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Research Article

Current Comparison of the Efficacy Vero And BHK-21 Cell Lines in the Isolation of *Chlamydia Abortus*

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Abstract

Chlamydia abortus is an obligate intracellular, gram-negative bacterium and a significant zoonotic agent associated with abortion storms, particularly in small ruminants. Due to its intracellular lifestyle, conventional isolation is limited, necessitating specialized protocols. This study aimed to develop and evaluate an optimized isolation method for *C. abortus* by comparing the efficacy of three different cell lines: Vero, monolayer BHK-21 (m-BHK-21), and suspended BHK-21 (s-BHK-21). Samples were collected from PCR-positive materials, including a calf's stomach content, sheep vaginal swab, lamb fetal liver, and calf fetal liver. Each sample was inoculated into the three cell lines. Cell cultures were monitored using an inverted microscope equipped with a trinocular imaging system. Stamp staining and conventional PCR were employed for initial and intermediate detection. Final confirmation of *C. abortus* presence was performed via Stamp staining and quantitative analysis using Real-Time PCR. Bacterial growth was quantified using a cell counter, leveraging the bacterium's gram-negative staining properties. Isolation was successful across all cell lines using the calf stomach content sample, whereas the remaining samples yielded positive results in only one cell type. Among the tested lines, s-BHK-21 demonstrated superior performance, producing the highest bacterial load (4.7×10^7 cells/mL). A 94-fold increase in viable cell count was observed from initial inoculation to the final harvest. The s-BHK-21 cell line is recommended as the most effective platform for *C. abortus* isolation, providing enhanced bacterial yield and reduced processing time. Its application may improve diagnostic accuracy and facilitate further research into *C. abortus* pathogenesis and control strategies.

Keywords: *C. abortus*, Cell Culture, Isolation, Public Healthcare, Zoonotic

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Introduction

Chlamydia abortus is an obligate intracellular, gram-negative, immobile bacterium that causes abortion as a result of the reproductive system and systemic infection in farm animals, especially small ruminants, and mammals (Everett 2000, Essig and Longbottom 2015, Fayez et al 2021). In its life cycle, it proliferates in inclusion bodies in the cytoplasm of the host cell with its metabolically inactive-infective elementary body (EB) and metabolically active-reticulate body (RB) (Alvesalo et al 2006). Although there are studies on seroprevalence in small ruminants (Hireche et al 2016, Mamlouk et al 2020), more comprehensive and updated studies are needed (Hireche et al 2016). Although transmission is both vertical and horizontal, it occurs through the aborted animal and secretions of the aborted female (WOAH 2018). It is stated that after the agent is taken into the body through the oral route, it settles in the tonsils, and from there, it is transported to the organs via the blood or lymphatic system (Essig and Longbottom 2015). In pregnant ewes with chlamydiosis infection, clinical findings are not evident until the 90th day, and abortion occurs in an asymptomatic manner (Buxton et al 1990). Although it is assumed that the reason for this may be the hematoma developing on the 60th day of pregnancy in the fetal chorionic villus layer (Longbottom and Coulter 2003), the mechanism of abortion has not been explained. In immune animals, although metritis occurs (Longbottom and Coulter 2003, Stuen and Longbottom 2011) and recurrent miscarriage is not observed, the infected animal

continues to shed the agent (Papp et al 1994, Papp and Shewen 1996). While no abortions are observed in the herd in the first year of exposure, in the following period, so-called abortion storms, reaching 30% on a herd basis, are encountered (Seaman 1985, Longbottom and Coulter 2003, Aitken and Longbottom 2007, Essig and Longbottom 2015).

Although ELISA is widely used in diagnosis, in persistent animals, the agent is found in the tissue, not in the blood, and it cannot be diagnosed with serological tests until the 90th day of pregnancy (Salti-Montesanto et al 1997). PCR is preferred among molecular diagnostic methods, and McCoy, Buffalo Green Monkey (BGM) or Baby Hamster Kidney (BHK) cells are used for isolation in cell culture (WOAH 2018). However, cell culture is a method with variable specificity and sensitivity that is difficult, dangerous, expensive, time-consuming, and requires expertise and laboratory infrastructure (Duruel et al 2021). However, the gold standard is provided by culture and microscopic staining (WOAH 2018).

