



Assessing antibody stability in filter paper-preserved blood samples for wildlife disease surveillance in tropical forests

María Fernanda Menajovsky^a, Gabriela M. Ulloa^b, Julia E. Fa^{c,d,e}, Daniel Montes^f,
Stephan Funk^g, Pedro Mayor^{a,h,i,*}

^a Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Bellaterra, Spain

^b Grupo de Enfermedades Infecciosas Re-Emergentes, Universidad Científica del Sur, Villa el Salvador, Lima, Peru

^c Center for International Forestry Research – World Agroforestry (CIFOR-ICRAF), Jalan CIFOR, Situ Gede, Bogor Barat, Bogor, Indonesia

^d Department of Natural Sciences, Manchester Metropolitan University, Chester Street, Manchester, United Kingdom

^e University of Gibraltar, United Kingdom

^f Universidad Peruana de Ciencias Aplicadas, Lima, Peru

^g Nature Heritage, Jersey, Channel Islands, Jersey

^h ComFauna, Comunidad de Manejo de Fauna Silvestre en la Amazonía y en Latinoamérica, Iquitos, Peru

ⁱ Museo de Culturas Indígenas Amazónicas, Iquitos, Peru

ARTICLE INFO

Keywords:

Epidemiological surveillance

Serology

Dried blood spots

Storage conditions

Fungal contamination

Tropical forests

ABSTRACT

Dried blood spots (DBS) on filter paper are widely used for serological diagnosis because they require minimal blood volumes and enable storage and transport without refrigeration, making them particularly useful in remote and resource-limited settings. However, DBS-based serology may be compromised by methodological variability and the lack of standardized preservation protocols. We evaluated the effects of preservation factors and quality indicators on DBS serological performance for *Toxoplasma gondii*, a pathogen with a broad host range and limited seasonal variability. We analyzed 787 DB samples from 17 mammal species collected by subsistence hunters in an Indigenous community in the northern Peruvian Amazon between 2008 and 2020. Recorded variables included filter paper type, time to cold-chain initiation, time to analysis, blood saturation, fungal contamination, and colorimetric values of reconstituted blood. Linear models with Type II ANOVA and AIC-based model selection were used to identify factors associated with seropositivity. Filter paper type was the primary determinant of serological outcomes, while severe fungal contamination was associated with reduced seropositivity. Colorimetric values were significantly related to serological results and may serve as a simple indicator of sample quality. These findings highlight the importance of standardized preservation protocols to minimize false negatives under tropical conditions. DBS-based serological studies should prioritize Protein Saver cards, limit fungal growth, and incorporate basic quality assessments to improve the reliability of wildlife disease surveillance within a One Health framework.

1. Introduction

Dried blood spots (DBS) collected on filter paper were first introduced more than five decades ago for neonatal metabolic screening (Guthrie and Susi, 1963). Since then, DBS methodologies have undergone substantial refinement and are now widely applied across biomedical and epidemiological research, particularly for the serological diagnosis of infectious diseases (Aubry et al., 2012; Billinger et al., 2023; Corran et al., 2008; Panteleeff et al., 1999). DBS has been

validated for antibody detection against a broad range of pathogens of human and animal health relevance, including Aujeszky's disease virus, dengue virus, hepatitis B virus, HIV-1, influenza virus, *Leishmania donovani*, *Mycoplasma pneumoniae*, *T. gondii*, and *Trypanosoma* spp. (Holroyd et al., 2022; Mei et al., 2001). Enzyme-linked immunosorbent assays (ELISAs) performed on DBS samples show high concordance with conventional serum-based assays, even after prolonged storage under appropriate conditions (Billinger et al., 2023; Smit et al., 2014).

The practicality, reliability, and cost-effectiveness of DBS make it a

* Corresponding author at: Pedro Mayor, Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Edifici V, Bellaterra, E-08193 Barcelona, Spain.

E-mail address: pedroginés.mayor@uab.cat (P. Mayor).

<https://doi.org/10.1016/j.rvsc.2026.106153>

Received 5 January 2026; Received in revised form 7 March 2026; Accepted 20 March 2026

Available online 20 March 2026

0034-5288/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

particularly attractive alternative to serum-based sampling in resource-limited and remote settings. DBS requires only small volumes of blood and eliminates the need for refrigeration during storage and transport, substantially reducing logistical complexity and cold-chain dependency (Shabihkhani et al., 2014; Smit et al., 2014).

Within wildlife research, DBS offers a robust substitute for conventional blood sampling methods, which are often constrained by ethical, logistical, and financial considerations (Aston et al., 2014; Curry et al., 2011; Rojas et al., 2024). Reliable diagnostic outcomes have been reported from DBS-derived samples using multiple analytical techniques, including PCR, ELISA, and card agglutination tests across a wide range of wild taxa (Desquesnes et al., 2022; Morales et al., 2017; Smit et al., 2014). Crucially, DBS sampling facilitates the participation of trained local actors, such as subsistence hunters, in sample collection, thereby extending disease surveillance capacity into remote and underserved regions (Corran et al., 2008; Rojas et al., 2024). This decentralized approach has proven particularly effective in challenging environments, including marine systems (Hansen et al., 2014), Arctic ecosystems (Brook et al., 2009; Curry, 2009), Asian tropical rainforests (Chan and Kaneko, 2025; Fornace et al., 2022), and the Amazon rainforest (Aston et al., 2014; Menajovsky et al., 2025), where conventional wildlife and public health monitoring remains limited.

