Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Development of a recombinant MOMP-based indirect ELISA for the serodiagnosis of caprine Chlamydiasis

Miguel Ángel De la Rosa-Ramos
Universidad Nacional Autónoma de México: Universidad Nacional Autonoma de Mexico
Fernando M Guerra-Infante
Instituto Nacional de Perinatología: Instituto Nacional de Perinatologia
Ángela Roque-López
Universidad Nacional Autónoma de México: Universidad Nacional Autonoma de Mexico
Cinthya I Santiago-Barrientos
Universidad Nacional Autónoma de México: Universidad Nacional Autonoma de Mexico
Laura Cobos-Marín
Universidad Nacional Autónoma de México: Universidad Nacional Autonoma de Mexico
José Luis Gutiérrez-Hernández
Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias
Gabriela Palomares-Resendiz
Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias
Rigoberto Hernández-Castro
Hospital General Dr Manuel Gea Gonzalez
Beatriz Arellano-Reynoso
arerey@yahoo.com

Universidad Nacional Autónoma de México: Universidad Nacional Autonoma de Mexico https://orcid.org/0000-0002-3067-2719
Francisco Suárez-Güemes
Universidad Nacional Autonoma de Mexico

Research Article

Keywords: Chlamydia abortus, ompA, recombinant MOMP, ELISA, goat, diagnosis

Posted Date: May 8th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9323769/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published at Tropical Animal Health and Production on October 26th, 2026. See the published version at https://doi.org/10.1007/s11250-026-05420-z.

Abstract

Caprine chlamydiosis, caused primarily by Chlamydia abortus, is a major cause of infectious abortion in small ruminants worldwide, leading to significant economic losses and posing a zoonotic risk. In developing countries, the high cost of imported commercial diagnostic kits limits routine epidemiological surveillance. The ompA gene was cloned into the pLATE vector and expressed in E. coli. The rMOMP was purified by affinity chromatography and used as the diagnostic antigen. Performance was evaluated using 82 goat sera and compared with a commercial kit. Robustness, precision, and stability (freeze-thaw and thermal stress) were analyzed in accordance with international validation standards. The iELISA demonstrated excellent diagnostic performance, with an Area Under the Curve (AUC) of 0.965, a sensitivity of 91.4%, and a specificity of 89.4%. The assay was highly precise, with repeatability (11.13%) and intermediate precision (17.91%) within the 20% acceptance threshold. Robustness testing showed stability against thermal fluctuations but identified incubation time as a critical control variable. Stability studies confirmed that serum samples remain reliable for up to 7 days under refrigeration (4 °C), although aliquoting is recommended to avoid signal loss (approx. 40%) observed after the first freeze-thaw cycle.

The rMOMP-based iELISA is a robust, highly sensitive, and cost-effective tool for the serological diagnosis of C. abortus. Its performance exceeds several commercial alternatives, positioning it as a valuable primary epidemiological filter for disease surveillance and control programs in goats, especially in regions with limited access to specialized diagnostic infrastructure.

1. Introduction

Caprine chlamydiosis, also known as Enzootic Abortion of Small Ruminants (EAR), is an infectious disease that severely affects livestock production. It is caused by bacteria of the genus *Chlamydia*, primarily *C. abortus* and *C. pecorum*, and manifests clinically as abortions during the last trimester of gestation (Rodolakis and Mohamad 2010; SADER 2016), thereby highlighting its economic impact on goat production. In addition to its production relevance, caprine chlamydiosis has significant zoonotic importance, posing a risk to public health, especially for pregnant women who have direct contact with infected animals (Escalante 2001; Aldama et al., 2022; Guerra-Infante et al., 2025). Consequently, timely diagnosis and the use of effective detection tools are essential for implementing appropriate prevention and control strategies.

Currently, the diagnosis of caprine chlamydiosis relies on detecting *Chlamydia* DNA using molecular techniques such as PCR or qPCR, or on bacterial isolation in cell culture. While these methods offer high accuracy and sensitivity, their high cost and technical complexity severely limit their routine application in field conditions (Serrato 2014; Opta et al., 2015; Sachse et al., 2009). Furthermore, the availability of commercial serological kits is limited in certain regions, and their prohibitive prices are often unsustainable for most goat producers in developing countries (Rueda 2018).

Recombinant proteins have gained widespread acceptance in recent decades for the design of diagnostic tests and the production of vaccines (Guevara 2012). This acceptance stems from their ability to induce specific antibodies in the host and from the benefit of expressing these proteins at high levels, given the inherent robustness of the culture system in which they are induced.

The protein selected for this tool is the Major Outer Membrane Protein (MOMP) of *C. abortus* (encoded by the *ompA* gene and expressed in *Escherichia coli*). MOMP is considered the most abundant and antigenic protein of the outer membrane (Forsbach et al., 2010), making it a key component utilized in multiple diagnostic tests (Santiago 2019; Hagemann et al., 2016). Consequently, the present research project focuses on the development and standardization of an in-house ELISA based on a recombinant *C. abortus* OmpA protein.

2. Materials and Methods

2.1 Bacterial strains and growth conditions

Escherichia coli BL21(DE3) strain (Invitrogen, Carlsbad, CA, USA) was transformed with the entire *ompA* gene of *Chlamydia abortus*, labeled with a poly-His tag, in the pLATE31 vector (Thermo Fisher Scientific, Waltham, MA, USA). The procedure was according to the manufacturer's instructions. The bacteria were grown in Luria-Bertani (LB) agar (Sigma-Aldrich, St. Louis, MO, USA) containing ampicillin (50 mg/L). Once the colonies were obtained, the presence of the insert was verified by PCR using the primers LIC-F: 5'-TAATACGACTCACTATAGGG-3' and LIC-R: 5'-GAGCGGATAACAATTTCACACAGG-3' (Santiago, 2019).