There are two types of commercial vaccines against the agent: inactive and attenuated live vaccines. The former can grow at 35-39°C as a result of the mutation of the 1B chain structure of *C. abortus*, but they do not work due to the physiologically high body temperature of sheep (Rodolakis 1983, Essig and Longbottom 2015). For this reason, abortion is reported in animals vaccinated with live vaccines (Essig and Longbottom 2015). Although inactive vaccines, which are widely used in Europe, provide protection, they cannot prevent the transmission of the agent. With the current technology, the production of new-generation vaccines that are cheaper, easier to administer, and have a strong and stable immune system response come into prominence (Essig and Longbottom 2015, WOAH 2018).

The accepted cell lines that have been preferred for years lead researchers to choose a cell line for routine use that presents little or no virus-like particles in terms of the sensitivity and specificity required (Fong et al 1994). In addition to this, since the cell line is not economical, other cell lines were evaluated, and a trial was made in our study to produce alternative solutions. Comparing the efficiency obtained when unused cell lines are used is among the aims of the study.

Due to its morphological properties, the agent can be produced more easily in suspended cell lines. However, as a result of the equipment and cost required by suspended cells, such a study was needed in order to compare different cell lines and the incubation time of the factor produced in the monolayer cell line against the suspended cell.

This study aims to isolate *C.abortus* using different cell lines and to determine the strains showing the best growth curve and the cell line with the most efficient results through serial passages.

Material and Methods

Positive Samples Used In The Study

A total of 108 samples of vaginal swabs (n=40) from cattle and sheep that aborted at different periods of pregnancy and aborted fetuses (n=68) from different regions of Türkiye and sent to our laboratory for diagnostic purposes were used in this study. Our study was approved by the ethics committee of the Faculty of Veterinary Medicine at the University of Selcuk in Konya, Türkiye and ethics committee approval number 2020/070 was obtained. All DNA extractions were performed according to the Wizard® Genomic DNA Purification Kit (Promega, USA), and the DNA extracts were stored at -20 °C until used.

Briefly, the samples were submitted to extraction according to the Wizard® Genomic DNA Purification Kit (Promega, USA). The presence of *Chlamydia abortus* in bacterial DNA isolates obtained from stomach contents was examined by conventional PCR. The PCR mix was prepared as follows: 5 µl 5x master mix (SolisBiodyne, Estonia), 50 ng/ µl DNA, 5 pmol of each primer (CabortusF 5´-CTCACCATTGTCTCAGGTGGA-3´, CabortusR 5´-ACCGTAATGGGTAGGAGGGGT-3´) and completed to 25 µl with ultrapure water. For the PCR cycle, a protocol of 95°C 15 min pre-denaturation, 40 cycles (94°C 60 sec, 62°C 60 sec, 72°C 60 sec) and 72°C 10 min final extension was used. The PCR products of suspicious DNAs that in an agarose gel containing 20 µg/mL ethidium bromide showed a band at 821 bp under UV light and using a 1kb bp ladder (Solisbiodyne, Estonia) were evaluated as positive (Berri et al 2009). Positivity was detected with PCR in the stomach contents of 1 calf aborted fetus, 1 sheep vaginal swab, 1 lamb fetal liver and 1 calf fetal liver samples.

Cell Culture

Preparation Of The Samples

Of a total of 2 tissue samples, 1 g from each was taken and homogenised with a scalpel. All samples were diluted under sterile conditions to 5 ml with phosphate buffer saline (PBS) by addition to 1 ml of the stomach contents or to the vaginal swap sample. The isolation phase started after appropriate amounts of streptomycin (100 mg/50 mL) (Sigma, Merck, Germany) were added to the completed samples and incubated at 37 °C for 24 hours, and, finally, the samples were passed through a 0.45 µm millipore syringe filter (Millex, Merck, Germany).

