the agent *in vitro* with several purposes. In this study the tachyzoite yields of *N. caninum* were compared in various cell cultures as the host cell lines. Among the cell cultures tested, two presented good susceptibility to the agent: cell lines Vero and MA-104. SW742 and TLI (*in vitro* suspension culture of lymphoid cells infected with *Theileria lestoquardi*) showed moderate sensitivity. No viable tachyzoite were detected in the culture of MDCK and McCoy cell lines. These results demonstrate that MA-104 and SW742 cells present adequate susceptibility to *N. caninum* compared to Vero cells, which have been largely used to multiply the parasite *in vitro*. Moreover, these have easy manipulation, fast multiplication and relatively low nutritional requirements. In addition, the result of this study showed that TLI cell line as a suspension cell culture is susceptible to Nc-1 tachyzoites infection and could be used as an alternative host cell line for tachyzoites culture *in vitro* studies.

***Keywords:*** *Neospora caninum*, Vero, TLI, MA-104, SW742, McCoy, MDCK

## INTRODUCTION

*Neospora caninum* is an apicomplexan parasite, first described by Bjerka˚s et al. in 1984 and isolated and named by Dubey et al. (1988). *Neospora caninum* has a worldwide distribution and is a major cause of reproductive failure in cattle (Innes et al. 2001). Considerable economic losses are attributed to *N. caninum* in the farming industry, including the costs of still birth and neonatal mortality, increased calving interval resulting from early fetal death, increased culling, reduced milk production and reduced value of breeding stock (Dubey 2003). Since its discovery, the realization of the economical significance of this parasite has led to significant efforts in elucidating how *N. caninum* interacts with its host (Naguleswaran *et al* 2003, Dubey *et al* 2007). Principally, *N. caninum* spends its life cycle in infected host cells as an intracellular parasite. Therefore, these processes that lead to cell host invasion are of crucial importance in maintenance of infection by *N. caninum* (Hemphill 1999). Moreover, the production of antigenic materials and the study of the relationship between the parasites

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and host cells require *in vitro* cultivation of the protozoan. Also *in vitro* cultures are used to perform genetic manipulation on the parasite (Radke *et al* 2000) or to screen potential chemotherapeutic agents (Strohbusch *et al* 2008, Leepin *et al* 2008). Up to now, *N. caninum* has been successfully cultured in the different monolayer cell lines. Among these, Vero cells are the most widely used type in routine for the cultivation of the parasite tachyzoite *in vitro* (Dubey 2003, Vonlaufen *et al* 2004, Lei *et al* 2005, Kang *et al* 2008). In that sense, susceptibility of different cell lines to the parasite would be great interest for diagnostic and experimental usage of *N. caninum*.

In the present study the suitability of six cell lines including Vero, MA-104, McCoy, MDCK, SW742 (all of them as attachment cell lines) and TLI (as a suspension cell line) to support *in vitro* growth of *N. caninum* tachyzoites were compared.

## MATERIALS AND METHODS

**Parasites preparation.** *Neospora caninum* tachyzoites of the Nc-1 isolate (Dubey *et al* 1988) were cultured on a 24-hr-old monolayer of Vero cell line and maintained in DMEM Medium (Sigma Co., USA) supplemented with 2% fetal calf serum, penicillin/ml (10,000 U), streptomycin/ml (100 µg) and streptomycin (25 µg) (Invitrogen, USA) at 37°C with 5% CO2 ( Hemphil et al. 1996). Free tachyzoites were harvested from infected cell cultures when about 80-90% of the Vero host cells had lysed, as determined by microscopic examination of monolayers for cytopathic effects (CPE) and then counted using a Neubauer chamber.

**Cell lines.** SW742 (human colorectal epithelial- like cell) and McCoy (mouse synovial fluid fibroblast- like cell) cell lines were obtained from the National Cell bank of Iran (NCBI), Pasteur Institute of Iran. Also MDCK (canine kidney epithelial- like cell), MA-104 (African green monkey kidney epithelial- like cell), Vero (African green monkey kidney epithelial cell) and TLI (*Theileria lestoquardi*- infected lymphoid cell line) cell lines were provided by Razi Vaccine and Serum Research Institute, Karaj, Iran. TLI cell line had been infected and transformed *in vivo* (Hooshmand- Rad, 1985) which isolated from prescapular lymph nodes of sheep undergoing natural *T. lestoquardi* infection and was passaged 100 times and known as vaccinal strain. The cells were maintained in supplemented DMEM and 10% fetal bovine serum and added to $25cm^2$ tissue culture flasks. Only, McCoy cells were maintained in supplemented RPMI 1640. Before inoculation of parasites, host cells were incubated for 24 hr. All cells were maintained in the same condition (at 37 °C in an atmosphere of 95% air and 5% $Co_2$).