2.2 Protein expression and purification of the recombinant MOMP (rMOMP)

For protein expression, the transformed *E. coli* strain was cultured overnight in a 5 mL aliquot of LB broth with ampicillin, then subcultured into 800 mL of fresh LB broth with ampicillin. The transformed cells were incubated at 37 °C until the optical density ($OD_{600\ nm}$) reached 0.5–0.8. Protein expression was then induced with 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (Sigma-Aldrich, St. Louis, MO, USA) for 5 hours at 37 °C with orbital shaking at 200 rpm.

The cells were harvested by centrifugation at 3500×g for 15 minutes and resuspended in Phosphate-Buffered Saline (PBS). Total proteins were subsequently obtained using the Ready Protein Extraction kit (soluble/insoluble) (BIORAD, Hercules, CA, USA) according to the manufacturer's instructions. The poly-His-labeled protein was purified by FPLC (ÄKTA pure, GE Healthcare, Fairfield, Connecticut, USA), utilizing a HisTrap HP column (Cytiva, Uppsala, Sweden) according to the manufacturer's instructions.

2.3 SDS-PAGE and protein sequencing

Purified recombinant *C. abortus* rMOMP, treated with 2× Laemmli loading buffer (Laemmli, 1970), was boiled for 5 minutes and centrifuged for 5 minutes at 10,000×g. The samples were subsequently loaded into a 12% SDS-PAGE gel and stained with Coomassie Blue R (Sigma-Aldrich). Sequencing was later performed at the Biotechnology Institute of UNAM (Universidad Nacional Autónoma de México) (Supplementary Information SI1). The final protein concentration was determined using the Bradford assay (Bradford 1976). Samples were stored at -20 °C until analysis.

2.4 Animals and sera

This protocol was approved by the Internal Committee for the Care and Use of Animals (CICUA) of the FMVZ UNAM (Faculty of Veterinary Medicine and Zootechnics - UNAM), under protocol No. 142.

In this study, serum samples from 82 goats were used. Blood samples (approximately 5 mL) were collected via jugular venipuncture using a sterile vacuum system (BD Vacutainer, New Jersey, USA) without anticoagulant. To ensure complete clot formation, the tubes were maintained in a vertical position at room temperature for 45 minutes and subsequently transported under refrigeration (4 °C) to the laboratory. The samples were then centrifuged at 1,500×g for 10 minutes to separate the serum. The resulting supernatant was aliquoted into sterile 1.5 mL microcentrifuge tubes and stored at -20°C until further analysis.

Positive sera (n=35) were obtained from animals originating in areas with previous reports of caprine chlamydiosis, while negative sera (n=47) were collected from clinically healthy goats belonging to herds with no prior history of chlamydiosis or abortion diseases.

2.5 Detection of anti-*C. abortus* antibodies by the ELISA test

2.5.1 Commercial kit

The presence of specific antibodies against *C. abortus* in the collected goat sera was determined using the IDScreen® Chlamydia abortus Indirect Multi-species (CHLMS-MS ID-Vet) antibody test (ID-Vet, Grabels, France). This commercial indirect ELISA kit uses a synthetic antigen from the Major Outer Membrane Protein (MOMP) of *C. abortus*. The assay was performed according to the manufacturer's instructions. The optical density (OD) was measured at 450 nm using a microplate reader. For each sample, the S/P percentage (Sample/Positive ratio) was calculated. Following the kit's diagnostic criteria, samples with an S/P% ≤ 50% were considered negative, those between 50% and 60% were classified as doubtful, and those with an S/P% ≥ 60% were considered positive.

2.5.2 In-house-made ELISA (CA-iELISA)

Goat sera were analyzed using a house-made ELISA based on the method proposed by De Haro et al. (2019). Nunc™ C96 Maxisorp immunoadsorption plates (Thermo Scientific Inc., Rockford, IL, USA) were coated with 100 µL of 1 µg/mL recombinant rMOMP in 0.05 M carbonate-bicarbonate buffer (pH 9.6) and refrigerated overnight. The plates were washed three times with 0.05% Tween-20 in phosphate-buffered saline (PBS-T) (Sigma-Aldrich). The wells were blocked with 1% bovine serum albumin (BSA) in PBS-T at 200 µL per well for 1 h at room temperature, then washed three times with PBS-T. 100 µL of diluted goat serum (1:200 in PBS) was added to each well, and the plate was incubated for 1 h at 37 °C. The plates were washed three times with PBS-T. The rabbit anti-goat immunoglobulin secondary antibody conjugated with horseradish peroxidase (HRP**)** (Cappel, Solon, Ohio, USA) was diluted 1:15,000 in PBS-T. 100 µL of this diluted secondary antibody was added to each well and incubated for 1 h at 37 °C. Following incubation, the plates were washed three times with PBS-T and subsequently developed using the commercial TMB Substrate kit (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions. Optical Density (OD) was determined at 490 nm using an ELx808 (BioTek, Winooski, VT, USA).

For the analysis of the results, the following equation (1) was used.

(1) S/Co ratio = $(Abs_{sample} - Abs_{negative\ control}) / (Abs_{positive\ control} - Abs_{negative\ control})$

The signal-to-cutoff (S/Co) ratio was calculated as the quotient of the difference between the sample absorbance and the negative control absorbance, divided by the difference between the positive and negative control absorbances. Samples with an S/Co index equal to or greater than the cutoff value were considered positive.

2.6 CA-iELISA validation protocol

The cutoff point was established following a previously described method byFrey et al. (1998) and was obtained using the following equation (2).

$$(2)\ CUTOFF\ point = X + SD\ t\sqrt{1 + (\tfrac{1}{n})}.$$

Results exceeding this value were considered positive for *C. abortus* infection. Subsequently, the assay's performance accuracy was verified by calculating intermediate precision and repeatability in accordance with the recommendations of Andreasson et al. (2015). In this procedure, the coefficient of variation (%CV) was determined from a series of independent measurements taken on different days (intermediate precision) and from simultaneous measurements (repeatability).

The method's selectivity was evaluated using caprine sera positive for diseases other than chlamydiosis, specifically Caprine Arthritis Encephalitis (CAE) and Brucellosis, to assess potential cross-reactivity. To evaluate the stability of the samples, 19 aliquots of both positive and negative control serum were prepared and subjected to freeze-thaw cycles following the protocols described by Andreasson et al. (2015) and Palacios et al. (2021) (Supplementary Information SI2).