Inoculation Of Positive Samples

For the purpose of the study, monolayer Green Monkey Kidney Cell (Vero), monolayer Baby hamster kidney cells (m-BHK-21), and suspended BHK-21 (s-BHK-21) cell lines were used. The adaptation of the cells and inoculation of the agent was the first stage, and multiplication was the second stage. In order to compare the products obtained from Vero, m-BHK-21 and s-BHK-21, an isolation protocol from different tissues was developed. In order to compare the isolation rate from the prepared positive samples, only fetal stomach contents were simultaneously and separately inoculated into Vero, m-BHK-21, and s-BHK-21 cells. For each of the other positive samples, it was conducted in a single cell line -Vero, m-BHK-21, s-BHK-21. For this, 1 mL was inoculated into the cell lines and were incubated (Thermo Scientific BB15, USA) at 37°C containing 5% CO_2 for 24 hours. Afterwards, the petri dishes were centrifuged at 2500-3500 g x 30-60 min to remove the medium. The protocol followed was, briefly, as ensued: in the first stage, for obtaining Vero and BHK-21 monolayer cells, Glasgow Minimum Essential Medium (GMEM) containing 10% fetal bovine serum (FBS) (Merck, Germany), streptomycin/mL (100 µg) (Sigma, Merck, Germany) and 100 mg/50 mL candisept (Vem, Vem İlaç AŞ, İstanbul, Turkey) was used. When the stock cells removed from -80°C were approximately 75% thawed at 37°C, both cell lines were transferred separately to 60 mm x 15 mm cell culture Petri dishes containing 4 mL GMEM. The transferred cells were kept in an incubator (Thermo Scientific BB15, USA) at 37°C containing 5% CO_2 for 24 hours, and their growth was observed under an inverted microscope equipped with a trinocular imaging system (DMi1, Leica, Germany). After observing that approximately 80% of the petri dish was covered with cells, sample inoculation was started. In the second stage, GMEM prepared without FBS was added to the Petri dishes and incubated for 2-5 days for BHK-21 and 4-7 days for the Vero cell line.

During the incubation periods, samples were taken under sterile conditions and checked with the Stamp staining method (Arda 2015) and conventional PCR. After the samples taken for staining and PCR were aspirated with a sterile syringe tip, the cells were centrifuged at 400 rpm x 10 minutes, the supernatant was discarded, and control was done with the cells remaining at the bottom.

In the case of the suspended BHK-21 cell line, for the cells removed from the -80°C freezer, GMEM medium containing 10% fetal bovine serum (Merck, Germany), streptomycin/mL (100 µg) (Sigma, Merck, Germany) and 100 mg/50 mL Candisept (Vem, Vem İlaç AŞ, İstanbul, Turkey) was used. When the stock cells removed from the -80°C freezer were approximately 75% thawed at 37°C, 10 mL of GMEM fresh medium was added. The cells were homogenised by pipetting slowly and transferred to cell culture bottles. The transferred cells were kept in a 37°C, 120 rpm incubated benchtop shaker (Thermo Scientific BB15, USA) containing 5% CO_2 for 24 hours. On alternate days, under sterile conditions, samples were taken from the supernatant into centrifuge tubes and centrifuged; then, the growth curve and viability were checked with a cell counting device (Biorad- TC20 Automated Cell Counter, USA).

Once $5x10^5$ cells/mL were obtained in the suspended cell line, agent inoculation was performed. 1 mL of the positive samples prepared for inoculation was added into the suspended cell under sterile conditions. The cell line to which the agent was added was placed in an incubated benchtop shaker at 37°C and 120 rpm, containing 5% CO_2. After 24 hours, the cells containing the agent were taken into centrifuge tubes and centrifuged at 400 rpm x 10 min. Afterwards, in the second stage, the supernatant was discarded, and fetal bovine serum-free (FBS) GMEM was added and incubated under the same conditions for 36-72 hours (Güngör et al 1969, WOAH 2018). During the incubation period, samples were taken from the supernatant under sterile conditions and centrifuged at 400 rpm x 10 min; the Stamp staining method (Arda 2015) was then applied to the pellet and control was performed by PCR (WOAH 2018).

Determination Of The Presence, Count And Growth Curve Of The Agent

While checking the presence of the agent, the cells were removed from the cell culture with 25% trypsin-EDTA (Sigma, Merck, Germany) and centrifuged at 400 rpm x 10 min with soft acceleration and deceleration. Afterwards, 100 µl of the pellet remaining at the bottom and the supernatant were taken separately onto a slide and stained according to the Stamp staining method (Arda 2015), and the presence of the agent was observed under an inverted microscope.

The presence of the agent was detected by real-time PCR from the samples taken from the pellet after centrifugation.

