Kent Academic Repository

Ladjal, Somia, Al-Adilee, Yaseen Majid Salman, Dahmane, Abdeldjalil, Dashti, Alejandro, Sánchez, Sergio, Reghaissia, Nassiba, Samari, Houssem, Dif, Sofiane, Krioui, Abelhak, Baroudi, Djamel and others (2026) ***Molecular prevalence and genotyping of Enterocytozoon bieneusi in cattle from Central Algeria.*** **Acta parasitologica, 71 (48). ISSN 1230-2821.**

Downloaded from

https://kar.kent.ac.uk/113311/ The University of Kent's Academic Repository KAR

The version of record is available from

https://doi.org/10.1007/s11686-026-01229-w

This document version

Author's Accepted Manuscript

DOI for this version

Licence for this version

CC BY (Attribution)

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in ***Title of Journal*** , Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies).

Molecular prevalence and genotyping of *Enterocytozoon bieneusi* in cattle from central Algeria

Somia Ladjal[1], Yaseen Majid Salman Al-Adilee[2,3], Abdeldjalil Dahmane[4], Alejandro Dashti[5], Sergio Sánchez[5], Nassiba Reghaissia[6], Houssem Samari[7], Sofiane Dif[8], Abelhak Krioui[9], Djamel Baroudi[1], Anastasios D. Tsaousis[2], David Carmena[5,10], AbdElkarim Laatamna[11*]

[1]Food Hygiene and Quality Assurance System Research Laboratory, Higher National Veterinary School, Issad Abbas Street, Bab Ezzouar, 16000 Algiers, Algeria

[2]Laboratory of Molecular and Evolutionary Parasitology, School of Natural Sciences, University of Kent, Canterbury, Kent, UK

[3]Department of Medical Microbiology, College of Medicine, Ninevah University, Mosul, Ninevah, Iraq

[4]Department of Veterinary Sciences, Faculty of Natural and Life Sciences, and Earth and Universe Sciences, Mohamed Khider University of Biskra, BP 145 RP, 07000, Biskra, Algeria

[5]Parasitology Reference and Research Laboratory, Spanish National Centre for Microbiology, Health Institute Carlos III, Majadahonda, Spain

[6]Laboratory of Sciences and Living Techniques, Institute of Agronomic and Veterinary Sciences, University of Souk Ahras, Annaba Road 41000, Souk Ahras, Algeria

[7]Faculty of Sciences, University of M’sila, 28000 M’sila, Algeria;

[8]Vétérinaire Privé, Ouled Derradj, Wilaya de M'sila

[9]Vétérinaire Privé, Berhoum, Wilaya de M'sila

[10]Center for Biomedical Research Network (CIBER) in Infectious Diseases, Health Institute Carlos III, Madrid, Spain

[11]Laboratory of Exploration and Valorization of Steppic Ecosystems, Faculty of Nature and Life Sciences, University of Djelfa, Moudjbara Road, BP3117, Djelfa, Algeria

*Correspondence: AbdElkarim Laatamna, Faculty of Nature and Life Sciences, University of Djelfa, Moudjbara Road, BP 3117, Djelfa, Algeria. E-mail: a.laatamna@univ-djelfa.dz

ORCID: 0000-0003-0406-7467, Tel: +213(0)674416030, Fax: +213(0)35636832

Somia Ladjal and Yaseen Majid Salman Al-Adilee contributed equally

Anastasios D. Tsaousis, David Carmena, and AbdElkarim Laatamna shared the same position.

Abstract

Enterocytozoon bieneusi is an obligate intracellular eukaryotic parasite of the phylum Microsporidia that infects a wide range of hosts, including humans. In Algeria, epidemiological information on microsporidian infections caused by *E. bieneusi* is scarce, with only three molecular studies conducted in equines, cattle, and dromedary camels. Here, we investigated the prevalence and genotypic diversity of *E. bieneusi* in cattle (*n* = 288), including cows (*n* = 182) and their calves (*n* = 106), from 54 farms in central Algeria. The initial screening was conducted using a nested PCR targeting a partial sequence of the internal transcribed spacer (ITS) region. *Enterocytozoon bieneusi* was identified in 16.0% (46/288) of faecal samples and in 42.6% (23/54) of sampled farms. Infection rates varied from 10.9% to 17.7% across the study areas. Significant differences in prevalence were observed according to cattle age, sex, and the presence/absence of other livestock species within cattle farms. Conversely, study area, production system, and presence/absence of diarrhoea showed no significant associations. Out of 46 *E. bieneusi*-positive cattle, genotyping was successful for 56.5% (26/46) of isolates. Phylogenetic analyses revealed seven known *E. bieneusi* genotypes clustering within the zoonotic Group 1: Type IV (42.3%, 11/26), D (7.7%, 2/26), and Peru6 (3.8%, 1/26), and the ruminant-adapted Group 2: J (19.2%, 5/26), I (15.4%, 4/26), BEB6 (7.7%, 2/26), and CM7 (3.8%, 1/26). The present study provides valuable molecular data on *E. bieneusi* infections in cattle in Algeria. It highlights the important role that cattle can play as reservoirs of zoonotic *E. bieneusi* genotypes.

