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Popular Article

Unlocking Insights: The significance of *in vitro* cultivation for haemoprotozoan parasites of veterinary importance

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Abstract

Haemoprotozoan parasites are microscopic organisms that invade the blood of animals, leading to severe health issues in veterinary medicine. Understanding their biology, life cycle and interactions with the host is paramount for developing effective control and treatment strategies. *In vitro* cultivation, the controlled growth and maintenance of these parasites in a laboratory environment, plays a pivotal role in advancing our knowledge and capabilities. This article explores the key significance of *in vitro* cultivation for haemoprotozoan parasites, encompassing research, drug development, vaccine creation and insights into disease pathogenesis and host-parasite interactions. By leveraging in vitro cultivation, we can enhance our understanding of haemoprotozoan parasites, ultimately contributing to improved veterinary healthcare and management practices.

1. Introduction

In vitro cultivation refers to the process of growing and maintaining haemoprotozoan parasites in a controlled laboratory environment outside their natural host. This technique has revolutionized the study of these parasites, enabling researchers to investigate their lifecycle, pathogenesis, drug sensitivity and host-parasite interactions in a controlled setting. Cultivation is an important method for diagnosis of many clinically important parasites for example *Entamoeba histolytica*, *Trichomonas vaginalis*, *Leishmania* spp., *Strongyloides stercoralis* and free-living amoebae (Ahmed, 2014). RPMI-PY medium can be used for *Trypanosoma cruzi*, *Leishmania amazonensis*, *Leishmania major*, *and Leishmania tropica* species since in all the species except *Leishmania braziliensis*, the exponential growth of the parasite was observed, in many cases higher than conventional media (Castelli *et al*., 2023).

1.1 Enhanced Replication and Life Cycle Studies

In vitro cultivation allows for the controlled replication of haemoprotozoan parasites, providing researchers with a limitless supply of parasites for experimentation. By studying their life cycles in vitro, researchers can uncover critical stages of development, host-specific factors, and

environmental cues that influence parasite growth and transmission. The ethical concerns of using laboratory animals and the difficulty of readily obtaining parasite life stages highlight the need for in vitro models as viable alternatives for cultivating and maintaining protozoan parasites. In vivo models may offer insights into host-parasite interaction (Sutrave and Richter, 2021).

1.2 Accessibility for Experimental Manipulation

In vitro cultivation offers a unique advantage by providing accessibility and ease of manipulation compared to in vivo studies. Researchers can modify environmental conditions, such as temperature, pH and nutrient availability, to mimic specific host environments and study the effects on parasite growth and behaviour. This flexibility enables the investigation of intricate cellular processes that may be challenging to study within the complex host environment.

1.3 Drug Screening and Anti-parasitic Discovery

One of the most significant contributions of in vitro cultivation is its role in drug screening and antiparasitic discovery. By exposing in vitro-cultivated parasites to a variety of drugs, researchers can assess their efficacy and determine drug resistance patterns. This information is invaluable for developing effective treatment strategies and identifying new drug targets to combat haemoprotozoan infections in veterinary medicine.

1.4 Immune Response Studies

In vitro cultivation also allows for the investigation of host-parasite interactions and immune responses during infection. By culturing parasites with host immune cells, researchers can simulate the immune environment and study the mechanisms of immune evasion employed by haemoprotozoan parasites. These studies aid in understanding the complex dynamics between the host immune system and the parasites, leading to the development of targeted immunotherapeutic interventions.

1.5 Genetic Manipulation and Molecular Studies

Advancements in molecular biology techniques have facilitated genetic manipulation of haemoprotozoan parasites *in vitro*. Researchers can introduce genetic modifications, knockdown or knockout specific genes and study the resulting phenotypic changes. These molecular studies provide insights into essential parasite proteins, gene function, and their role in pathogenicity, further expanding our understanding of these parasites.

2. Techniques and Methodologies

Culturing *Trypanosoma evansi in vitro* involves maintaining the parasite in a controlled laboratory environment outside of the host. Materials and reagents required are *T. evansi* culture flask, culture medium (e.g., Hirumi-9 or similar medium), Fetal bovine serum (FBS), Penicillin-streptomycin antibiotics, CO_2 incubator, Microscope and cell counting chamber. **Steps for in vitro culture of *T. evansi* are:**

1. Preparation of Culture Medium: Prepare a suitable culture medium for *T. evansi*. Hirumi-9

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medium is commonly used and can be supplemented with 10% FBS and penicillin-streptomycin antibiotics.