In order to check the viability of the cells in the monolayer and suspended cell line, in addition to monitorisation with a microscope, they were counted with the TC20 Automatic Cell Counter (Biorad, USA) cell counting device. For cell counting, after the cells propagated as monolayers were checked under an inverted microscope, the cells were detached with trypsin-EDTA and centrifuged; then, under sterile conditions, a sample for the cell counting device was taken from the pellet remaining at the bottom. A cell sample of 20 µl and 20 µl of trypan blue dye were mixed, then 10 µl of this mixture were placed on the slide of the counting device and the results were displayed on the device.

For the suspended cell line, a sample was taken under sterile conditions, and after centrifugation, the pellet remaining at the bottom was homogenised again, and 20 µl of the sample along with 20 µl of trypan blue were placed on a cell count slide and counted on the device.

Real-time PCR was used to demonstrate the presence of the agent and the growth curve in the samples taken from the cell cultures. For real-time PCR, the samples taken to an Eppendorf tube from the cell culture under sterile conditions were extracted from the pellet after centrifugation according to the Wizard® Genomic DNA Purification Kit (Promega, USA) and the DNA of the agent was obtained (WOAH 2018, Esmaeili 2024).

The reaction mixture was prepared with a total of 10 µl of 2x SsoAdvanced Universal SYBR Green Supermix (Biorad, USA), 0.2 µl of each primer (CabortusF and CabortusR), and 9.6 µl of DNA, making a total of 20 µl. The primer for RT-PCR (CabortusF 5'-CTCACCATTGTCTCAGGTGGA -3', CabortusR 5'-ACCGTAATGGGTAGGAGGGGT-3') was designed as the same primer used in conventional PCR (Berri et al 2009). The prepared cycle was applied in the Bio-Rad CFX96 Touch Real-Time PCR thermal cycler device with a protocol of 10 minutes at 95°C, then 45 seconds at 94°C, and 45 seconds at 62°C, for a total of 40 cycles. Both the positive and negative controls obtained from the Culture Collection of the Department of Microbiology (Selcuk University, Faculty of Veterinary Medicine) were included in the cycle. Analysis results were evaluated according to Ct (cycle threshold) data along with positive and negative controls. Amplification observed in the sigmoidal curve and Ct value ≤ 37 were considered positive. Amplifications where no curve was observed, or this threshold value was higher than 37 were considered negative (Nordentoft et al 2011, Yeni and Balevi 2023). PCR products were also confirmed by imaging on agarose gel.

Results

According to the results obtained, the isolation of 4 strains was successfully isolated in cell culture. The presence and number of the obtained strains were confirmed by the Stamp staining conventional and real-time PCR methods. Among these, the strains showing the best multiplication curves were determined based on the number of agents in cell culture samples taken at certain periods. While adapting and comparing the gastric fluid samples of these 4 positive agents for all 3 cell lines, the remaining 3 samples were passed one into the Vero cell line, one into the monolayer BHK-21, and one into the suspended BHK-21 cell line. The findings obtained as a result of the adaptation of the gastric fluid sample were explained in detail and evaluated along with the real-time PCR assay taken from the last stage of the remaining samples.

Vero Cell Line

For the Vero cell line, a sample was taken for staining 24 hours after the inoculation, at the stage when it was centrifuged, and the medium was replaced with medium without FBS. Before the inoculation, $5.4x10^5$ live cells /mL were found according to the cell counting device. After staining, a few agents were observed inside the cell in the Vero cell line, and a few agents were observed outside the cell on the floor of the staining area.

After the third day of incubation in a medium without FBS, both staining and conventional PCR were performed. In the staining, it was observed that the number of cells not containing the agent decreased and that in the stained cells, the presence of the agent was in greater density and abundance. At this stage, no band from the agent was observed on the performed conventional PCR. Samples were taken for real-time PCR and compared with growth in other cell lines.

In the staining performed after the 7th day in the medium without FBS, similarly to the previous staining, agents were observed inside the cell, and those that could not enter the cell or completed the reproductive cycle and had exited the cell, on the floor of the staining area. At this stage, the cells were counted with a cell counting device, finding 1x106 live cells/mL. The positive result in the conventional PCR performed on the sample taken confirmed the presence of the agent also by PCR (WOAH 2018) (Figure 1, Figure 2, Figure 3).

Monolayer BHK-21 Cell Line

Similarly, for the monolayer BHK-21 cell line, samples were taken for staining 24 hours after inoculation. Before the inoculation, $2.1x10^5$ live cells/mL were found by the cell counting device. During the staining, the presence of sporadic agents was observed in the cells and on the staining surface.