Keywords: Distribution, *Enterocytozoon bieneusi*, genotypes, cattle, Algeria

Introduction

Microsporidia are a fungi-related ubiquitous group of obligate intracellular eukaryotic parasites that infect humans and most major animal groups. Among them, *E. bieneusi* is the most clinically significant species, being the main cause of human microsporidiosis [1, 2]. This parasite has also been widely reported in both farmed and wild animals [3]. Infections by *E. bieneusi* can present with or without clinical manifestations in immunocompetent individuals, whereas in immunocompromised hosts (e.g., HIV-infected patients), it often causes severe disease, most notably chronic diarrhoea [4]. The species exhibits remarkable genetic diversity, with more than 600 valid genotypes identified worldwide based on the sequence analysis of the ribosomal internal transcribed spacer (ITS) genetic marker [3, 5, 6]. These genotypes cluster into 15 distinct groups, with Groups 1 and 2 comprising genotypes with zoonotic potential, whereas Groups 3–15 consist mainly of host-adapted genotypes found in non-human animals [7–9]. Cattle are recognised as important reservoirs of *E. bieneusi* and play a key role in its epidemiology. To date, at least 97 genotypes have been detected in bovids (cattle, yak, and water buffalo), 15 of which have also been reported in humans [6, 10].

The prevalence of *E. bieneusi* in cattle varies considerably across countries worldwide and can reach up to 52.0% [6, 10–12]. However, molecular epidemiological data on bovine microsporidiosis remain limited in North Africa. In Algeria, only a few investigations have previously addressed the prevalence and molecular diversity of *E. bieneusi* in livestock, including equines, cattle, and dromedary camels [13–15].

The present study investigates the molecular prevalence, associated risk factors, and genotype distribution of *E. bieneusi* in cows and their calves from different areas in central Algeria. In this region, the epidemiology of this pathogen is unknown.

Material and methods

Ethical statement

This study was conducted in accordance with Algerian legislative guidelines (RD 08/1988). The Ethics Committee of the Laboratory of Exploration and Valorization of Steppic Ecosystems from the Faculty of Nature and Life Sciences (Ziane Achour University-Djelfa, Algeria) approved the collection of cattle faecal samples (approval no. 005 EVES/FSNV/2021). Farm owners were individually contacted, informed about the objectives of the study and the procedures involved, and written informed consent for faecal sample collection was obtained from each participant.

Study areas and sample collection

The present study was carried out between June 2022 and April 2023 in three provinces from central Algeria, namely Algiers, Tipaza, and M'sila (Fig. 1). Algiers and Tipaza are coastal regions with a Mediterranean humid climate, while M'sila has a steppe environment marked by an arid climate. In Algiers, industrial and tourism-related activities dominate, whereas in Tipaza, agriculture, fishing, and tourism serve as the main economic sectors. Cattle breeding is mainly found around Algiers and Tipaza, with sheep breeding being less prominent in these provinces. Conversely, the pastoralism of small ruminants is a key agricultural activity in M'sila, while cattle breeding and the rearing of other livestock such as dromedary camels are less common.

A total of 288 faecal samples were collected from 54 farms, including 46 samples from one farm in Algiers, 72 samples from 24 farms in Tipaza, and 170 samples from 29 farms in M'sila (Fig. 1). From the three studied areas, farm selection and sampling were conducted randomly. The herd size on the examined farms ranged from 4 to 46 animals, with cattle ages varying from 13 days to 11 years (median: 24 months). The sampled farms were intended for dairy and/or meat productions. Sample collection was carried out once from each farm without repeated sampling.

Faecal samples were collected directly from the rectum of cattle, placed in individual sterile plastic containers, and transported to the laboratory in an isotherm box. For each animal, information was recorded on locality, sex, age, presence or absence of diarrhoea at the time of sampling, production systems, and presence or absence of other livestock species (mainly sheep and goats). The presence or absence of diarrhoea was assessed by clinical examination of sampled cattle. All samples were preserved at +4 °C in 2.5% potassium dichromate until molecular analysis.

Genomic DNA extraction and purification

Total DNA was extracted from 200 mg of each faecal sample using the PureLink™ Microbiome DNA Purification Kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The DNA was eluted in 100 µL of elution buffer and stored at –20 °C until PCR analysis.