Despite these advantages, the interpretation of DBS-based serological data remains constrained by methodological heterogeneity and the absence of standardized guidelines for antibody quantification and optimal storage conditions (Amini et al., 2021; Smit et al., 2014). Sample integrity and analytical accuracy can be influenced by multiple factors, including temperature and humidity, particularly in tropical rainforest environments, where high humidity can affect the drying process of blood samples, along with filter paper type, blood volume, spot size, storage conditions, and elution protocols (Amini et al., 2021; Berezky et al., 2005; Färnert et al., 1999; Mei et al., 2001).

Against this background, the present study evaluates how preservation conditions and color-based quality indicators influence the serological performance of DBS for the detection of *T. gondii* antibodies in wild mammals from the Amazon. This study focused on the serological detection of *T. gondii*, a protozoan parasite of major One Health relevance due to its broad host range, ecological persistence, and zoonotic potential. *T. gondii* was selected because its prevalence is relatively stable across years and exhibits limited seasonal variation in transmission dynamics within tropical forest systems (Barroso et al., 2020; Bier et al., 2020).

By addressing key methodological uncertainties, this work contributes to improving the reliability and interpretability of wildlife disease surveillance data, thereby strengthening efforts to understand pathogen circulation at the human–animal–environment interface within a One Health framework.

2. Materials and methods

2.1. Sample collection

We analyzed 787 dB samples obtained from free-ranging populations of 17 mammal species, including ungulates, rodents, and primates. Samples were collected between 2008 and 2020 by multiple ($n = 54$) local subsistence hunters as part of a long-term wildlife conservation and monitoring program in Nueva Esperanza, a Yaguas Indigenous community in the northern Peruvian Amazon. All participating hunters received standardized training in blood collection and filter paper handling to ensure consistency in sampling procedures.

Blood was collected *post-mortem* from the cranial or caudal vena cava and applied directly to one of three filter paper types: FTA cards (Flinders Technology Associates), Whatman 903 Protein Saver cards (hereafter “Protein Saver”), and Whatman 3MM filter paper. The filter paper containing the blood sample was dried in the shade for a minimum of 3 h. When the blood was dry, the filter paper was sealed in individual

plastic bags with desiccant and stored at room temperature during the stay in Nueva Esperanza before being transferred to -70°C for preservation.

All DBS samples were processed and analyzed simultaneously on a single analytical date using a standardized laboratory protocol. A homogeneous section of 132 mm^2 area of blood-soaked of each DBS was excised and eluted in $400\text{ }\mu\text{l}$ phosphate-buffered saline (PBS). Eluates were subsequently tested for anti-*T. gondii* IgG antibodies using the ID Screen® Toxoplasmosis IgG Indirect Multispecies ELISA kit (IDvet, Montpellier, France), following the manufacturer's instructions.

2.2. Sample-associated variables

Each DBS sample was accompanied by metadata describing preservation conditions and indicators of sample quality. Preservation-related variables included: (a) filter paper type, (b) time elapsed (in days) between blood collection and initiation of cold-chain storage, (c) time elapsed between blood collection and serological analysis (in years), (d) degree of blood saturation on the filter paper, and (e) presence of fungal contamination.

Blood saturation and fungal contamination were assessed visually using semi-quantitative ordinal scales. Blood saturation was rated on a Likert scale from 1 (light saturation) to 3 (heavy saturation). Fungal contamination was scored from 0 (no visible contamination) to 3 (high contamination). Both assessments were conducted independently by two researchers for all samples.

Sample quality was further characterized using objective color metrics derived from the Red–Green–Blue (RGB) values of reconstituted blood spots. RGB values were extracted using Adobe Photoshop® to quantify color intensity and degradation objectively (Süsstrunk et al., 1999). Lower RGB values corresponded to darker coloration of the reconstituted blood and were interpreted as indicative of better-preserved samples.

The response variable for serological analyses was *T. gondii* antibody detection, expressed as percent positivity. Percent positivity was calculated for each sample relative to the optical density (OD) of the plate-specific positive control, in accordance with the ELISA kit manufacturer's guidelines.

2.3. Statistical analysis

Data preparation involved converting categorical variables into factors and evaluating data completeness. Prior to multivariable modelling, pairwise correlations among explanatory variables were assessed. Time in storage (years) was strongly correlated with filter paper type ($p < 0.001$); to avoid multicollinearity, time in storage was excluded from subsequent models, and filter paper type was retained as the representative variable.

Multiple linear regression models were fitted to examine associations between *T. gondii* percent positivity and variables related to sample preservation and quality. The significance of individual predictors was assessed using analysis of variance (ANOVA) with Type II sums of squares.

To identify the most informative predictors of serological outcomes, three classes of regression models were constructed and compared: (1) RGB metrics only, (2) preservation factors only, and (3) combined models incorporating both RGB metrics and preservation factors. Model selection was based on bidirectional stepwise selection using Akaike's Information Criterion (AIC), and model performance was evaluated using adjusted R^2 , AIC, and Bayesian Information Criterion (BIC). For color-based analyses, a composite RGB index was calculated as the mean of the red, green, and blue channel values to capture overall color variation in reconstituted blood samples.