After the 2nd day of incubation in medium without FBS - that is, on the 3rd day after the inoculation - staining and conventional PCR were performed. In the staining, the agents were observed within the cell more abundantly and more clearly compared to the Vero cell line. A weak band was seen as a result of the conventional PCR of the sample. The results were interpreted by taking samples for real-time PCR on the 3rd day of incubation in the media without FBS.

Staining and conventional PCR were repeated on the monolayer BHK-21 cell line at the end of the 5th day. As a result of the staining, it was determined that there was a qualitative increase in the number of agents both entering the cell and on the ground, compared to the previous one. The cell count performed at this stage revealed $3x10^6$ live cells/mL. The conventional PCR result was also found positive (Figure 4).

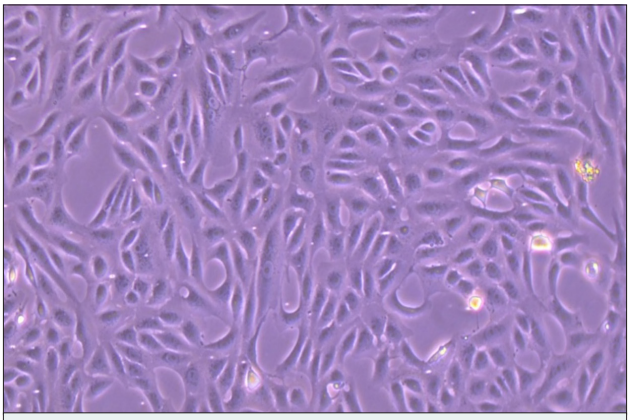

Figure 1. Day 0 before inoculating the Vero cell line (x100 magnification) (Section: 3.1. Vero cell line)

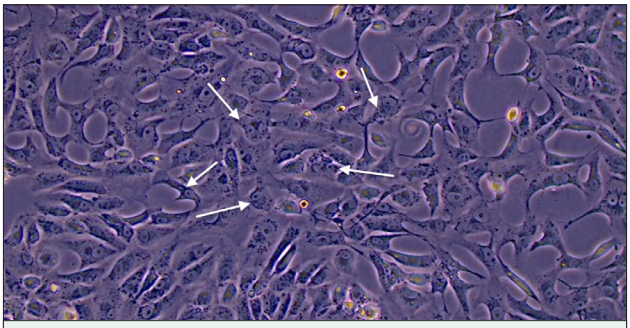

Figure 2. 24 hours after inoculating the Vero cell line, before centrifugation. Arrow; the agent is seen as small dots around the cell line (x100 magnification) (Section: 3.1. Vero Cell Line)

Suspended BHK-21 Cell Line

For the suspended BHK-21 cell line, staining was performed 24 hours after inoculating $5x10^5$ live cells/mL, just before transferring to an FBS-free medium. After staining, a few active cells and plenty of empty cells were observed on the slide.

On the second day of the transition to an FBS-free medium, 50 mL of the medium was taken and centrifuged, and then staining was performed from the pellet remaining at the bottom. After the staining, the cells, along with the agent both inside and outside the cells, on the staining area were observed. Positivity was after observed in the samples taken for conventional PCR.

After staining on the 3rd day without FBS, agents were observed inside the cells and scattered on the floor of the staining area along with death cell debris. In the cell count performed at this stage, $4.7x10^7$ live cells/mL were found. Positivity was also determined in the conventional PCR performed. A sample was taken for real-time PCR, and all of these three cell lines were submitted to real-time PCR and the results were interpreted.

Real-Time PCR

Real-time PCR was performed to the sample of gastric fluid which was adapted to each cell line -Vero, monolayer BHK-21, and suspended BHK-21- on the third day without FBS along with the other positives samples also on the third day without FBS and which were adapted to separate cell lines. As a result, is it found that the cell line in which the agent reproduces in the highest amount in the shortest time is suspended BHK-21, and these results are compatible with the staining. Apart from this, it is observed that reproduction is also achieved in the Vero cell line and the monolayer BHK-21 cell line, but the number of agents produced in the same period of time is less than in the suspended cell line.