PCR detection of *Enterocytozoon bieneusi*

Detection of *E. bieneusi* was carried out using a nested PCR targeting a partial sequence of the internal transcribed spacer (ITS) region, along with parts of the flanking large and small subunit of the ribosomal RNA gene, as previously described [16]. In the primary PCR, a fragment of approximately 435 bp was amplified using the primers EBITS3 (5′–GGTCATAGGGATGAAGAG–3′) and EBTIS4 (5′TTCGAGTTCTTTCGCGCTC–3′). The secondary PCR amplified a fragment of approximately 390 bp using the primers EBITS1 (5′–GCTCTGAATATCTATGGCT–3′) and EBITS2.4 (5′–ATCGCCGACGGATCCAAGTG–3′).

Primary and secondary PCR reactions (50 µL) contained 2 µL of template DNA, 1 µM of each primer, and 25 µL of 2× PCRBIO Taq Mix Red (PCR Biosystems Ltd, UK). In all cases, cycling conditions were as follows: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 40 s, annealing at 53 °C for 45 s, and extension at 72 °C for 45 s; followed by a final extension at 72 °C for 4 min. An *E. bieneusi*-positive isolate and nuclease-free water were included as positive and negative controls, respectively, in each PCR run.

Gel electrophoresis

PCR amplicons of *E. bieneusi* were electrophoresed on 2% agarose (ZellBio GmbH, Germany) gels stained with ethidium bromide and visualised under ultraviolet illumination. PCR products of the expected size were subsequently purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific, CA, USA) following the manufacturer's instructions, and bidirectionally sequenced at Eurofins (Cologne, Germany).

Sequence and phylogenetic analyses

Raw sequences were visualized and edited using the software Chromas Lite 2.1 (http://chromas-lite.software.informer.com/2.1). The generated consensus sequences were assembled, then compared against GenBank entries via BLAST, and further analysed in MEGA X to confirm species identity and assign genotypes [17].

The evolutionary history of the ITS sequences generated in this study was inferred using the Maximum Likelihood (ML) method under the General Time Reversible (GTR) model [18]. Sequences from representative genotypes of human, animal, and/or environmental origin, covering all 15 groups of *E. bieneusi*, were included in the analysis for comparative purposes. Initial trees for the heuristic search were obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated using the Maximum

Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA X [17].

The representative sequences of *E. bieneusi* generated in this study have been deposited in GenBank under accession numbers PX617831–PX617837.

Statistical analysis

All molecular analysis data were recorded in an Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA), and statistical analyses were performed using SPSS v.22.0 (IBM SPSS Inc., Chicago, IL, USA). Differences in infection rates of *E. bieneusi* by cattle attributes (independent variables) were assessed using the Chi-square test (two-tailed) and odds ratios (OR) with 95% confidence intervals (CI). Variables found to be significant in the univariable analysis were subsequently included in a binary logistic regression model to identify independent risk factors. Additionally, to evaluate whether the presence of *E. bieneusi* in faecal samples differed between cow–calf pairs, an exact McNemar's test for paired proportions was used to compare the discordant groups (cow-only positive and calf-only positive pairs). The difference in proportions was calculated with a 95% CI to determine whether it was statistically significant. The test assesses whether infection status tends to differ systematically between cows and calves within pairs — i.e., whether calves are more likely to be infected than their mothers.

Results

Prevalence of *Enterocytozoon bieneusi*

Out of 288 PCR-screened cattle, *E. bieneusi* was detected in 16.0% (46/288; 95% CI: 11.9–20.7) of faecal samples and in 42.6% (23/54; 95% CI: 29.2–56.8) of sampled farms.

Enterocytozoon bieneusi prevalence varied from 10.9% to 17.7% in the three study areas (Table 1).

Risk association analysis

Table 1 shows the distribution of *E. bieneusi* infections in the surveyed cattle population according to the epidemiological (location, age, sex, production system, cohabitation) and clinical (diarrhoea) variables considered in the present study.

The prevalence of *E. bieneusi* varied significantly according to cattle age, sex, and the presence/absence of other livestock species within cattle farms. *Enterocytozoon bieneusi* infections were more prevalent in calves younger than six months, while cattle over five years old showed the lowest prevalence (P = 0.029). Males were significantly more frequently infected than females (P = 0.005), with a 0.395-fold increased risk. Additionally, *E. bieneusi* infections were more prevalent in cattle from farms without other livestock compared to mixed farms (P = 0.010). Conversely, no significant differences in prevalence were observed based on location (study area), production system, or the presence or absence of diarrhoea in the infected cattle.