2.7 Statistical method

The sensitivity and specificity of the CA-iELISA test were evaluated using the 2 × 2 contingency tables, comparing the results against the reference commercial kit. The diagnostic performance of the assay was further analyzed using Receiver Operating Characteristic (ROC) curves to determine the Area Under the Curve (AUC) as an indicator of global accuracy.

All experimental data, including the evaluation of precision and stability, were analyzed using one-way ANOVA followed by Tukey's post-hoc tests for multiple comparisons. A p-value < 0.05 was considered statistically significant. Statistical analyses and ROC curve generation were performed using IBM SPSS Statistics version 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1 *ompA* gene and recombinant MOMP identified from transformed *E. coli*

An amplification product of 1,346 bp was obtained using the LIC-F and LIC-R primer sets in the transformed *E. coli*. This bacterium was cultured for 5 h in LB broth supplemented with IPTG to produce recombinant MOMP (rMOMP). The purification and presence of rMOMP were demonstrated by 12% SDS-PAGE, which showed a ~40 kDa protein. The identity of the rMOMP protein was confirmed by sequencing at the Instituto de Biotecnología – UNAM (Supplementary Information SI1).

3.2 Establishment of indirect CA-iELISA

Eighty-two caprine serum samples were used to identify the sensitivity and specificity of CA-iELISA. These sera were previously analyzed in the commercial kit ID Screen® *Chlamydia abortus* Indirect Multi-species, of which 35 sera were positive, and 47 sera were negative.

To identify IgG antibodies against *C. abortus* in these sera using the CA-iELISA assay, an antigen concentration of 1 µg/well and a serum dilution of 1:200 were used. The optimal S/Co cutoff value was 0.2762 OD; with this value, 37 positive and 45 negative samples were obtained (Table 1).

The sensitivity and specificity of the CA-iELISA assay relative to the ID Screen® *Chlamydia abortus* Indirect Multi-species test were 91.4% and 89.4%, respectively (Table 1).

Fig. 1 shows the cutoff point of the CA-iELISA assay at 490 nm of OD. A sample was considered positive when the absorbance value was higher than or close to 0.28, while a negative sample was considered when the value was below 0.28. The sensitivity and specificity were evaluated using receiver operating characteristic (ROC) curves based on the results of the ID Screen® *Chlamydia abortus* Indirect Multi-species test (Fig. 2). The sensitivity was 91.4%, the specificity was 89.4%, and the area under the curve (AUC) was 0.965 ($p < 0.0001$).

3.3 CA-iELISA validation

The CA-iELISA assay precision was determined by evaluating repeatability and intermediate precision. Repeatability (intra-assay precision) yielded a mean value of 11.13%, while intermediate precision (inter-assay precision), calculated from 25 independent measurements over five days, was 17.91%.

Robustness. Following the protocols established by Andreasson et al. (2015), assay robustness was evaluated against variations in incubation temperature (±5 °C) and time (±5 min). One-way analysis of variance (ANOVA) for positive sera showed no statistically significant differences in thermal variation relative to the standard temperature of 37 °C ($p > 0.05$). However, differences were detected between the extremes of 32 °C and 42 °C. In contrast, negative sera showed highly significant differences across all temperature comparisons ($p < 0.001$).

Regarding the time factor, positive sera remained stable when incubation was reduced by 5 min, but showed a significant decrease in absorbance when incubation was prolonged (+5 min). For negative sera, any deviation from the standard time resulted in significant changes ($p<0.05$); specifically, shortening the incubation time increased optical density (OD) values, shifting them closer to the cut-off point.

Sample stability was assessed with 19 aliquots under different conditions, with a sample stored at –20 °C for one month as the reference. After the first freeze-thaw cycle, signal recovery decreased to 59.96%, stabilizing between 73% and 84% in subsequent cycles.

Under refrigeration (4 °C), recovery levels ranged from 85.38% to 108.90% over a 168-hour period. Conversely, samples maintained at room temperature exhibited fluctuating recovery rates, ranging from 72.2% to 98.3%. Based on the criteria of Andreasson et al. (2015), the assay demonstrated optimal stability at 4 °C under refrigeration and effective long-term preservation at –20 °C.

Selectivity. Selectivity testing identified a cross-reaction (false positive) in sera from animals infected with lentivirus (the causative agent of caprine arthritis-encephalitis). At the same time, no reactivity was recorded in the remaining samples evaluated.

4. Discussion

The presumptive diagnosis of *C. abortus* infection is based on the herd's clinical history and the characteristic placental lesions of EAR. However, due to its similarity to other reproductive pathologies, a definitive diagnosis requires laboratory confirmation (Sachse et al. 2009). The detection of *Chlamydia* spp. PCR or qPCR enables direct identification in clinical samples (Kaltenboeck et al. 1997; Yoshida et al. 1998; Pantchev et al. 2010), often targeting the *ompA* gene. Although this methodology represents a fundamental diagnostic standard (Pospischil 2006; Longbottom et al. 2002; Opota et al. 2015), it has significant limitations, including the need for specialized infrastructure, high operational costs, and highly trained personnel.

In contrast, serology remains the most widely used method for detecting anti-*Chlamydia* antibodies. Although post-isolation immunofluorescence is considered the gold standard, it is a labor-intensive technique subject to observer interpretation and high inter-laboratory variation (Longbottom and Coulter 2003; Baud et al. 2010). Furthermore, the complement fixation (CF) test, based on LPS, has limited specificity; low titers may result from *C. pecorum* infections or cross-reactions with Gram-negative bacteria such as *E. coli* and *Salmonella* spp. (Aitken and Longbottom 2004). Additionally, its application is unfeasible in hemolytic or anticomplementary sera, and its analytical efficacy is reduced by the inability of certain immunoglobulin isotypes to fix complement (Cross and Clafin 1963; Schmeer et al. 1987).