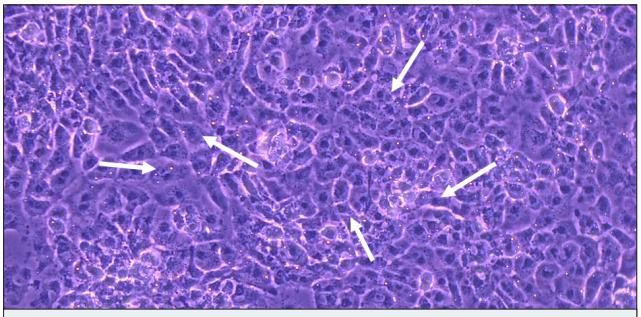

Figure 3. Transition phase in the Vero cell line to FBS-free medium after centrifugation at 24th hour. Arrows; Many C.abortus enter the cell after the process (x100 magnification) (Section: 3.1. Vero Cell Line)

As a result of the adaptation of different samples in different cell lines, it is seen that the reproduction of the agent is successful, but the highest reproduction curve is again in the suspended BHK-21 cell line (Figure 5).

It has been determined that, rather than the generally preferred cell line, the monolayer Vero cell line yields efficient results in terms of *C. abortus* reproduction and is the most economically suitable cell line for both routine researchers and the cell culture laboratory equipment used; nevertheless, the BHK-21 cell line is preferable in terms of the short reproduction period of the agent. Considering the time, equipment and financial resources of the researchers, it is demonstrated that the Vero cell line is the most financially suitable for *C. abortus*. It is concluded that the suspended BHK-21 cell may be preferred for the production of a higher number of agents, requiring a shorter time span and less equipment.

The fact that, after many years, *C. abortus* can be adapted from tissue and propagated into cell culture implies, as preliminary research for new studies to be conducted, that the most efficient reproduction from different tissue samples is obtained from gastric fluid and liver tissue in suspended BHK-21.

Discussion

Although Hela and McCoy are the preferred cell lines when isolating *C. trachomatis* in cell culture, McCoy is generally the preferred cell line for the isolation of *C. abortus* (Fong et al 1994). The McCoy cell line was first obtained from the synovial fluid of a human knee joint in

Figure 4. Cell and the burgundy stained factor indicated by the arrowhead Stamp staining of the monolayer BHK-21 cell line (x100 magnification) (Section: 3.2. Monolayer BHK-21 cell line)

1955 (Hsu and Moorhead 1957). In studies performed over time, it has been reported that the cell line derived from laboratory mice and modified to become the cell line known by being sensitive to Chlamydia spp and, thus, nowadays the cell line of preference is McCoy. However, in studies conducted, virus-like particles were detected in McCoy cells and Type-A and Type-C tumour viruses were identified, and it was thought that these tumours were the result of contamination with laboratory mice suffering from leukaemia (Kajima et al 1967). Although it is widely preferred today, the biological properties of this cell line and the fact that it contains endogenous retroviruses are ignored by most researchers. Reverse transcriptase testing is performed on commercial cell lines to confirm species origin and demonstrate the presence of endogenous retrovirus (Fong et al 1994).

The Vero cell line was first used in Japan in 1962 (Yasumura 1963) and was soon found to be sensitive to many viruses such as polyomavirus, measles, rubella, arbo- and adenoviruses, and bacterial toxins such as diphtheria toxin and Shiga-like toxin (Sasaki et al 1964, Liebhaber et al 1967, Rhim and Schell 1967, Simizu et al 1967, Rhim et al 1969, Miyamura et al 1974, Konowalchuk et al 1977, Speirs et al 1977, Remis et al 1984).

The BHK-21 cell line was obtained in 1961 from kidney fibroblasts of one-day-old baby hamsters and is sensitive to many viruses such as smallpox virus, herpes virus, rabies, pseudorabies, and foot-and-mouth virus (Stoker and Macpherson 1964).

While there are no isolation studies on *C. abortus* in Türkiye, there are studies from the past years on C. psitacci on embryonated chicken eggs, McCoy and BHK-21 cell lines (Türütoğlu 1995). In another study, it was reported that in our country, the prevalence of *C. abortus* within herds was 40%, and the prevalence between herds was up to 80%, but there is currently no data on isolation (Bulut et al 2004, Öztürk et al 2016, Aras et al 2017, Tuzcu et al 2022, Yur et al 2022).