The binary logistic regression model, applied to variables (age, sex, and cohabitation with other livestock species) significant in the univariable analysis, identified cohabitation as the only factor significantly associated with *E. bieneusi* infection. The prevalence rate in cattle from farms without other livestock was higher compared to cattle from mixed farms (P = 0.042), with OR of 0.503 (95% CI: 0.260–0.974). Age and sex of cattle showed no significant association with *E. bieneusi* infection, with ORs of 0.736 (95% CI: 0.505–1.073, P = 0.111) and 1.642 (95% CI: 0.752–3.586, P = 0.214), respectively.

Among the 65 examined cow–calf pairs, 7 (10.8%) were cow-only positive and 16 (24.6%) were calf-only positive for *E. bieneusi*. The presence of *E. bieneusi* varied between cow-calf pairs in these discordant samples (*n* = 23). Only two pairs (3.1%) had *E. bieneusi* in both cows and their calves. The exact McNemar's test showed that this difference was not statistically significant (*P* = 0.097). The estimated difference in proportions was 0.39 (95% CI: 0.13–0.66). Although the difference was not statistically significant (*P*= 0.097), it was observed that calves were more often infected when their mother cows were negative.

Genotyping of *Enterocytozoon bieneusi* isolates

A total of 46 cattle isolates tested positive for *E. bieneusi* at the ITS locus. Of these, high-quality sequencing data allowing genotype identification were obtained for 56.5% (26/46). In the remaining 43.5% (20/46), sequencing quality was insufficient to determine the genotype, although the presence of *E. bieneusi* was confirmed. Sequence analyses revealed seven known genotypes of *E. bieneusi*. The most frequently detected genotype was Type IV (42.3%, 11/26), followed by J (19.2%, 5/26), I (15.4%, 4/26), BEB6 and D (7.7%, 2/26 each), and CM7 and Peru6 (3.9%, 1/26 each). Phylogenetic analysis showed that all seven genotypes clustered within zoonotic Groups 1 (Type IV, D, and Peru6) and 2 (J, I, BEB6, and CM7) (Fig. 2, Table 2).

Table 3 displays the distribution of *E. bieneusi* genotypes in the examined cattle based on epidemiological factors (location, age, sex, production system, cohabitation) and clinical status (diarrhoea) considered in this study. Geographically, the M'sila region showed both the highest frequency (10.6%, 18/170) and the greatest diversity (100%, 7/7) of *E. bieneusi* infections by the seven known genotypes. Type IV was the only genotype detected across all three surveyed regions. The highest infection rates were observed in cattle older than 60 months (23.3%, 7/30) and in males (10.4%, 7/67). Interestingly, 10 out of 11 Type IV isolates were detected in females. Farms raising both dairy and beef cattle exhibited the highest infection rate (21.3%, 10/47). Genotypes CM7 and D were exclusively identified in nondiarrhoeic cattle, while 80.0% (4/5) and 90.9% (10/11) of the isolates characterised as genotypes J and Type IV (respectively) were also found in asymptomatic animals. In one cow-calf pair, both animals were infected by the same *E. bieneusi* genotype J.

Discussion

The epidemiology of microsporidiosis in cattle is poorly documented in North African countries including Algeria, particularly regarding molecular data. Here we report molecular prevalence and genotyping data of *E. bieneusi* in cows and their calves from central Algeria. The main strengths of this study were: (i) understanding of the distribution of *E. bieneusi* genotypes in cattle, (ii) demonstration of the role that cattle can play as important reservoirs of zoonotic genotypes of *E. bieneusi* in central Algeria.

In the present study, the PCR-based prevalence of *E. bieneusi* was estimated at 16.0%. A recent meta-analysis covering 37 studies across different geographical and epidemiological settings estimated a global cattle prevalence of 14.0% under comparable diagnostic conditions [11]. Therefore, our prevalence aligns with the global average reported for this pathogen. However, our prevalence is slightly higher than that reported in a previous Algerian study, which documented a prevalence of 10.8% [14]. Similar prevalence rates (14.0–18.0%) have been reported in South Africa, Brazil, Argentina, and South Korea [19–22]. In Turkey, Iran, the USA, and China, higher prevalence rates (29.0–52.0%) were reported [23–26]. Conversely, lower rates (0.6–10.0%) were documented in Spain, China, Austria, and Thailand [27–30]. These comparisons underscore the wide epidemiological variability of *E. bieneusi* infections, influenced by factors such as host characteristics, study design (e.g., sample size), local husbandry systems, and diagnostic methods.