**Yield of Parasite Derived *In Vitro*.** Equal number of Nc-1 tachyzoites (obtained from *in vitro* described above) were seeded to 24-hr-old cell lines (for each flask 1ml of suspension of *N. caninum* tachyzoites containing $2\times10^6$ NC/ ml were adjusted to $1\times10^6$ cell/ ml) which added to $25cm^2$ tissue culture flasks and maintained in 10 ml DMEM medium with 2% FBS, 2.30 mg/ml NaHCO3, 2.38 mg/ml HEPES, 2 mm glutamine, 50 U/ml penicillin, and 50 mg/ml streptomycin at 37 °C with 5% CO2 (LV et al, 2010 with some modifications). Whole development process of *N. caninum* tachyzoites in each cell line was observed daily under an inverted phase-contrast microscope (XDS-1B, China). The incubation was continued until the maximum numbers of tachyzoites were released from all of the host cells (for 5 consecutive days post infection). The numbers of extracellular tachyzoites from 10 fields in each flask were counted and the daily average number was calculated and growth curves were drawn (Lv *et al* 2010).

**Statistical analysis.** A one- way ANOVA was used for the comparison of the yield of parasite harvested from all of the cell cultures in different days post infection and differences were considered significant when $P<0.05$, using computer software SPSS version 16 for windows (SPSS, Chicago, IL, USA).

## RESULTS

The whole development process of *N. caninum* tachyzoites *in vitro* in all cell cultures was observed

daily for 5 consecutive days by inverted microscope (Table 1).

**Table 1.** Average number of *Neospora caninum* tachyzoites (in five determinations) obtained in the six various cell cultures ± Standard Error of Mean (SEM). No viable tachyzoite was detected in the culture of MDCK and McCoy cells on day 4.

| cell line | 1st dpi $\times10^4$ | 2nd dpi $\times10^4$ | 3th dpi $\times10^4$ | 4th dpi $\times10^4$ | Average $\times10^4$ |
|---|---|---|---|---|---|
| **Vero** | 3.5±0.50 | 6.25±1.3 | 21.25±3.7 | 24.2±2.2 | 13.8 |
| **TLI** | 2.25±0.25 | 4.5±0.95 | 19.75±3.5 | 20.85±1.7 | 11.83 |
| **MA-104** | 2.75±0.47 | 5.25±0.85 | 20±2.1 | 22.1±0.67 | 12.52 |
| **SW742** | 2.25±0.47 | 5±0.91 | 20.75±2.3 | 21.2±2.1 | 12.3 |
| **McCoy** | 1.75±0.47 | 1.25±0.47 | 0.75±0.47 | 0 | 0.93 |
| **MDCK** | 2±0.40 | 1±0.40 | 0.25±0.25 | 0 | 0.81 |

In this study, the numbers of the released parasites in Vero, TLI, MA-104 and SW742 cell cultures increased at a slow to moderate rate on the first 3 days and multiplied rapidly on day 4 which reached the maximum number. At the end of the fourth day, released parasites reach to the plateau. The numbers of the extracellular tachyzoites started to decrease on day 5. However, in McCoy and MDCK cell lines the numbers of extracellular parasites decrease at a moderate rate on day 2. No viable tachyzoite were detected in the both cultures on day 4 (Figure 1). There were no significant differences between the average tachyzoite yields harvested from Vero, MA-104, TLI and SW742 cell cultures in every 4 consecutive days post infection ($P>0.05$). However, the average tachyzoite yields harvested from McCoy and MDCK cell cultures were significantly smaller than the tachyzoites harvested from Vero, MA-104, TLI and SW742 cell cultures ($P<0.05$). At the second day post infection started significant differences between tachyzoites counted in these groups.