In addition to modelling serological outcomes, RGB values were also treated as response variables to evaluate the extent to which preservation factors influenced sample coloration. This analysis aimed to

determine whether simple color metrics could serve as practical indicators of DBS quality before laboratory reconstitution and testing.

All statistical analyses were conducted in R (v4.4.3) using RStudio (v2024.12.1). A Type I error rate of 0.05 was adopted for hypothesis testing. Data processing and visualization were performed using the tidyverse, dplyr, and ggplot2 packages, and the Excel dataset containing serological results and associated metadata was imported using the readxl package.

2.4. Ethics

The research protocols for the sampling of wildlife were approved by the Peruvian Forest and Wildlife Service (N° 258–2019-MINAGRI-SERFOR-DGGSPFFS; 27/05/2019) and the Institutional Animal Use Ethics Committee of the Universidad Peruana Cayetano Heredia (ref. 102,142; 14/05/2019). Dried blood samples on filter paper from wildlife were exported with the approval of the Peruvian Forestry and Wildlife Service (N° 003258/SP-Peru, N° 003260/SP-Peru, N° 003568-SERFOR-Peru, N° 003579-SERFOR-Peru, BB-00017 20I-Spain and BB-00018 20I-Spain).

3. Results

3.1. Effects of preservation factors on serological outcomes

Analysis of variance revealed a strong effect of filter paper type on *T. gondii* percent seropositivity ($F = 30.83$, $p < 0.0001$; Fig. 1). Linear model estimates indicated that, relative to FTA cards, samples collected on Whatman 3MM paper exhibited significantly lower percent seropositivity (estimate = -0.286 , $p < 0.0001$), whereas samples collected on Protein Saver cards showed significantly higher percent seropositivity (estimate = 0.197 , $p = 0.009$).

Observed seropositivity differed markedly among filter paper types, with values of 8.8% for Whatman 3MM ($n = 307$), 24.6% for FTA cards ($n = 130$), and 31.7% for Protein Saver cards ($n = 350$), highlighting substantial variability attributable to sample substrate.

Fungal contamination showed a negative, though not statistically significant, association with percent seropositivity at the highest contamination level (level 3; estimate = -0.33 , $p = 0.08$). Corresponding seropositivity rates across increasing fungal contamination

categories (0–3) were 21.7% ($n = 651$), 38.7% ($n = 62$), 12.9% ($n = 31$), and 0.0% ($n = 19$), respectively, suggesting a progressive decline in detectable antibody signal with increasing contamination. No significant associations were detected for other preservation-related variables.

Type II ANOVA based on the fitted linear model confirmed filter paper type as the dominant explanatory variable, accounting for the largest proportion of variance in *T. gondii* percent seropositivity (partial $\eta^2 = 0.10$, $p < 0.0001$; Fig. 2).

3.2. Color as a quality predictor

Analysis of RGB color metrics revealed a statistically significant association between color intensity of reconstituted DBS samples and *T. gondii* percent seropositivity. Specifically, lower RGB-average values, which indicate darker coloration and higher color intensity, were linked to higher serological percentages (estimate = -0.004 , $p < 0.0001$; Fig. 3). Despite this significant relationship, the explanatory power of the RGB-average metric was limited. The model explained only a modest proportion of the variance in serological outcomes (adjusted $R^2 = 0.106$), indicating that color intensity alone accounts for approximately 10.6% of the observed variability. These results suggest that RGB-based color metrics may function as a weak proxy for antibody degradation and are insufficient as a standalone indicator of DBS serological reliability.

3.3. Integration of preservation factors and quality indicators

Models integrating preservation factors with color-based quality indicators outperformed models based on either component alone (adjusted $R^2 = 0.150$), compared with RGB-only (adjusted $R^2 = 0.105$) and preservation/quality-only models (adjusted $R^2 = 0.108$). Stepwise model selection identified filter paper type, fungal contamination, and the RGB-average value as the principal predictors of *T. gondii* serological outcomes.

Among these predictors, the use of Whatman 3MM filter paper (standardized $\beta = -0.38$) and higher RGB-average values (standardized

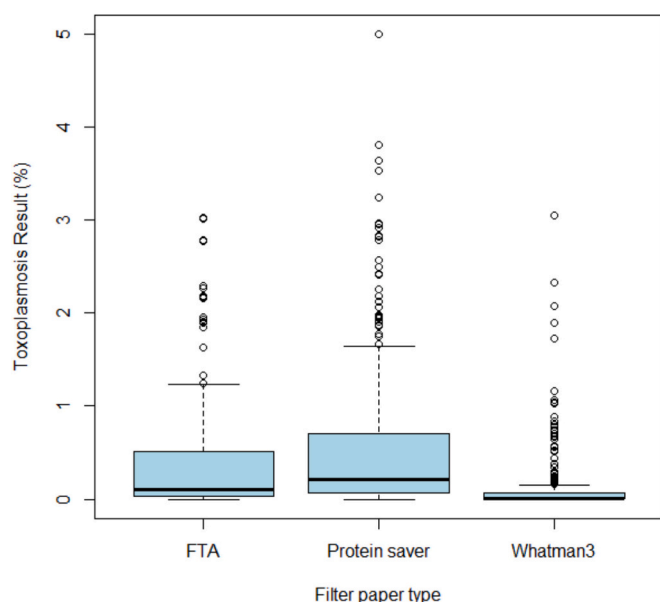


Fig. 1. *Toxoplasma gondii* percentual serological results according to filter paper type, based on DBS samples ($n = 787$) collected from wild animals between 2008 and 2020 in the northern Peruvian Amazon.