The molecular prevalence of *E. bieneusi* varied significantly by age group in the present study, with a higher infection rate in calves compared to adults. This pattern aligns with previous studies [31–34] and reflects age-dependent susceptibility stemming from both physiological and behavioural factors. Calves' susceptibility to *E. bieneusi* may be linked to immature immune function [34]. However, age-related differences are not always observed; for example, some studies in China found no significant correlation [12, 35], while in Turkey it was reported that older (more than three years) cattle were more prone to infection [23]. Such discrepancies suggest that both young and adult animals can contribute to transmission dynamics, either as susceptible hosts or as chronic asymptomatic shedders. A sex-related difference was also noted in this study, with males being significantly more infected than females. Similar results have been reported [34], whereas other studies documented opposite findings [36], or no sex-related differences at all [20, 23]. The susceptibility of males or females to *E. bieneusi* infections remains unclear, and further research is necessary to clarify whether sex factors influence this susceptibility.

Although infection rates of *E. bieneusi* varied insignificantly between M'sila, Tipaza, and Algiers, the variation of prevalence rates observed among these areas can be the result of several factors including regional sampling imbalance, or local differences in sources of infection or transmission pathways.

Farm management also appeared to influence the risk of infection with *E. bieneusi*. Cattle raised without contact with other ruminant species were 2.28 times more likely to be infected than those reared alongside sheep or goats. Various cattle-related factors such as management practices, stocking density, and hygienic conditions within cattle farms could increase the risk of *E. bieneusi* infections in cattle populations without co-habitation and co-grazing with small ruminants. However, the relationship between cattle susceptibility to *E. bieneusi* and their cohabitation with other livestock species (including potential cross-species transmission with sheep and goats) remains unclear and requires further investigation.

In this study, *E. bieneusi* detection differed slightly between cows and their calves, with calves showing a higher infection frequency (16 calf-only-positive pairs) than their cows (7 cow-only-positive pairs). Although this difference was not statistically significant, the observed pattern suggests that young animals may be more susceptible to *E. bieneusi* infection than their older counterparts. In addition to the immature immune system of calves, this greater susceptibility could be related to environmental exposure to contaminated faecal material or stress associated with early life management conditions. The environmental transmission of *E. bieneusi* could play an important role on the recurrence of infections in calves. These findings highlight the need for attention to hygiene practices, manure management, and calf-rearing environments to reduce potential exposure and transmission of *E. bieneusi* within herds.

Genotyping analysis identified seven potentially zoonotic *E. bieneusi* genotypes, including Type IV (42.3%), J (19.2%), I (15.4%), BEB6 and D (7.7% each), and CM7 and Peru6 (3.9% each). This diversity surpasses that reported by Baroudi et al. (2017) [14], who detected six genotypes (BEB3, BEB4, BEB6, I, J, and PtEb IX) in Algerian cattle. Compared with Tipaza and Algiers regions, M'sila exhibited both the highest prevalence and the greatest diversity of *E. bieneusi* genotypes. This regional difference is likely attributable to the larger and more representative sample size obtained in M'sila. The recurrence of genotypes BEB6, I, and J in both Algerian studies suggests long-term circulation and potential host adaptation within local cattle populations. The predominance of Type IV (a zoonotic genotype with broad host range) in this study contrasts with the cattle-adapted BEB4 reported previously in Algeria. This shift may reflect regional or temporal changes in genotype epidemiology, with potential implications for cross-species transmission. The identification of CM7, D, and Peru6 genotypes (unreported in Algerian cattle to date) may suggest recent introduction or local emergence, possibly linked to livestock trade or changing environmental conditions. Globally, *E. bieneusi* genotypes BEB4, I, and J, were most frequently detected in cattle [11, 29, 37, 38], but many of the variants identified here (BEB6, CM7, D, Peru6, Type IV) have also been found in humans, non-human primates, domestic animals, and wildlife [3, 6, 39, 40]. The detection of such zoonotic genotypes highlights the public health relevance of cattle as reservoirs of *E. bieneusi* and underscores the importance of integrating veterinary and human surveillance within a One Health framework.

Genotype determination was unsuccessful for approximately half (43.5%) of the *E. bieneusi* PCR-positive samples due to suboptimal sequence quality, most likely associated with low DNA concentrations. The inability to genotype all PCR-positive samples represents a limitation of the present study and restricts a more comprehensive understanding of the distribution of *E. bieneusi* genotypes in cattle populations. Consequently, the molecular findings reported here may not fully reflect the overall epidemiological situation of *E. bieneusi* in Algeria.

In conclusion, our findings indicate a globally consistent prevalence and significant genetic diversity of *E. bieneusi* in Algerian cattle, including zoonotic genotypes reported for the first time in Algeria. These results strengthen the evidence of cattle as potential reservoirs contributing to environmental contamination and zoonotic transmission of *E. bieneusi*, highlighting the importance of coordinated monitoring strategies across animal, human, and environmental health sectors.