1. Inoculation of Culture Flask: Start with a culture flask containing the appropriate volume of culture medium. The flask should be sterile. Add a small amount of *T. evansi* culture (usually a few drops or a small volume of previously cultured parasites) to the medium.
2. Incubation: Place the culture flask in a suitable incubator. *T. evansi* can be cultured at 37°C in a CO_2 incubator, but it can also grow at room temperature.
3. Monitoring and Feeding: Regularly monitor the culture under a microscope to check for parasite growth. *T. evansi* typically multiplies by binary fission. Periodically feed the culture by adding fresh culture medium to replenish nutrients and remove waste products.
4. Subculturing (Passaging): When the parasite density becomes too high or the culture medium becomes depleted, it's time to subculture the parasites. This involves transferring a portion of the culture to a new flask containing fresh medium. Sub-culturing prevents overgrowth and maintains the health of the culture.
5. Parasite Counting: Use a microscope and a cell counting chamber to estimate the parasite density in the culture. This helps determine when to subculture and how much culture to transfer.
6. Maintaining Sterility: Ensure all procedures are performed using aseptic techniques to prevent contamination of the culture.
7. Experimental Manipulations (if necessary): If you are conducting experiments with *T. evansi*, you can introduce specific conditions or treatments into the culture to study their effects on the parasites.
8. Record Keeping: Maintain accurate records of the culture's age, density, subculture dates, and any experimental data.
9. Storage (if necessary): If you need to store *T. evansi* for a longer duration, consider cryopreservation using suitable cryo-protectants, as mentioned in a previous response.

In vitro culture of *Babesia equi*, a protozoan parasite responsible for causing equine piroplasmosis, requires specialized techniques and culture conditions. The variety of media and sera from various host species is employed and the composition of the medium ingredients relies on the Babesia sp. that has to be cultivated (Schuster, 2002). Materials and Reagents required are *B. equi*-infected horse blood, culture medium (e.g., RPMI 1640), fetal bovine serum (FBS), antibiotics (e.g., penicillin-streptomycin), gas mixture (5% CO_2, 5% O_2, and 90% N_2), microscope and cell counting chamber, culture flasks or plates, CO_2 incubator.

Steps for in vitro culture of *B. equi* are:

1. Collection of Infected Blood: Collect blood from a *Babesia equi*-infected horse. This blood

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will serve as the source of parasites.

2. Processing of Blood Sample: Centrifuge the blood to separate the red blood cells (RBCs) from the plasma and buffy coat. The parasites are typically found within the RBCs.
3. Washing RBCs: Wash the RBCs multiple times with a suitable buffer (e.g., phosphate-buffered saline or PBS) to remove plasma and residual contaminants.
4. Preparation of Culture Medium: Prepare a culture medium, such as RPMI 1640, supplemented with 10-20% FBS and antibiotics (e.g., penicillin-streptomycin). The medium should be warmed to 37°C.
5. Inoculation of RBCs: Mix the *Babesia equi*-infected RBCs with the prepared culture medium in a culture flask or plate. The initial parasite concentration should be determined based on previous knowledge or microscopic examination.
6. Incubation: Place the culture flask or plate in a CO_2 incubator with a controlled gas mixture (5% CO_2, 5% O_2, and 90% N_2) and maintain it at 37°C.
7. Monitoring: Regularly monitor the culture under a microscope to check for the presence of *Babesia equi* parasites. They will be observed within the RBCs.
8. Feeding and Maintenance: Periodically replenish the culture medium with fresh medium to provide nutrients and remove waste products. This can be done every 1-2 days or as needed.
9. Subculturing (Passaging): As the parasite density increases, perform subculturing by transferring a portion of the culture to a new flask with fresh medium. This prevents overcrowding and maintains parasite health.
10. Parasite Counting: Use a microscope and a cell counting chamber to estimate the parasite density in the culture. This helps determine when to subculture and the appropriate parasite-to-RBC ratio.
11. Storage (if necessary): If you need to store *B. equi* cultures for an extended period, consider cryopreservation using suitable cryoprotectants and protocols.
12. Record Keeping: Maintain detailed records of the culture's age, density, subculture dates, and any experimental data.