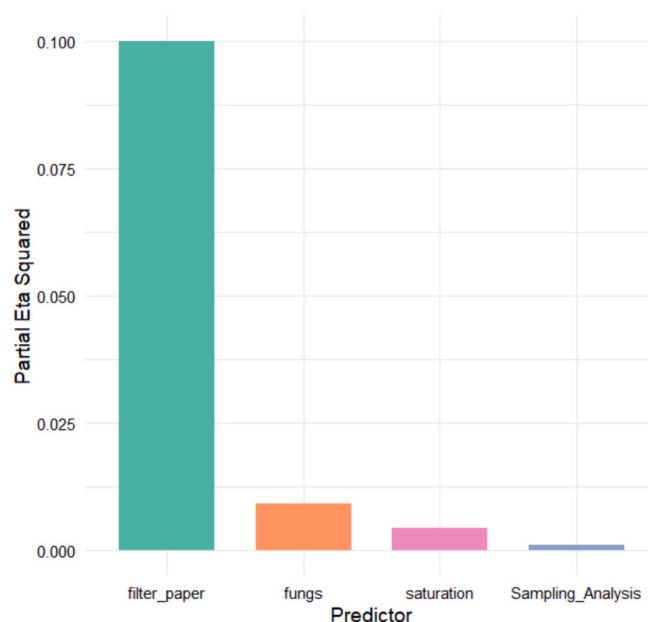


Fig. 2. Effect sizes of sample preservation factors on *Toxoplasmosis gondii* percentual serological results, based on partial eta squared (η^2) values from the ANOVA model using DBS samples ($n = 787$) collected from wild animals between 2008 and 2020 in the northern Peruvian Amazon. Partial Eta-squared values indicate the relative contribution of each predictor to the variation in serological outcomes.

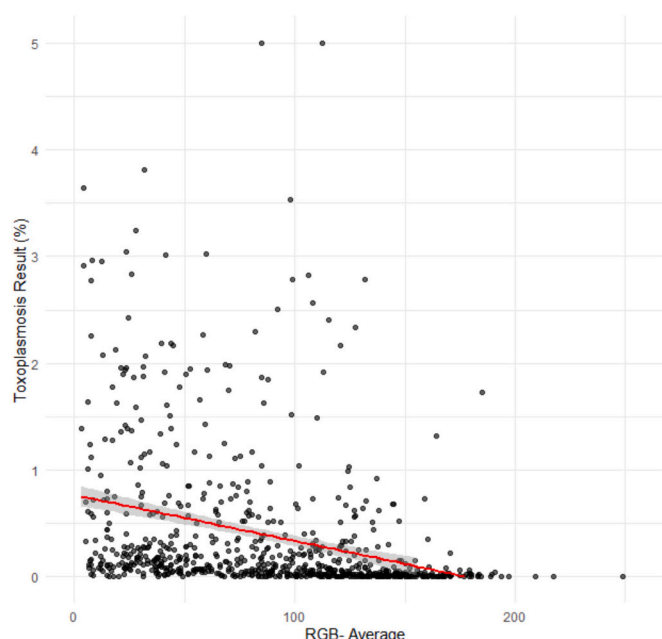


Fig. 3. Relationship between the RGB-average value of reconstituted blood and the percentage of *Toxoplasma gondii* serological results. Data are based on DBS samples ($n = 787$) collected from wild animals between 2008 and 2020 in the northern Peruvian Amazon. A linear regression line (in red) with a 95% confidence interval is shown to indicate the overall trend. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$\beta = -0.26$) exhibited the strongest negative effects on percent seropositivity, indicating substantially reduced antibody detectability (Fig. 4). Moderate and high levels of fungal contamination (levels 2 and 3) were associated with smaller but consistently negative effects (standardized $\beta = -0.02$ and -0.05 , respectively; Fig. 4).

Overall, these results demonstrate that both substrate-related

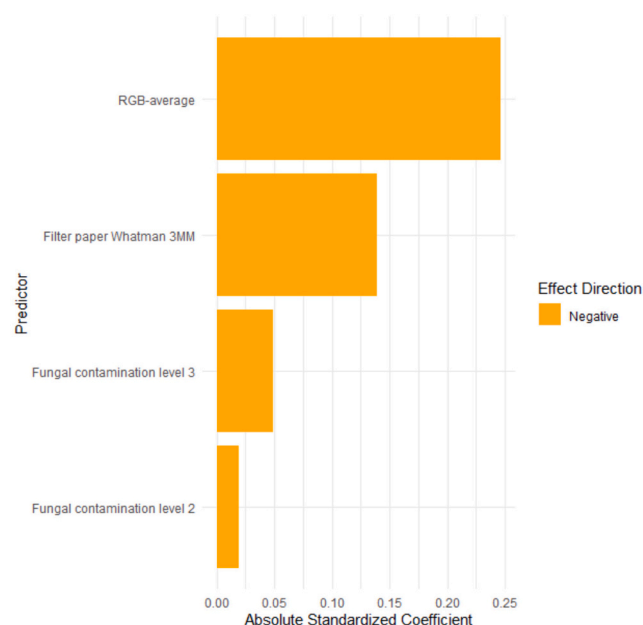


Fig. 4. Predictors with adverse effects on *Toxoplasma gondii* serological results, based on DBS samples ($n = 787$) collected from wild animals between 2008 and 2020 in the northern Peruvian Amazon. Whatman 3MM filter paper and RGB-average showed the strongest negative associations with antibody detection, while fungal contamination levels 2 and 3 had weaker adverse effects.

preservation factors and color-based quality indicators contribute to variability in DBS serological performance, with filter paper type emerging as the dominant determinant of antibody detection efficiency.