Acknowledgements

We thank all farm owners for their help during the sampling process.

Author Contributions

S.L. Conceptualization, sample collection, and manuscript drafting. Y.M.S.A. Executing laboratory experiments and manuscript drafting. A.D. Data analysis and manuscript drafting. A.D. Molecular and phylogenetic analysis, reviewing and editing. S.S. Molecular and phylogenetic analysis. N.R. Sample collection and executing laboratory experiments. H.S. Sample collection. S.D. Sample collection. A.K. Sample collection. D.B. Manuscript drafting. A.D.T. Supervising, reviewing and editing. D.C. Supervising, checking molecular analysis, reviewing and editing. A.K.L. Manuscript drafting, supervising, checking molecular analysis, reviewing and editing.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability

Not applicable.

Ethical Approval

Faecal samples were collected from cattle after obtaining informed consent from the farm owners.

Competing Interests

The authors declare no competing interests.

References

1. Didier ES, Stovall ME, Green LC, Brindley PJ, Sestak K, Didier PJ (2004) Epidemiology of microsporidiosis: sources and modes of transmission. *Vet Parasitol* 126(1–2):145–166. https://doi.org/10.1016/j.vetpar.2004.09.006
2. Didier ES, Weiss LM (2006) Microsporidiosis: current status. *Curr Opin Infect Dis* 19(5):485–492. https://doi.org/10.1097/01.qco.0000244055.46382.23
3. Santín M, Fayer R (2011) Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. *Res Vet Sci* 90(3):363–371. https://doi.org/10.1016/j.rvsc.2010.07.014
4. Matos O, Lobo ML, Xiao L (2012) Epidemiology of *Enterocytozoon bieneusi* infection in humans. *J Parasitol Res* 2012:981424. https://doi.org/10.1155/2012/981424
5. Santín M, Fayer R (2009) *Enterocytozoon bieneusi* genotype nomenclature based on the internal transcribed spacer sequence: A consensus. *J Eukaryot Microbiol* 56(1):34–38. https://doi.org/10.1111/j.1550-7408.2008.00380.x
6. Koehler AV, Zhang Y, Gasser RB (2022) A perspective on the molecular identification, classification, and epidemiology of *Enterocytozoon bieneusi* of animals. *Experientia Supplementum* 114:389–415. https://doi.org/10.1007/978-3-030-93306-7_14
7. Thellier M, Breton J (2008) *Enterocytozoon bieneusi* in human and animals: focus on laboratory identification and molecular epidemiology. *Parasite* 15:349–358. https://doi.org/10.1051/parasite/2008153349
8. Jiang Y, Tao W, Wan Q, Li Q, Yang Y, Lin Y, Zhang S, Li W (2015) Zoonotic and potentially host-adapted *Enterocytozoon bieneusi* genotypes in sheep and cattle in Northeast China and an increasing concern about the zoonotic importance of previously considered ruminant-adapted genotypes. *Appl Environ Microbiol* 81(10):3326–3335. https://doi.org/10.1128/AEM.00328-15
9. Jiang S, Yu S, Feng Y, Zhang L, Santín M, Xiao L, Li W (2024) Widespread distribution of human-infective *Enterocytozoon bieneusi* genotypes in small rodents in

northeast China and phylogeny and zoonotic implications revisited. *Acta Trop* 253:107160. https://doi.org/10.1016/j.actatropica.2024.107160

10. Qin Y, Chen C, Qin YF, Yang XB, Li MH, Meng XZ, Zhao ZY, Ma N, Cai Y, Zhang Y, Zhao Q (2022) Prevalence and related factors of *Enterocytozoon bieneusi* in cattle: a global systematic review and meta-analysis. *Prev Vet Med* 208:105775. https://doi.org/10.1016/j.prevetmed.2022.105775
11. Taghipour A, Bahadory S, Abdoli A (2022) A systematic review and meta-analysis on the global prevalence of cattle microsporidiosis with focus on *Enterocytozoon bieneusi*: an emerging zoonotic pathogen. *Prev Vet Med* 200:105581. https://doi.org/10.1016/j.prevetmed.2022.105581
12. Zhao JQ, Fan YY, Lei YD, Liu D, Wang JW, Yang X, Song JK, Zhao GH (2024) Molecular characterization of common zoonotic protozoan parasites and bacteria causing diarrhea in dairy calves in Ningxia Hui Autonomous Region, China. *Parasite* 31:60. https://doi.org/10.1051/parasite/2024059
13. Laatamna AE, Wagnerová P, Sak B, Květoňová D, Xiao L, Rost M, McEvoy J, Saadi AR, Aissi M, Kváč M (2015) Microsporidia and *Cryptosporidium* in horses and donkeys in Algeria: detection of a novel *Cryptosporidium hominis* subtype family (Ik) in a horse. *Vet Parasitol* 208(3–4):135–142. https://doi.org/10.1016/j.vetpar.2015.01.007
14. Baroudi D, Khelef D, Hakem A, Abdelaziz A, Chen X, Lysen C, Roellig D, Xiao L (2017) Molecular characterization of zoonotic pathogens *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in calves in Algeria. *Vet Parasitol Reg Stud Rep* 8:66–69. https://doi.org/10.1016/j.vprsr.2017.02.005
15. Baroudi D, Zhang H, Amer S, Khelef D, Roellig DM, Wang Y, Feng Y, Xiao L (2018) Divergent *Cryptosporidium parvum* subtype and *Enterocytozoon bieneusi* genotypes in