In this regard, the ELISA assay has established itself as a high-sensitivity, high-specificity alternative, driving the commercialization of assays based on synthetic peptides or recombinant proteins targeting the Major Outer Membrane Protein (MOMP) (Mygind et al., 2000; Bandehpour et al., 2006; Baud et al., 2010). However, many of these commercial kits have failed to achieve the expected diagnostic performance for identifying active infections in complex cases (Land et al., 2003; Baud et al., 2010). This highlights the need to develop assays with optimized analytical parameters to improve diagnostic precision.

In the present study, the MOMP of *C. abortus* was selected as the target for the iELISA because it is a highly immunogenic antigen with an approximate molecular weight of 40 kDa and is the bacterium’s primary immunodominant component. Its selection is based on its prominent surface exposure, representing nearly 60% of the total protein mass of the outer membrane, and its content of both genus- and species-specific epitopes. Furthermore, it plays a critical role in the interaction between the bacterium and the host cell (Baehr et al. 1988; Salti-Montesanto et al. 1997; Vretou et al. 2007;

McCauley et al. 2007; Forsbach-Birk et al. 2010; Feher et al. 2013). These characteristics are fundamental, as they facilitate the induction of a robust humoral immune response in goats, a key factor in optimizing the assay's diagnostic sensitivity.

The use of rMOMP expressed in *E. coli* offers significant advantages over native antigens derived from cell cultures. Traditional methods for obtaining *Chlamydia* antigens are labor-intensive and costly, and they involve biosafety risks associated with handling live infectious agents. The purification and reconstitution of native MOMP are complex due to the dense network of disulfide bonds in the outer membrane and the presence of the Chlamydia Outer Membrane Complex (COMC). Specifically, MOMP has been identified to contain conserved cysteine residues that form intramolecular and intermolecular disulfide bonds with other COMC proteins, such as OmcA and OmcB (Hatch et al. 1984; Yen et al. 2005). This structural conformation drastically reduces the protein's solubility under native conditions, thereby hindering its purification and characterization (Wang et al. 2006). In contrast, recombinant production in *E. coli*, a system with a well-characterized genome and metabolism, enables a standardized, safe, and high-yield manufacturing process, reaching production scales of grams per liter (Georgiou and Segatori, 2005; Demain and Vaishnav, 2009).

Our results demonstrate that the rMOMP produced by this expression system retains its antigenic integrity and the ability to effectively discriminate between positive and negative caprine sera. This finding is consistent with previous reports proposing recombinant proteins as a cost-effective and accessible alternative for serological diagnosis, particularly in developing regions where access to cell culture technologies is limited (Schumann and Ferreira 2004; Khan et al. 2016).

In this study, the pLATE/ompA vector was constructed by integrating the full-length gene sequence to ensure an effective and specific immune response. DNA sequencing of the rMOMP construct confirmed the presence of the *ompA* gene and demonstrated full identity with the reference nucleotide and amino acid sequences of *C. abortus*. The molecular weight, estimated *in silico* at 40 kDa based on the amino acid sequence, was corroborated by affinity chromatography purification; SDS-PAGE analysis revealed a single band with the expected electrophoretic mobility. Based on these findings, the isolated protein was confirmed as *C. abortus* rMOMP.

Subsequently, the rMOMP was used to develop an indirect ELISA for detecting anti-*C. abortus* IgG antibodies in goats. The initial critical parameter was determining the cut-off point, as establishing a reliable value is essential for diagnostic discrimination between infected and non-infected individuals. Various methods have been proposed to optimize this threshold (López-Ratón et al. 2014), all of which require well-characterized positive and negative controls to maximize the assay's sensitivity and specificity.

Typically, in the development of in-house ELISA assays, cut-off values are estimated using independent negative sera included in the titration plates. A conventional formula for this calculation uses the mean $(\bar{x})$

, and standard deviation (SD) of these control readings; however, in such cases, multipliers are often set arbitrarily (e.g., defining the cut-off as two or three times the mean absorbance of the negative controls). In contrast, Frey et al. (1998) propose a more rigorous statistical approach in which the cut-off point is determined using a probabilistic method. This approach provides greater statistical certainty in defining positivity than traditional empirical methods, thereby optimizing diagnostic reliability (Lardeux et al. 2016).

Afterward, the overall performance of the developed CA-iELISA was evaluated through ROC curve analysis, yielding an Area Under the Curve (AUC) of 0.965. This value indicates excellent discriminative capacity (Cerda and Cifuentes 2012), falling within the ranges reported for serological tests based on recombinant *Chlamydia* antigens. Although reports indicate AUC values exceeding 0.80 with the MOMP protein, the performance observed in this study is highly competitive. Minor variations in specificity may be attributed to heterogeneity in the immune response under field conditions and to potential prior exposure of animals to other *Chlamydia* species, such as *C. pecorum*, which can influence the assay's cross-reactivity (Rodolakis and Laroucau 2015; Hernández-Ramírez et al.2025).

The selection of this threshold prioritizes diagnostic sensitivity, a strategic decision considering that caprine chlamydiosis is a disease with high reproductive impact and zoonotic potential. In infectious disease control programs, it is essential to use screening tests with high detection rates (few false negatives), to enable the timely identification of seropositive animals and the implementation of biosafety measures. The observed specificity (89.4%), while moderate, is competitive with other local diagnostic methods and highlights the test's value as a primary epidemiological filter in regions where access to commercial kits is limited.

Minor variations in specificity may be attributed to heterogeneity in the immune response under field conditions and to potential prior exposure of animals to other *Chlamydia* species, such as *C. pecorum*, which can influence the assay's cross-reactivity (Rodolakis and Laroucau 2015; Hernández-Ramírez et al. 2026). However, a limitation of the present study was the inability to test the CA-iELISA against a characterized panel of sera from animals confirmed to be infected with other *Chlamydia* species or related pathogens, which would have allowed for a more precise evaluation of analytical specificity. Furthermore, since the samples were obtained from field conditions, the infection stage of the seropositive animals, whether in a latent phase or during active shedding, could not be confirmed via gold-standard methods such as bacterial isolation or qPCR. Future research involving experimentally infected animals and a broader range of cross-reactive pathogens is necessary to further refine the diagnostic accuracy of this assay.