3.4. Corrected seropositivity based on quality assessment

Observed seropositivity varied substantially depending on the sample quality criteria. Of the 787 dB samples analyzed, 170 tested positive for *T. gondii*, yielding an overall seropositivity of 21.6% (Table S1). When samples identified as low quality in the statistical analyses - specifically those with high fungal contamination (level 3) and those collected on Whatman 3MM filter paper were excluded, a subset of 471 high-quality samples remained. Within this subset, 143 samples were seropositive, corresponding to a seropositivity of 30.4%.

This increase indicates that inclusion of lower-quality samples resulted in a marked underestimation of *T. gondii* exposure in the full dataset, on the order of approximately nine percentage points. Such underestimation is consistent with reduced antibody detectability associated with suboptimal preservation conditions.

When the dataset was further restricted to samples collected exclusively on Protein Saver cards, with all samples exhibiting level 3 fungal contamination excluded, 345 samples remained, of which 111 were seropositive. This yielded a seropositivity of 32.1%, reinforcing the conclusion that sample substrate and preservation quality substantially influence DBS-based serological estimates.

4. Discussion

This study examined key factors influencing sample integrity in DBS-based serology under tropical field conditions. The results demonstrate that filter paper type is the primary determinant of serological performance, while severe fungal contamination can further compromise sample integrity and increase the likelihood of false-negative results. In addition, the association between RGB color metrics of reconstituted blood and reduced seropositivity suggests that color-based indicators may provide a practical, albeit limited, tool for identifying samples of compromised quality.

Different filter paper types are designed for distinct diagnostic purposes, which strongly influences their suitability for serological applications (Li et al., 2024). Whatman 903 Protein Saver cards are specifically manufactured for biological sample collection and optimized for protein and antibody preservation, consistently yielding high antibody recovery and performance comparable to serum-based ELISA testing (Hegazy et al., 2020; Mei et al., 2010; Smit et al., 2014). In contrast, Whatman 3MM is a general-purpose laboratory filter paper not designed for biological sampling, which likely contributes to the reduced and inconsistent antibody recovery observed in this study, despite the scarcity of direct comparative evaluations (Fachiroh et al., 2008; Smit et al., 2014). FTA cards, although primarily developed for molecular and genetic applications, are chemically treated to inhibit microbial growth, denature proteins, and limit enzymatic degradation (Cardona-Ospina et al., 2019; Marchand et al., 2021). Our findings indicate that these properties also confer partial protection for antibodies, resulting in better serological performance than Whatman 3MM, albeit inferior to Protein Saver cards.

These findings are consistent with previous studies indicating superior antibody recovery from filter papers specifically designed for biological sampling, compared to general-purpose paper substrates (Lange et al., 2017; Smit et al., 2014). However, few field studies have quantitatively demonstrated the effect of filter paper type on seropositivity rates, especially under tropical conditions, highlighting the added value of the present study.

Environmental conditions, particularly temperature and humidity, are known to strongly influence DBS stability, significantly reducing the sensitivity of the tests performed (Rojas et al., 2024; Smit et al., 2014), highlighting the importance of rapid and complete desiccation and

appropriate storage of filter paper samples in tropical environments (Aston et al., 2014; Corran et al., 2008). Inadequate moisture control favours fungal proliferation, which can degrade proteins and other biomolecules and thus reduce antibody detectability (Li et al., 2005; Mei et al., 2001). Although fungal contamination emerged as a negative factor in our analyses, only high contamination levels were consistently associated with marked signal loss, indicating that moderate fungal growth does not invariably preclude antibody detection. This suggests that sample degradation is not driven solely by humidity and fungal presence but results from the interaction of multiple preservation-related factors.

Contrary to expectations, the time elapsed between blood collection and the start of cold chain storage did not appear to influence the serological results. Previous studies have indicated that samples maintained and processed at room temperature for periods of less than 24 h have optimal quality (Rojas et al., 2024), and that subsequent direct exposure to sunlight, high humidity, and elevated temperatures, especially during prolonged storage, can negatively affect sample integrity (Lange et al., 2017). However, DBS samples are generally considered stable for up to 28 days at room temperature under controlled conditions (Amini et al., 2021; Batterman and Chernyak, 2014; Corran et al., 2008), and long-term preservation is most reliably achieved at -20°C (Bevins et al., 2016; Corran et al., 2008; Hwang et al., 2012). Furthermore, other studies suggest that the most critical nucleic acid degradation processes can occur rapidly after sampling (Li et al., 2024). While most previous studies have focused on nucleic acids, and direct evidence for antibodies remains limited, antibody degradation can also begin shortly after biological sampling. Therefore, these findings underscore the importance of implementing effective preservation measures as early as possible in tropical environments (Smit et al., 2014).