dromedary camels in Algeria. *Parasitol Res* 117(3):905–910. https://doi.org/10.1007/s00436-017-5734-1

16. Buckholt MA, Lee JH, Tzipori S (2002) Prevalence of *Enterocytozoon bieneusi* in swine: an 18-month survey at a slaughterhouse in Massachusetts. *Appl Environ Microbiol* 68(5):2595–2599. https://doi.org/10.1128/AEM.68.5.2595-2599.2002
17. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGAX: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol* 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096
18. Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York. https://global.oup.com/academic/product/molecular-evolution-and-phylogenetics-9780195135855
19. Abu Samra N, Thompson PN, Jori F, Zhang H, Xiao L (2012) *Enterocytozoon bieneusi* at the wildlife/livestock interface of the Kruger National Park, South Africa. *Vet Parasitol* 190(3–4):587–590. https://doi.org/10.1016/j.vetpar.2012.06.031
20. Fiuza VRS, Lopes CW, Oliveira FC, Fayer R, Santín M (2016) New findings of *Enterocytozoon bieneusi* in beef and dairy cattle in Brazil. *Vet Parasitol* 216:46–51. https://doi.org/10.1016/j.vetpar.2015.12.008
21. Del Coco VF, Córdoba MA, Bilbao G, de Almeida Castro P, Basualdo JA, Santín M (2014) First report of *Enterocytozoon bieneusi* from dairy cattle in Argentina. *Vet Parasitol* 199:112–115. http://dx.doi.org/10.1016/j.vetpar.2013.09.024
22. Lee JH (2007) Prevalence and molecular characteristics of *Enterocytozoon bieneusi* in cattle in Korea. *Parasitol Res* 101:391–396. https://doi.org/10.1007/s00436-007-0468-0
23. Bilgin T, Usluğ S, Karademir GK, Okur M, Yetişmiş G, Yıldırım A (2020) Molecular prevalence and phylogenetic characterization of *Enterocytozoon bieneusi* in healthy

cattle. *Turkiye Parazitol Derg* 44(1):36–42. https://doi.org/10.4274/tpd.galenos.2020.6851

24. Rahimi HM, Mirjalali H, Zali MR (2021) Molecular epidemiology and genotype/subtype distribution of *Blastocystis* sp., *Enterocytozoon bieneusi*, and *Encephalitozoon* spp. in livestock: concern for emerging zoonotic infections. *Sci Rep*11:17467. https://doi.org/10.1038/s41598-021-96960-x
25. Santín M, Dargatz D, Fayer R (2012) Prevalence and genotypes of *Enterocytozoon bieneusi* in weaned beef calves on cow-calf operations in the USA. *Parasitol Res* 110(5):2033–2041. https://doi.org/10.1007/s00436-011-2732-6
26. Zhao A, Zhang K, Xu C, Wang T, Qi M, Li J (2020) Longitudinal identification of *Enterocytozoon bieneusi* in dairy calves on a farm in Southern Xinjiang, China. *Comp Immunol Microbiol Infect Dis* 73:101550. https://doi.org/10.1016/j.cimid.2020.101550
27. Abarca N, Santín M, Ortega S, Maloney JG, George NS, Molokin A, Cardona GA, Dashti A, Köster PC, Bailo B, Hernández-de-Mingo M, Muadica AS, Calero-Bernal R, Carmena D, González-Barrio D (2021) Molecular detection and characterization of *Blastocystis* sp. and *Enterocytozoon bieneusi* in cattle in northern Spain. *Vet Sci* 8(9):191. https://doi.org/10.3390/vetsci8090191
28. Gao H, Liang G, Su N, Li Q, Wang D, Wang J, Zhao L, Kang X, Guo K (2023) Prevalence and molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in diarrheic and non-diarrheic calves from Ningxia, northwestern China. *Animals* 13:1983. https://doi.org/10.3390/ani13121983
29. Lichtmannsperger K, Harl J, Roehl SR, Schoiswohl J, Eibl C, Wittek T, Hinney B, Wiedermann S, Joachim A (2023) *Enterocytozoon bieneusi* in fecal samples from calves and cows in Austria. *Parasitol Res* 122:333–340. https://doi.org/10.1007/s00436-022-07733-y