A fundamental aspect in validating this CA-iELISA was ensuring the reproducibility and robustness of the results. According to the criteria of Andreasson et al. (2015), the precision values obtained confirm that the detection system is stable against typical variations in the analytical environment. Although literature suggests that intermediate precision should not exceed twice the repeatability, this criterion is flexible depending on the method's nature; therefore, a coefficient of variation (%CV) of less than 20% was adopted as the acceptance threshold (Jacobson 1998). The achieved levels demonstrated effective minimization of inter-batch and inter-operator variability, thereby indirectly validating the success of the rMOMP purification process and the protocol’s standardization.

Regarding robustness, the assay showed stability against accidental thermal variations, minimizing interference from incubator fluctuations. However, incubation time proved to be a critical variable showing statistically significant differences and requiring strict adherence to established times by the operator.

Stability testing revealed that to preserve analyte integrity in the field, samples should be kept at 25 °C for less than four hours or refrigerated for up to 24 hours prior to processing. While long-term freezing (one month) maintained reactivity, moderate sensitivity to physical stress due to ice crystal formation was observed. Consequently, to ensure maximum diagnostic fidelity, aliquoting of original samples is recommended to avoid repeated freeze-thaw cycles. Furthermore, the stability of the rMOMP antigen used for plate coating is a key factor for assay reproducibility. In this study, the recombinant protein remained stable when stored at -20 °C in a carbonate-bicarbonate buffer. However, it is well-documented that the secondary and tertiary structures of the MOMP, which are rich in cysteine residues, are susceptible to oxidative stress and temperature-induced aggregation. Therefore, while our results demonstrate consistent performance over three months, long-term evaluation of rMOMP antigenic stability and the use of specialized protein stabilizers are recommended to ensure the shelf-life of the pre-coated plates.

Several studies have evaluated the humoral response to the *C. abortus* rMOMP protein in sera from naturally and experimentally infected animals by ELISA. In this regard, Hagemann et al. (2016) reported a robust antibody response in sheep beginning in the second week post-infection, which persisted for up to 47 weeks; significantly, this assay showed no cross-reactivity with sera positive for *C. pecorum* or *C. psittaci*, nor did it yield false positives in *C. abortus*-negative animals. Similarly, the use of recombinant antigens has demonstrated high diagnostic fidelity, as illustrated by Forsbach-Birk et al. (2010), who achieved 83% sensitivity and 100% specificity in an ELISA developed with the *C. trachomatis* rMOMP protein. Recently, our research group used this *C. abortus* rMOMP protein in an ELISA system to identify IgG anti-*C. abortus* in fertile patient sera. The results showed 10.7% positivity; of these, 7% were due to cross-reactivity with *C. trachomatis*

rMOMP. With these results, the *C. abortus* rMOMP protein can be used to identify patients who have been exposed to this bacterium.

The CA-iELISA developed in this study demonstrated a sensitivity of 91.4% and a specificity of 89.4%, superior to that of the reference commercial test (CHLMS-MS, IDvet, France). The difference in specificity may be due to the commercial kit detecting only IgG, whereas our system identifies total immunoglobulins, thereby broadening the detection spectrum. Furthermore, the higher sensitivity of the CA-iELISA compared to the commercial assay may stem from the use of the full-length rMOMP protein, which retains its four immunodominant variable regions.

MOMP-based commercial assays have historically shown greater precision than those based on LPS; for instance, studies in sheep report sensitivities of up to 94.74% with MOMP-based kits, compared with lower values with LPS-based kits (LSI Vet, Thermo Fisher Scientific, USA). The ELISA in this study showed competitive sensitivity, exceeding the levels reported for other commercial MOMP-based kits, such as MVD-Enfer (MVD, Uruguay) and IDvet, with 78.95% and 73.68%, respectively. Finally, our specificity was significantly higher than that reported by McCauley et al. (2007), who obtained 70.4% using synthetic MOMP peptides; this difference suggests that using the full-length protein in our assay minimizes the detection of cross-reactive immunoglobulins observed in peptide-based methods (Vretou et al., 2007; O'Neill et al., 2018).

A critical aspect of serological diagnosis is cross-reactivity, particularly in assays that use LPS as an antigen, where co-detection between *C. pecorum* and *C. abortus* is common, alongside cross-reactions with Gram-negative bacteria (*Salmonella* spp. and *Acinetobacter* spp.) that share structural epitopes in their LPS. In this context, the use of recombinant proteins such as rMOMP is a superior strategy for enhancing specificity. However, although the presence of *Chlamydia* species other than *C. abortus* was not confirmed in this study, any eventual cross-reactivity with other pathogenic *Chlamydiae* in small ruminants could represent a diagnostic advantage under field conditions. Since the reproductive disease complex in these species can be caused by various members of the genus, a broad-detection assay would facilitate comprehensive flock diagnosis. This would enable the implementation of sanitary control and management measures which, in clinical practice, are similar across different *Chlamydia* infections (Hernández-Trejo et al., 2014; Rahman et al., 2018; Hernández-Ramírez et al., 2026).

The primary strength of this iELISA lies in its high sensitivity and specificity, which outperform those of several commercial kits. However, a limitation of the present study was the lack of reactivity assessment in specific subpopulations, such as primiparous animals or those with recurrent infections. For commercial scalability, the assay must consistently detect antibodies across different stages of the production cycle.

In this regard, the kinetics of anti-MOMP antibodies must be carefully considered; although they are long-lasting surface markers, their persistence in small ruminants with normal parturition may be limited. Furthermore, literature indicates that anti-MOMP titers typically increase significantly around the time of abortion or parturition and decrease gradually thereafter (Pawlikowska and Deptuła, 2008). Therefore, the sampling timeframe relative to the reproductive event is a critical factor for diagnostic interpretation. In conclusion, the relevance of this test lies in its potential as an epidemiological surveillance tool, a biomarker of disease progression, and a platform for evaluating the immunogenicity of new vaccine candidates.