Our study has several limitations. First, the multi-year collection could have affected positivity rates due to progressive antibody degradation. Unfortunately, the commercial availability of different filter papers during the study period resulted in the oldest samples being collected predominantly on Whatman 3MM paper, followed chronologically by Protein Saver and FTA cards. Therefore, there is a collinearity between the sampling year and the type of filter paper used, preventing us from isolating these two factors in the multivariable models. Consequently, it was not possible to fully disentangle the independent effects of sample age and filter paper type, introducing temporal confounding that must be considered when interpreting our results and explicitly addressed in future prospective studies. Secondly, although direct comparison of the same samples using different substrates should provide greater robustness to the conclusions, the logistical and financial constraints referred to above, due to the prolonged sampling collection over 12 years, have conditioned our experimental design. Third, the inclusion of a standardized detection method would improve the interpretation and reproducibility of the results. However, the Sabin-Feldman staining test, the gold standard for detecting IgG antibodies against *T. gondii*, requires live parasites, which entails greater risks and technical complexity. On the other hand, molecular tests such as real-time PCR (RT-PCR) have limitations due to the low parasite load, the intermittent nature of parasitemia, and the difficulty in obtaining samples from the active site of infection. For this reason, we used ELISA serological tests, which are the most widely used standard screening method due to their high sensitivity and automation.

The implications of these findings are particularly relevant for wildlife disease surveillance and One Health research in remote tropical regions. In such settings, logistical constraints often limit the feasibility of conventional sampling approaches, contributing to substantial gaps in epidemiological knowledge. DBS sampling offers a practical solution for pathogen surveillance under difficult conditions. While this methodology may be practical for serologically confirming the circulation of pathogens, our results demonstrate that the inclusion of low-quality samples may result in an underestimation of pathogen seropositivity rates, and may silence the circulation of low-frequency pathogens at the

wildlife-human interface.

5. Conclusions

Overall, these findings demonstrate that standardized preservation protocols are critical for minimizing false negatives and ensuring the reliability of DBS-based serology in tropical field settings. Protein Saver cards consistently outperformed other substrates, and even FTA cards preserved antibody integrity more effectively than general-purpose filter papers. Although RGB-based color metrics explained only a limited proportion of variation in serological outcomes, they may nonetheless provide a pragmatic preliminary screening tool to identify potentially degraded samples before laboratory analysis. Such metrics, however, cannot replace robust procedures for sample collection, storage, and handling.

DBS-based serological surveillance in tropical wildlife systems should therefore prioritize the use of Protein Saver cards, minimize moisture exposure and fungal growth, and incorporate basic quality assessments where feasible. Implementing these measures will substantially improve the accuracy and interpretability of wildlife disease surveillance data, thereby strengthening One Health efforts to characterize pathogen circulation and assess zoonotic spillover risk in remote ecosystems.

CRedit authorship contribution statement

María Fernanda Menajovsky: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gabriela M. Ulloa:** Writing – review & editing, Methodology, Investigation, Data curation. **Julia E. Fa:** Writing – review & editing, Writing – original draft. **Daniel Montes:** Writing – review & editing, Methodology, Investigation. **Stephan Funk:** Writing – review & editing, Software, Investigation, Formal analysis. **Pedro Mayor:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The research protocols for the sampling of wildlife were approved by the Peruvian Forest and Wildlife Service (N° 258–2019-MINAGRI-SERFOR-DGGSPFFS) and the Institutional Animal Use Ethics Committee of the Universidad Peruana Cayetano Heredia (ref. 102,142). Dried blood samples on filter paper from wildlife were exported with the approval of the Peruvian Forestry and Wildlife Service (N° 003258/SP-Peru, N° 003260/SP-Peru, N° 003568-SERFOR-Peru, N° 003579-SERFOR-Peru, BB-00017 20I-Spain, and BB-00018 20I-Spain).

Funding

This work was supported by ERANet-LAC (ERANet17/HLH-0271), research projects (Contract N° 136-2018-FONDECYT; AC18/00054 Instituto de Salud Carlos III; 400800/2019-5 CNPq). MFM was supported by FI-SDUR EMC/3345/2020.

Declaration of competing interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Acknowledgments

We sincerely thank all the people from Nueva Esperanza who actively participated in data collection, which shows that communal participation is an important step in the development of wildlife management. We also extend our thanks to R. Bodmer, P. E. Puertas, and P. Pérez for their kind assistance during the fieldwork. MFM acknowledges a doctoral scholarship from the Catalan Agency for Management of University and Research Grants [scholarship FI-SDUR EMC/3345/2020]. GU received two grants from the CNPq PPGSPAA program in GD modality (140312/2020-0) and SWE modality (201546/2020-5).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rvsc.2026.106153>.

Data availability

The data used to support the findings of this study are available at <https://doi.org/10.34810/data2892>.