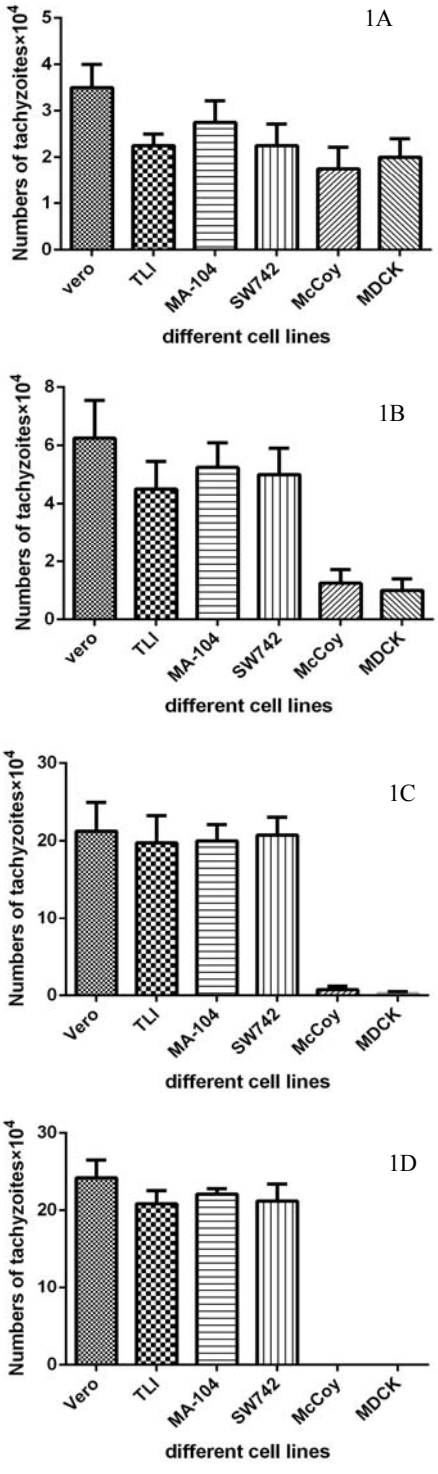


**Figure 1.** Kinetics of production of *Neospora caninum* tachyzoites in various cell cultures with SEM on different consecutive days post infection (dpi). *Neospora caninum* tachyzoites were added with equal concentration to 25 cm2 tissue culture flasks and average number of tachyzoites obtained in the six various cell cultures counted on first dpi (A); on second dpi (B); on third dpi (C) and on fourth dpi (D).

## DISCUSSION

In the recent years, numerous host cells have been successfully used for the laboratory maintenance and

propagation of *N. caninum* tachyzoites. After These cells include Vero cell (Angela 2002, François *et al* 2002, Naguleswaran *et al* 2002, Okeoma *et al* 2004), bovine mononuclear cell (Tuo *et al* 2005), bovine angioendothelial cell (Omata *et al* 2005), cat and dog fibroblast cell (Lei et al. 2005), cat kidney cell (Lei *et al* 2005), murine epidermal keratinocytes (Vonlaufen *et al* 2002), rat astrocytes (Pinherio *et al* 2006), cancer cell lines of human such as MCF-7 (Lv *et al* 2010) and trophoblastic (BeWo) and uterine cervical (HeLa) cells (Carvalho *et al* 2010). Among these, the Vero cell line, as an attachment surface, is the most commonly used for the spread of *in vitro* (Dubey 2003). inoculation of *N. caninum* tachyzoites to these cell lines, propagation of tachyzoites was compared *in vitro* which the highest tachyzoite yields were obtained from Vero cell line. But it was not significant difference in tachyzoite yields harvested from MA-104, SW742 and TLI cell cultures and these cell lines could be used as alternative host cells for *in vitro* studies. Sometimes, the cultivation of attachment cells presents difficulties, especially with regard to the speed of propagation and because the most cell lines are adherent cells.Therefore, the availability of non-adherent cell lines (suspension culture) susceptible to *N. caninum* would be of great interest in the mass production of *N. caninum* tachyzoites. *N. caninum* is similar in biological aspects to *T. gondii* and some researchers reported that the HeLa and LLC cells invariably produced better results than Vero cells in the continuous production of *T. gondii* (Evans *et al* 1999). The result of this study showed that TLI cell line as suspension cell culture is a suitable host cell for cultivating *N.caninum* which did not show any significant differences in average released number of tachyzoite compared to Vero cell line. In the present study, SW742 (human colorectal epithelial- like cells) was successfully used to culture *N. caninum* tachyzoites. In similar study, Lv et al. (2010) showed that MCF-7 human breast carcinoma cells are susceptible to Nc-1 tachyzoites infection and could be used as an alternative host cell line for tachyzoites culture *in vitro* studies. The results here demonstrated that *N. caninum* had the ability to infect various host cells including bovine, ovine, dog, mouse, monkey and human cells. Therefore, these data support that *N. caninum* tachyzoites can invade and grow in human cells. However, whether *N. caninum* is a human pathogen is unclear yet. Recently, *N. caninum* tachyzoites and bradyzoites infected primary canine intestinal cells an *in vitro* model (Hemphill *et al* 2009). Also, a method has been developed for the *in vitro* culture of *N. caninum* bradyzoites containing tissue cysts (Hemphill 2007). In McCoy and MDCK cell lines the numbers of extracellular parasites decrease at a moderate rate on day 2 and no viable tachyzoites were detected in the both cultures on day 4. According to this result, it seems that *N. caninum* tachyzoites did not have the ability to multiply and grow in these 2 cell cultures. In another study, Cadore et al. (2009) did not detect any visible tachyzoites in the culture of MDCK cells, which confirm our results.

In conclusion, these results demonstrate that MA-104, SW742 and TLI cells present adequate susceptibility to *N. caninum* compared to Vero cells and could be used to multiply the parasite *in vitro*. These have easy manipulation, fast multiplication and relatively low nutritional requirements.

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