Declarations

Acknowledgements

Miguel A. De la Rosa-Ramos acknowledges the support from the “Secretaría de Ciencia, Humanidades, Tecnología e Innovación, México” through the 'Formación y Consolidación de las y los Investigadores por México' program. The authors

are also grateful to Miriam Marleni García-Clara, and Omar Cortéz-Hernández for their valuable technical support and assistance.

Funding

This research was funded by Dirección General de Asuntos del Personal Académico, Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (DGAPA-PAPIIT), grant number IT202224.

Conflict of interest

The author(s) declare that they have no competing interests.

Credit authorship contribution statement

Miguel A De la Rosa-Ramos: Investigation, Formal analysis, Visualization, Validation, Data curation, Writing – original draft, **Fernando M Guerra-Infante:** Conceptualization, Supervision, Writing – review & editing, **Ángela Roque-López:** Investigation, Formal analysis, Validation, Data curation, Writing – review & editing, **Cinthya I Santiago-Barreto:** Investigation, Formal analysis, Validation. **Laura Cobos-Marín:** Investigation, Validation, Data curation, **José Luis Gutiérrez-Hernández:** Investigation, Validation, Data curation, **Gabriela Palomares-Resendiz:** Investigation, Validation, Data curation, **Rigoberto Hernández-Castro:** Investigation, Formal analysis, Validation, **Beatriz Arellano-Reynoso:** Conceptualization, Supervision, Investigation, Visualization, Funding acquisition, Writing, review & editing, **Francisco Suárez-Güemes:** Conceptualization, Supervision, Visualization, Funding acquisition. All authors read and approved the final manuscript.

Ethics approval

This protocol was approved by the Internal Committee for the Care and Use of Animals (CICUA) of the FMVZ UNAM (Faculty of Veterinary Medicine and Zootechnics - UNAM), under protocol No. 142.

Data availability statement

All data supporting the findings of this study are available within the paper and its Supplementary Information.

References

1. Aitken ID, Longbottom D (2004) Enzootic abortion of ewes (ovine chlamydiosis). In: OIE Biological Standards Commission (eds) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Office International des Epizooties, Paris, pp 635–641
2. Aldama FJ, Montes de Oca R, Varela JA (2022) Diagnóstico, prevención y control de enfermedades causadas por *Chlamydia* en pequeños rumiantes. Revisión. Rev Mex Cienc Pecu 13(3):725–742. https://doi.org/10.22319/rmcp.v13i3.5564
3. Andreasson U, Perret-Liaudet A, van Waalwijk van Doorn LJ et al. (2015) A practical guide to immunoassay method validation. Front Neurol 6:179. https://doi.org/10.3389/fneur.2015.00179
4. Baehr W, Zhang YX, Joseph T, Su H, Nano FE, Everett KD, Caldwell HD (1988) Mapping antigenic domains expressed by *Chlamydia trachomatis* major outer membrane protein genes. Proc Natl Acad Sci USA 85(11):4000–4004. https://doi.org/10.1073/pnas.85.11.4000
5. Bandehpour M, Seyed N, Shadnoush M, Pakzad P, Kazemi B (2006) Using recombinant *Chlamydia* major outer membrane protein (MOMP) in ELISA diagnostic kit. Iran J Biotechnol 4:239–244. http://www.ijbiotech.com/article_6978.html
6. Baud D, Regan L, Greub G (2010) Comparison of five commercial serological tests for the detection of anti-*Chlamydia trachomatis* antibodies. Eur J Clin Microbiol Infect Dis 29:669–675. https://doi.org/10.1007/s10096-010-0912-4

7. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3
8. Cerda J, Cifuentes L (2012) Using ROC curves in clinical investigation: theoretical and practical issues. Rev Chilena Infectol 29(2):138–141. https://doi.org/10.4067/S0716-10182012000200003
9. Cross RF, Clafin RM (1963) The incidence and removal of procomplementary activity in swine serum. Am J Vet Res 4:334–336
10. Demain AL, Vaishnav P (2009) Production of recombinant proteins by microbes and higher organisms. Biotechnol Adv 27(3):297–306. https://doi.org/10.1016/j.biotechadv.2009.01.008
11. De Haro-Cruz MJ, Guadarrama-Macedo SI, López-Hurtado M, Escobedo-Guerra MR, Guerra-Infante FM (2019) Obtaining an ELISA test based on a recombinant protein of *Chlamydia trachomatis*. Int Microbiol 22:471–478. https://doi.org/10.1007/s10123-019-00074-4
12. DOF (2016) Acuerdo mediante el cual se dan a conocer en los Estados Unidos Mexicanos las enfermedades y plagas exóticas y endémicas de notificación obligatoria de los animales terrestres y acuáticos. Diario Oficial de la Federación, 4 de mayo de 2016. http://www.dof.gob.mx/nota_detalle.php?codigo=5436016&fecha=04/05/2016
13. Escalante OC, Lazcano A, Soberón M (2001) *Chlamydophila* spp. como agente zoonótico en México: reporte de un caso. In: Abstracts of XXXVII Reunión Nacional de Investigación Pecuaria, Tuxtla Gutiérrez, Chiapas, México, p. 41. https://abanicoacademico.mx/revistasabanico/index.php/abanico-veterinario/article/view/321/853
14. Feher VA, Randall A, Baldi P, Bush RM, de la Maza LM, Amaro RE (2013) A 3-dimensional trimeric β-barrel model for *Chlamydia* MOMP contains conserved and novel elements of Gram-negative bacterial porins. PLoS ONE 8(7): e68934. https://doi.org/10.1371/journal.pone.0068934
15. Forsbach-Birk V, Simnacher U, Pfrepper KI, Soutschek E, Kiselev AO, Lampe MF, Meyer T, Straube E, Essig A (2010) Identification and evaluation of a combination of chlamydial antigens to support the diagnosis of severe and invasive *Chlamydia trachomatis* infections. Clin Microbiol Infect 16(8):1237–1244. https://doi.org/10.1111/j.1469-0691.2009.03003.x
16. Foster CN, Rossi UA, Rossetti CA (2025) Validation of an in house iELISA for serodiagnosis of caprine brucellosis and evaluation of the performance of a *B. neotomae* lysate. Vet Microbiol *302:110389*. https://doi.org/10.1016/j.vetmic.2025.110389
17. Frey A, Di Canzio J, Zurakowski D (1998) A statistically defined endpoint titer determination method for immunoassays. J Immunol Methods 221(1-2):35–41. https://doi.org/10.1016/s0022-1759(98)00170-7
18. García-Perdomo HA (2016) Asociación de infección por *Chlamydia trachomatis* con el diagnóstico de cervicitis aguda. MedUNAB 19(3):190–191. https://doi.org/10.29375/01237047.2631
19. Georgiou G, Segatori L (2005) Preparative expression of secreted proteins in bacteria: status report and future prospects. Curr Opin Biotechnol 16(5):538–545. https://doi.org/10.1016/j.copbio.2005.07.008
20. Guerra-Infante FM et al (2025) Comparative evaluation of recombinant *Chlamydia abortus* and *Chlamydia trachomatis* Major Outer Membrane Proteins for diagnosing human chlamydial infection. Microbiol Res 16:159. https://doi.org/10.3390/microbiolres16070159
21. Hagemann JB et al (2016) Analysis of humoral immune responses to surface and virulence-associated *Chlamydia abortus* proteins. J Clin Microbiol 54(7):1883–1890. https://doi.org/10.1128/JCM.00351-16
22. Hatch TP, Allan I, Pearce JH (1984) Structural and polypeptide differences between envelopes of infective and reproductive life cycle forms of *Chlamydia spp.* J Bacteriol 157(1):13–20. https://doi.org/10.1128/jb.157.1.13-20.1984
23. Hernández-Ramírez PA et al (2025) Presence of *Chlamydia suis* and *Chlamydia abortus* in pigs from Mexico: first report. Accepted Rev Colomb Cienc Pecu. https://doi.org/10.17533/udea.rccp.e361091
24. Kaltenboeck B et al (1997) Use of synthetic antigens improves detection by ELISA of antibodies against abortigenic *Chlamydia psittaci* in ruminants. J Clin Microbiol 35(9):2293–2298. https://doi.org/10.1128/jcm.35.9.2293-2298.1997

25. Khan S et al (2016) Role of recombinant DNA technology to improve life. Int J Genomics 2016:2405954. https://doi.org/10.1155/2016/2405954
26. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685. https://doi.org/10.1038/227680a0
27. Land JA et al (2003) Performance of five serological chlamydia antibody tests in subfertile women. Hum Reprod 18(12):2621–2627. https://doi.org/10.1093/humrep/deg479
28. Lardeux F, Torrico G, Aliaga C (2016) Calculation of the ELISA's cut-off based on the change-point analysis method. Mem Inst Oswaldo Cruz 111(8):501–504. https://doi.org/10.1590/0074-02760160119
29. Longbottom D, Coulter LJ (2003) Animal chlamydioses and zoonotic implications. J Comp Pathol 128(4):217–244. https://doi.org/10.1053/jcpa.2002.0629
30. Longbottom D et al (2002) Serological diagnosis of ovine enzootic abortion by ELISA with recombinant POMP90. J Clin Microbiol 40(11):4235–4243. https://doi.org/10.1128/JCM.40.11.4235-4243.2002
31. McCauley LM et al (2007) Comparison of ELISA and CFT assays. Aust Vet J 85(8):325–328. https://doi.org/10.1111/j.1751-0813.2007.00189.x
32. Mygind P, Christiansen G, Persson K, Birkelund S (2000) Detection of *Chlamydia trachomatis*-specific antibodies. J Med Microbiol 49:457–465. https://doi.org/10.1099/0022-1317-49-5-457
33. O'Neill LM et al (2018). Comparison of three commercial serological tests for the detection of *Chlamydia abortus* infection in ewes. Irish Vet J 71, 13. https://doi.org/10.1186/s13620-018-0124-2
34. Opota O et al (2015) Improving the molecular diagnosis of *Chlamydia psittaci* and *Chlamydia abortus*. J Med Microbiol 64(10):1174–1185. https://doi.org/10.1099/jmm.0.000139
35. Palacios-Rodríguez V et al (2021) Development and validation of two immunoassays for detection of *Brucella canis*. Abanico Vet 11:e404. https://doi.org/10.21929/abavet2021.31
36. Pantchev A et al (2010) Detection of all *Chlamydophila* spp. using species-specific real-time PCR. Comp Immunol Microbiol Infect Dis 33(6):473–484. https://doi.org/10.1016/j.cimid.2009.08.002
37. Pawlikowska M, Deptuła W. (2008). Limfocyty B i ich produkty u ssaków w chlamydiozach i chlamydofilozach [B limphocytes and their products in mammals in Chlamydiosis and Chlamydophilosis]. Adv Agric Sci, 12(1), 43-54.
38. Pospischil A (2006) Enzootic abortion in ewes: a review of recent developments. Small Rumin Res 62(1-2):113–115. https://doi.org/10.1016/j.smallrumres.2005.08.005
39. Vretou E, et al (2007) Evaluation of two commercial assays for the detection of *Chlamydophila abortus* antibodies. Vet Microbiol 123(1–3): 153-161. https://doi.org/10.1016/j.vetmic.2007.02.023.
40. Rodolakis A, Mohamad KY (2010) Zoonotic potential of *Chlamydophila*. Vet Microbiol 140(3–4):382–391. https://doi.org/10.1016/j.vetmic.2009.03.014
41. Rodolakis A., Laroucau K (2015). Chlamydiaceae and chlamydial infections in sheep or goats. Vet Microbiol 181(1-2), 107–118. https://doi.org/10.1016/j.vetmic.2015.07.010
42. Rueda Garcés YI (2018) Seroprevalencia de enfermedades abortivas en cabras. Tesis de Especialidad, Universidad Autónoma de Querétaro. https://ri-ng.uaq.mx/jspui/bitstream/123456789/1455/1/RI004529.pdf
43. Sachse K et al (2009) Recent developments in the laboratory diagnosis of chlamydial infections. Vet Microbiol 135(1-2):2–21. https://doi.org/10.1016/j.vetmic.2008.09.040
44. Salti-Montesanto V et al (1997) Diagnosis of ovine enzootic abortion using a competitive ELISA. Am J Vet Res 58(3):228–235
45. Santiago Barrientos CI (2019) Expresión de OmpA recombinante de *Chlamydia abortus* en *Escherichia coli*. Tesis de maestría, UNAM. https://ru.dgb.unam.mx/bitstream/20.500.14330/TES01000795408/3/0795408.pdf