References

- Amini, F., Auma, E., Hsia, Y., Bilton, S., Hall, T., Ramkhalawon, L., Heath, P.T., Le Doare, K., 2021. Reliability of dried blood spot (DBS) cards in antibody measurement: a systematic review. *PLoS One* 16 (3), e0248218.
- Aston, E.J., Mayor, P., Bowman, D.D., Mohammed, H.O., Liotta, J.L., Kwok, O., Dubey, J. P., 2014. Use of filter papers to determine seroprevalence of toxoplasma gondii among hunted ungulates in remote Peruvian Amazon. *Int. J. Parasitol. Parasites. Wildl.* 3 (1), 15–19.
- Aubry, M., Roche, C., Dupont-Rouzeyrol, M., Aaskov, J., Viallon, J., Marfel, M., Lalita, P., Elbourne-Duituturaga, S., Chanteau, S., Musso, D., Pavlin, B.I., Harrison, D., Kool, J. L., Cao-Lormeau, V.M., 2012. Use of serum and blood samples on filter paper to improve the surveillance of dengue in Pacific Island countries. *J. Clin. Virol.* 55 (1), 23–29.
- Barroso, P., García-Bocanegra, I., Acevedo, P., Palencia, P., Carro, F., Jiménez-Ruiz, S., Almería, S., Dubey, J.P., Cano-Terriza, D., Vicente, J., 2020. Long-term determinants of the seroprevalence of toxoplasma gondii in a wild ungulate community. *Animals* 10 (12), 2349.
- Batterman, S., Chernyak, S., 2014. Performance and storage integrity of dried blood spots for PCB, BFR and pesticide measurements. *Sci. Total Environ.* 494, 252–260.
- Bereczky, S., Martensson, A., Gil, J.P., Farnert, A., 2005. Rapid DNA extraction from archive blood spots on filter paper for genotyping of plasmodium falciparum. *Am. J. Trop. Med. Hyg.* 72 (3), 249–251.
- Bevins, S., Pappert, R., Young, J., Schmit, B., Kohler, D., Baeten, L., 2016. Effect of storage time and storage conditions on antibody detection in blood samples collected on filter paper. *J. Wildl. Dis.* 52 (3), 478–483.
- Bier, N.S., Stollberg, K., Mayer-Scholl, A., Johne, A., Nöckler, K., Richter, M., 2020. Seroprevalence of *Toxoplasma gondii* in wild boar and deer in Brandenburg, Germany. *Zoonoses Public Health* 67 (6), 601–606.
- Billinger, K., Okai, C.A., Russ, M., Koy, C., Röwer, C., Opuni, K.F., Glocker, M.O., 2023. Dried serum spots on pre-punched filter paper discs are ready-to-use storage and shipping devices for blood-borne antigens and antibodies. *J. Immunol. Methods* 519, 113519.
- Brook, R.K., Kutz, S.J., Veitch, A.M., Popko, R.A., Elkin, B.T., Guthrie, G., 2009. Fostering community-based wildlife health monitoring and research in the Canadian North. *EcoHealth* 6 (2), 266–278.
- Cardona-Ospina, J.A., Villalba-Miranda, M.F., Palechor-Ocampo, L.A., Mancilla, L.I., Sepúlveda-Arias, J.C., 2019. A systematic review of FTA cards® as a tool for viral RNA preservation in fieldwork: are they safe and effective? *Prev. Vet. Med.* 172, 104772.
- Chan, C.W., Kaneko, A., 2025. Malaria control and elimination in Vanuatu and Papua New Guinea: multidisciplinary and collaborative research with island communities, 1985–2024. *Parasitol. Int.* 109, 103111.
- Corran, P.H., Cook, J., Lynch, C., Leendertse, H., Manjurano, A., Griffin, J., Cox, J., Abeku, T., Bousema, T., Ghani, A.C., Drakeley, C., Riley, E., 2008. Dried blood spots as a source of anti-malarial antibodies for epidemiological studies. *Malar. J.* 7, 1–12.
- Curry, P., 2009. Caribou herds and arctic communities: exploring a new tool for caribou health monitoring. *Arctic* 62 (4), 495–499.
- Curry, P.S., Elkin, B.T., Campbell, M., Nielsen, K., Hutchins, W., Ribble, C., Kutz, S.J., 2011. Filter-paper blood samples for ELISA detection of Brucella antibodies in caribou. *J. Wildl. Dis.* 47 (1), 12–20.
- Desquesnes, M., Gonzatti, M., Sazmand, A., Thévenon, S., Bossard, G., Boulangé, A., Gimonneau, G., Truc, P., Herder, S., Ravel, S., Sereno, D., Jamonneau, V., Jittapalpong, S., Jacquet, P., Solano, P., Berthier, D., 2022. A review on the diagnosis of animal trypanosomoses. *Parasit. Vectors* 15 (1), 64.
- Fachiroh, J., Prasetyanti, P.R., Paramita, D.K., Prasetyawati, A.T., Anggrahini, D.W., Haryana, S.M., Middeldorp, J.M., 2008. Dried-blood sampling for epstein-barr virus immunoglobulin G (IgG) and IgA serology in nasopharyngeal carcinoma screening. *J. Clin. Microbiol.* 46 (4), 1374–1380.
- Färnert, A., Arez, A.P., Correia, A.T., Björkman, A., Snounou, G., do Rosário, V., 1999. Sampling and storage of blood and the detection of malaria parasites by polymerase chain reaction. *Trans. R. Soc. Trop. Med. Hyg.* 93 (1), 50–53.