Culturing *B. equi* in vitro can be challenging due to the parasite's specific requirements and sensitivity to environmental conditions.

In vitro culture of *Theileria annulata*, a protozoan parasite responsible for causing tropical theileriosis in cattle, requires specialized techniques and culture conditions. The reduction of virulence during in vitro maintenance is a significant characteristic of some Babesia and Theileria spp. in vitro cultures, but not all of them. As a result, *T. parva* and *Babesia* spp., among other possible sources of attenuated parasites, might be employed for vaccine purposes (Shkap and Pipano, 2000). Materials and Reagents are *Theileria annulata*-infected blood, culture medium (e.g., RPMI 1640), fetal bovine

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serum (FBS), penicillin-streptomycin antibiotics, CO_2 incubator, microscope and cell counting chamber, culture flasks or plates and cryoprotectant (e.g., glycerol or DMSO, if long-term storage is needed).

Steps for *in vitro* culture of *Theileria annulata* are as follows:

1. Collection of Infected Blood: Collect blood from a *T. annulata*-infected cow. This blood will serve as the source of parasites.
2. Processing of Blood Sample: Centrifuge the blood to separate the red blood cells (RBCs) from the plasma and buffy coat. The parasites are typically found within the leukocytes and RBCs.
3. Isolation of Leukocytes: Carefully collect the buffy coat layer, which contains leukocytes and infected cells, and transfer it to a separate sterile container.
4. Washing Leukocytes: Wash the isolated leukocytes several times with a suitable buffer (e.g., phosphate-buffered saline or PBS) to remove plasma and residual contaminants.
5. Preparation of Culture Medium: Prepare a culture medium, such as RPMI 1640, supplemented with 10-20% FBS and antibiotics (e.g., penicillin-streptomycin). Warm the medium to 37°C.
6. Inoculation of Leukocytes: Mix the washed *T. annulata*-infected leukocytes with the prepared culture medium in a culture flask or plate. The initial parasite concentration should be determined based on previous knowledge or microscopic examination.
7. Incubation: Place the culture flask or plate in a CO_2 incubator and maintain it at 37°C with a controlled atmosphere (5% CO_2) to simulate the physiological conditions of the host.
8. Monitoring: Regularly monitor the culture under a microscope to check for the presence of *T. annulata* parasites within the leukocytes. Parasite morphology and division can be observed.
9. Feeding and Maintenance: Periodically replenish the culture medium with fresh medium to provide nutrients and remove waste products. This can be done every 1-2 days or as needed.
10. Subculturing (Passaging): As the parasite density increases, perform subculturing by transferring a portion of the culture to a new flask with fresh medium. This prevents overcrowding and maintains parasite health.
11. Parasite Counting: Use a microscope and a cell counting chamber to estimate the parasite density in the culture. This helps determine when to subculture and the appropriate parasite-to-leukocyte ratio.
12. Storage (if necessary): If you need to store *T. annulata* cultures for an extended period, consider cryopreservation using suitable cryoprotectants and protocols.
13. Record Keeping: Maintain detailed records of the culture's age, density, subculture dates, and any experimental data.

3. Challenges and Future Directions

Despite the numerous advantages, in vitro cultivation also presents challenges. This section

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explores limitations such as the inability to replicate the complete host environment, difficulties in mimicking the immune response accurately, and potential variations among different parasite strains. Moreover, it highlights ongoing research efforts and future directions, including the integration of in vitro and in vivo studies, the application of three-dimensional (3D) cell culture models, and the use of microfluidic systems for more accurate replication of the host environment.

4. Conclusion

In vitro cultivation has proved to be an invaluable tool for understanding haemoprotozoan parasites of veterinary importance. Its contributions to the field have enhanced our knowledge of parasite biology, pathogenesis, drug sensitivity, and immune interactions. Continued advancements in in vitro techniques hold promise for addressing veterinary challenges related to these parasites, ultimately leading to improved diagnostics, therapeutics and disease management strategies.

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