46. Schmeer N et al (1987) Analysis of caprine IgG subclass responses to *Chlamydia psittaci*. Vet Microbiol 14(2):125–135. https://doi.org/10.1016/0378-1135(87)90004-6
47. Schumann W, Ferreira LCS (2004) Production of recombinant proteins in *Escherichia coli*. Genet Mol Biol 27:442–453. https://doi.org/10.1590/S1415-47572004000300022
48. Serrato Díaz A et al (2014) PCR: reacción en cadena de la polimerasa. En: Cornejo Romero A et al. (eds) *Herramientas moleculares aplicadas en ecología*. SEMARNAT–INECC–UAM-I. https://www.researchgate.net/publication/266856169_PCR_reaccion_en_cadena_de_la_polimerasa
49. Vretou E et al (2007) Evaluation of two commercial assays for detection of *Chlamydophila abortus* antibodies. Vet Microbiol 123(1-3):153–161. https://doi.org/10.1016/j.vetmic.2007.02.023
50. Wang Y et al (2006) Identification of surface-exposed components of MOMP of *Chlamydia trachomatis*. Protein Sci 15(1):122–134. https://doi.org/10.1110/ps.051616206
51. WOAH (World Organisation for Animal Health) (2022) Principios y métodos de validación de las pruebas de diagnóstico. En: *Manual de las pruebas de diagnóstico y de las vacunas para los animales terrestres*. WOAH, París. https://www.woah.org/fileadmin/Home/esp/Health_standards/temporary_esp/2022/1.1.02_VALIDATION_Spanish.pdf
52. Yen TY, Pal S, de la Maza LM (2005) Characterization of the disulfide bonds and free cysteine residues of the *Chlamydia trachomatis* major outer membrane protein. Biochemistry 44(16):6250–6256. https://doi.org/10.1021/bi047775v
53. Yoshida H, Kishi Y, Shiga S, Hagiwara T (1998) Differentiation of *Chlamydia* species by combined use of PCR and restriction endonuclease analysis. Microbiol Immunol 42(5):411–414. https://doi.org/10.1111/j.1348-0421.1998.tb02303.x

Table 1

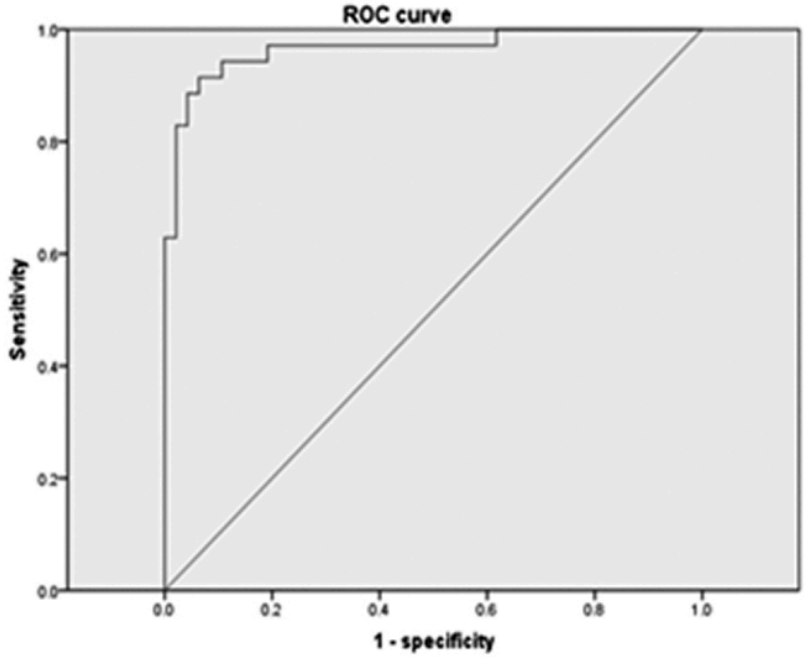
Table 1 Sensitivity and specificity for the determination of anti-*C. abortus* antibody by ELISA assays

		CHLMS-MS ID-Vet test		
		Positive	Negative	Total
CA-iELISA	Positive	32	5	37
	Negative	3	42	45
	Total	35	47	82
	Sensitivity = 91.4%		PPV = 86.5 %	
	Specificity = 89.4%		NPV = 93.3%	

PPV: Positive Predictive Value

NPV: Negative Predictive Value

Figures



553

Area under the curve

Area	Standard error	Asymptotic Significance	95% Asymptotic confidence interval	
			Lower limit	Upper limit
.965	.020	.000	.915	1.000

Figure 2

Anti-*C. abortus* antibodies in CA-iELISA in receiver operating characteristic (ROC) analyses. The antibody consensus of 37 *C. abortus* infection-positive and 45 *C. abortus* infection-negative sera was used as the categorical variable known a priori, and the signal intensity (S/Co) values observed for serum reactivity in an ELISA test were used as predictor variables forthe anti-*C. abortus* antibody status. IgG's average sensitivity from *C. abortus* was 91.4%, and the specificities were 89.4%. The area under the curve (AUC) was 0.965 ($p < 0.05$)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterialDelaRosaTAHP.docx