30. Udonsom R, Prasertbun R, Mahittikorn A, Chiabchalard R, Sutthikornchai C, Palasuwan A, Popruk S (2019) Identification of *Enterocytozoon bieneusi* in goats and cattle in Thailand. *BMC Vet Res* 15:308. https://doi.org/10.1186/s12917-019-2054-y

31. Santín M, Fayer R (2009) A longitudinal study of *Enterocytozoon bieneusi* in dairy cattle. *Parasitol Res* 105(1):141–144. https://doi.org/10.1007/s00436-009-1374-4

32. Li J, Luo N, Wang C, Qi M, Cao J, Cui Z, Huang J, Wang R, Zhang L (2016) Occurrence, molecular characterization and predominant genotypes of *Enterocytozoon bieneusi* in dairy cattle in Henan and Ningxia, China. *Parasites Vectors* 9:142. https://doi.org/10.1186/s13071-016-1425-5

33. Tang C, Cai M, Wang L, Guo Y, Li N, Feng Y, Xiao L (2018) Genetic diversity within dominant *Enterocytozoon bieneusi* genotypes in pre-weaned calves. *Parasites Vectors* 11:170. https://doi.org/10.1186/s13071-018-2768-x

34. Liu Y-Y, Qin R-L, Mei J-J, Zou Y, Zhang Z-H, Zheng W-B, Liu Q, Zhu X-Q, Gao W-W, Xie S-C (2022) Molecular detection and genotyping of *Enterocytozoon bieneusi* in beef cattle in Shanxi province, north China. *Animals* 12, 2961. https://doi.org/10.3390/ani12212961

35. Sun TB, Lin XY, Pan SM, Chen LF, Shi QM, Li WC, Wang QY (2023) Prevalence of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in beef cattle in the Hebei Province of China. *Med Weter* 79(3):142–147. http://dx.doi.org/10.21521/mw.6748

36. Song HY, Wang KS, Yang JF, Mao HM, Pu LH, Zou Y, Ma J, Zhu XQ, Zou FC, He JJ (2021) Prevalence and novel genotypes identification of *Enterocytozoon bieneusi* in dairy cattle in Yunnan province, China. *Animals* 11:3014. https://doi.org/10.3390/ani11113014

37. Li W, Feng Y, Santin M (2019) Host specificity of *Enterocytozoon bieneusi* and public health implications. *Trends Parasitol* 35(6):436–451. https://doi.org/10.1016/j.pt.2019.04.004

38. Wang MY, Zhang S, Zhang ZS, Qian XY, Chai HL, Wang Y, Fan WJ, Yi C, Ding YL, Han WX, Zhao L, Liu YH (2024) Prevalence and molecular characterization of *Cryptosporidium* spp., *Enterocytozoon bieneusi*, and *Giardia duodenalis* in dairy cattle in Ningxia, Northwestern China. *Vet Res Commun* 48:2629–2643. https://doi.org/10.1007/s11259-024-10364-6

39. Sak B, Brady D, Pelikánová M, Květoňová D, Rost M, Kostka M, Tolarová V, Hůzová Z, Kváč M (2011) Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. *J Clin Microbiol* 49(3):1064–1070. https://doi.org/10.1128/JCM.01147-10

40. Wang L, Xiao L, Duan L, Ye J, Guo Y, Guo M, Liu L, Feng Y (2013) Concurrent infections of *Giardia duodenalis*, *Enterocytozoon bieneusi*, and *Clostridium difficile* in children during a cryptosporidiosis outbreak in a pediatric hospital in China. *PLoS Negl Trop Dis* 7(9):e2437. https://doi.org/10.1371/journal.pntd.0002437

Figure legends

Fig. 1 The map of study areas in Algeria where faecal samples from cattle were collected.

Fig. 2 Phylogenetic relationship among *Enterocytozoon bieneusi* genotypes inferred based on partial sequences of the internal transcribed spacer (ITS) region using the Maximum likelihood method implemented in MEGA X. The tree shows the phylogenetic relationship among sequences generated from the present study and reference sequences retrieved from GenBank. Genotype PtEb XI (belonging to Group 11) was used as outgroup to root the tree. Sequences generated in this study are represented with green filled circles.