- Fornace, K., Manin, B.O., Matthiopoulos, J., Ferguson, H.M., Drakeley, C., Ahmed, K., Chua, T.H., 2022. A protocol for a longitudinal, observational cohort study of infection and exposure to zoonotic and vector-borne diseases across a land-use gradient in Sabah, Malaysian Borneo: a socio-ecological systems approach. *Wellcome Open Research* 7, 63.
- Guthrie, R., Susi, A., 1963. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatr* 32 (3), 338–343.
- Hansen, C.M., Hueffer, K., Gulland, F., Wells, R.S., Balmer, B.C., Castellini, J.M., O'Hara, T., 2014. Use of cellulose filter paper to quantify whole-blood mercury in two marine mammals: validation study. *J. Wildl. Dis.* 50 (2), 271–278.
- Hegazy, M.M., Hegazy, M.K., Azab, M.S., Nabih, N., 2020. Validation of dried blood spots in monitoring toxoplasmosis. *Pathog. Glob. Health.* 114 (5), 242–250.
- Holroyd, T.A., Schiaffino, F., Chang, R.H., Wanyiri, J.W., Saldanha, I.J., Gross, M., Hayford, K., 2022. Diagnostic accuracy of dried blood spots for serology of vaccine-preventable diseases: a systematic review. *Expert Rev. Vaccines* 21 (2), 185–200.
- Hwang, J., Jaroensuk, J., Leimanis, M.L., Russell, B., McGready, R., Day, N., Snounou, G., Nosten, F., Imwong, M., 2012. Long-term storage limits PCR-based analyses of malaria parasites in archival dried blood spots. *Malar. J.* 11, 1–7.
- Lange, B., Roberts, T., Cohn, J., Greenman, J., Camp, J., Ishizaki, A., Easterbrook, P., 2017. Diagnostic accuracy of detection and quantification of HBV-DNA and HCV-RNA using dried blood spot (DBS) samples—a systematic review and meta-analysis. *BMC Infect. Dis.* 17 (Suppl. 1), 693.
- Li, C.C., Seidel, K.D., Coombs, R.W., Frenkel, L.M., 2005. Detection and quantification of human immunodeficiency virus type 1 p24 antigen in dried whole blood and plasma on filter paper stored under various conditions. *J. Clin. Microbiol.* 43 (8), 3901–3905.
- Li, J., Ulloa, G.M., Mayor, P., Santolalla Robles, M.L., Greenwood, A.D., 2024. Nucleic acid degradation after long-term dried blood spot storage. *Mol. Ecol. Resour.* 24, e13979.
- Marchand, A., Roulland, I., Semence, F., Ericsson, M., 2021. EPO transgene detection in dried blood spots for antidoping application. *Drug Test Anal.* 13 (11–12), 1888–1896.
- Mei, J.V., Alexander, J.R., Adam, B.W., Hannon, W.H., 2001. Innovative non-or minimally-invasive technologies for monitoring health and nutritional status in mothers and young children use of filter paper for the collection and analysis of human whole blood specimens 1. *J. Nutr.* 131 (5), 1631–1636.
- Mei, J.V., Zobel, S.D., Hall, E.M., De Jesús, V.R., Adam, B.W., Hannon, W.H., 2010. Performance properties of filter paper devices for whole blood collection. *Bioanalysis* 2 (8), 1397–1403.
- Menajovsky, M.F., Mayor, P., Bodmer, R., Pérez-Peña, P., Ulloa, G.M., Greenwood, A.D., Montero, S., Lescano, A.G., Santolalla, M.L., Segalés, J., Sibila, M., Cabezon, O., Espunyes, J., 2025. Monitoring of selected swine viral diseases in Peruvian Amazon peccaries. *EcoHealth* 22 (1), 69–78.
- Morales, E.A., Mayor, P., Bowler, M., Aysanoa, E., Perez-Velez, E.S., Perez, J., Ventocilla, J.A., Valdeviano, G.C., Lescano, A.G., 2017. Prevalence of Trypanosoma cruzi and other trypanosomatids in frequently-hunted wild mammals from the Peruvian Amazon. *Am. J. Trop. Med. Hyg.* 97 (5), 1482.
- Panteleeff, D.D., John, G., Nduati, R., Mbori-Ngacha, D., Richardson, B., Kreiss, J., Overbaugh, J., 1999. Rapid method for screening dried blood samples on filter paper for human immunodeficiency virus type 1 DNA. *J. Clin. Microbiol.* 37 (2), 350–353.
- Rojas, A., Germitsch, N., Oren, S., Sazmand, A., Deak, G., 2024. Wildlife parasitology: sample collection and processing, diagnostic constraints, and methodological challenges in terrestrial carnivores. *Parasit. Vectors* 17 (1), 127.
- Shabihkhani, M., Lucey, G.M., Wei, B., Mareninov, S., Lou, J.J., Vinters, H.V., Singer, E. J., Cloughesy, T.F., Yong, W.H., 2014. The procurement, storage, and quality assurance of frozen blood and tissue biospecimens in pathology, biorepository, and biobank settings. *Clin. Biochem.* 47 (4–5), 258–266.
- Smit, P.W., Elliott, I., Peeling, R.W., Mabey, D., Newton, P.N., 2014. An overview of the clinical use of filter paper in the diagnosis of tropical diseases. *Am. J. Trop. Med. Hyg.* 90 (2), 195.
- Süstrunk, S., Buckley, R., Swen, S., 1999. Standard RGB color spaces. In: *Proceedings 7th IS&T/SID Color Imaging Conference 1999. Society of Imaging Science & Technology*, pp. 127–134.