As a result of our study, reproduction was achieved in Vero and BHK-21 cell lines, and the Vero cell line was evaluated to be more economical compared to other cell lines in laboratory. When the studies conducted so far are evaluated, the cell lines used for the isolation of *C. abortus* are mostly HeLa 229 (Campos-Hernández et al 2014), McCoy (Rekiki et al 2002, Maley et al 2009, Ortega et al 2015), BHK-21 (Jones et al 1995).

Although the replication period of the agent is stated to be 3-4 days in total according to the cell lines included in World Organisation for Animal Health (WOAH) 2018, in line with this current study, it was determined as 4-7 days with the Vero cell line, while 3-5 days in the monolayer BHK-21 cell line and 3-4 days in the suspended BHK-21 cell line. Considering all these, as a result of the study, which has not previously had such economic data, it is proven that the Vero cell line is more efficient in financial terms and the BHK-21 cell line in terms of time.

In addition, the Vero cell line was compared as a monolayer cell, while the BHK-21 cell line was compared as both suspended and monolayer. While more cells and this one

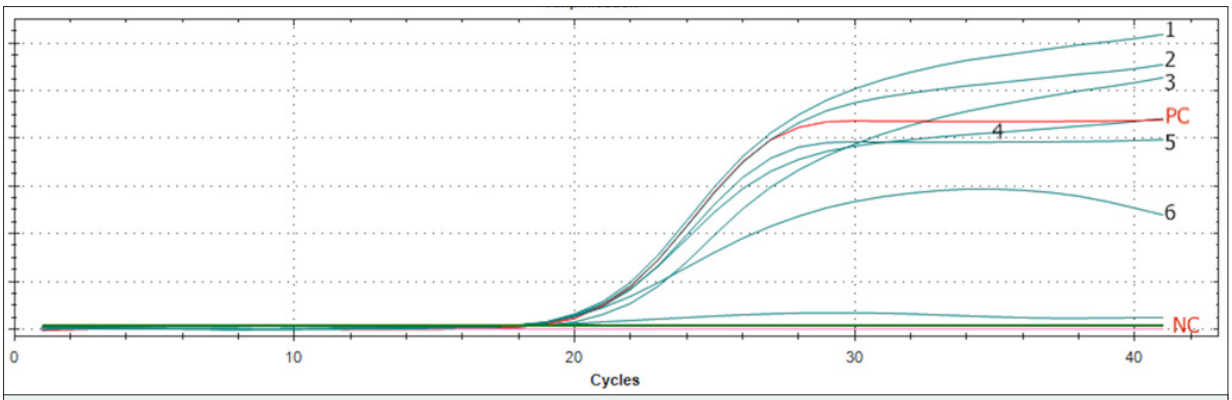

Figure 5; PC: Positive control, NC: Negative control, 1: Agent from gastric fluid adapted to suspended BHK-21, 2: Agent from fetal liver tissue adapted to suspended BHK-21, 3: Agent from gastric fluid adapted to monolayer BHK-21, 4: Agent from gastric fluid adapted to Vero cell line, 5: Agent from vaginal swap adapted to monolayer BHK-21, 6: Agent from bovine liver sample adapted to Vero cell (Section: 3.4 Real-Time PCR Results).

are produced in a shorter time in the suspended cell line, additional equipment, such as a magnetic stirrer or an incubated benchtop shaker, is required for the suspended cell line. While additional time is required for the same number of this one and cells in the monolayer cell line, the possibility of production in a petri dish or flask without the need for additional equipment is also cited as one of its advantages.

Conclusion

Vero cells were found to be the most cost-effective option for *C. abortus* isolation, providing good results with less need for equipment. Although BHK-21 cells allowed faster and more efficient reproduction, they may require more technical resources. Among the tested tissues, gastric fluid and liver samples showed the best results, especially in suspended BHK-21 cells. These findings show that *C. abortus* can still be adapted and grown in cell culture, and this method can be improved for future studies.

Declarations

Competing Interests

Authors declare that there are no conflicts of interest related to the publication of this article.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

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Ethical Statement

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine at the University of Selcuk in Konya, Türkiye (Grant number: 2020-70).

Author Contributions

Motivation/Concept: AB; Design: EET; Control/Supervision: OE, ZS; Data Collection and Processing: OD; Analysis and Interpretation: AI; Literature Review: EET, BP; Writing the Article: EET, BP; Critical Review: AB, ZS, OE

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