Comparing Performance of Copro-Diagnostics for Monitoring Intestinal Parasitic Infections in The Golden Jackal (Canis aureus)

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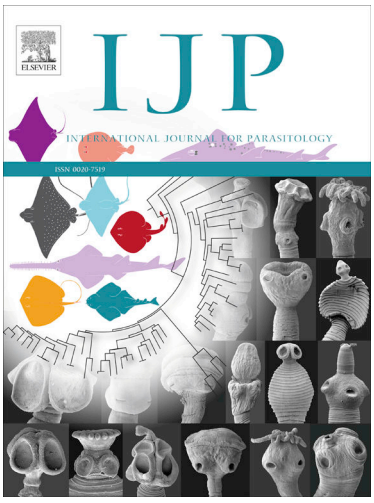

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Comparing Performance of Copro-Diagnostics for Monitoring Intestinal Parasitic Infections in The Golden Jackal (*Canis aureus*)

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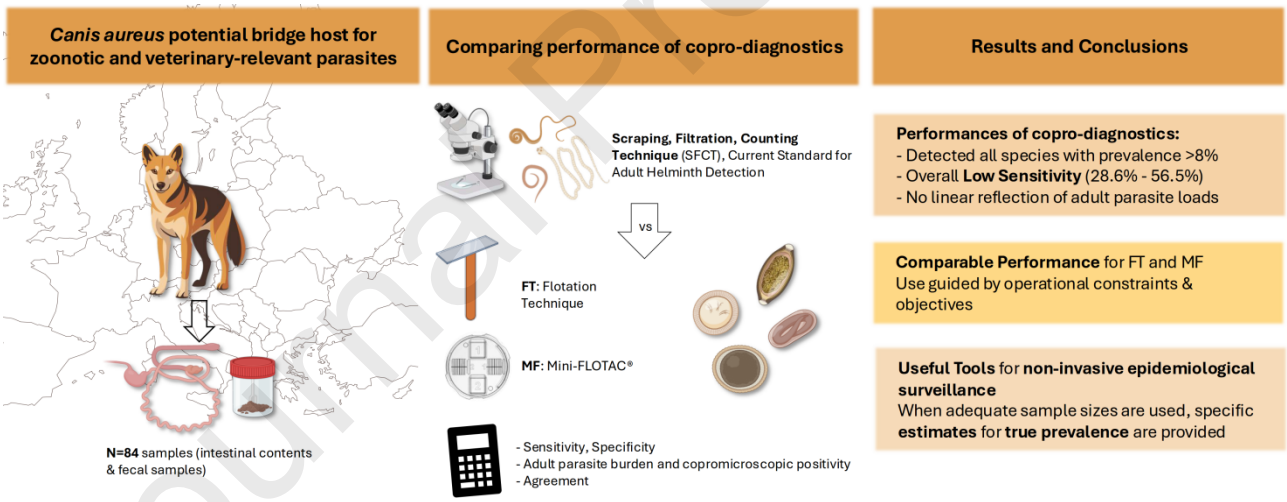

Highlights

- Copromicroscopic techniques show low-medium sensitivity but high specificity
- Adult parasite burden does not predict copromicroscopic positivity in golden jackals
- Fecal surveys can be used for monitoring if prevalence estimates are adjusted for test sensitivity

Abstract

The golden jackal *Canis aureus* is regarded as a potential bridge host for emerging parasites in Europe to domestic animals, owing to its adaptability to live in human-dominated landscapes. As the species continues to expand in Europe, copromicroscopy offers a non-invasive alternative to necropsy-based methods to obtain epidemiology data, but its performance in the species has never been assessed. We compared the performance of two copromicroscopic tests, flotation technique (FT) and Mini-FLOTAC (MF), to the Scraping, Filtration and Counting technique (SFCT), current standard for helminth detection, and assessed their agreement.

The intestinal contents and corresponding fecal samples from 84 golden jackals were examined. Sensitivity and specificity of FT and MF relative to SFCT were estimated for each detected taxon (*Toxocara canis*, *Trichuris vulpis*, strongylids, *Mesocestoides* spp., and *Taenia* spp.). Associations between adult parasite burden and copromicroscopic positivity were explored using non-parametric tests and logistic regression models. Agreement between FT and MF was assessed using concordance and Gwet's γ(AC1).

Both FT and MF detected the most prevalent helminth species at SFCT, but showed low sensitivity, ranging from 28.6% to 56.5%. Parasite intensity did not significantly influence copromicroscopic positivity for most taxa, and quantitative MF results did not linearly reflect parasite loads. The two methods showed comparable performance, suggesting that their use may be guided by operational constraints and study objectives.

Despite their limitations, copromicroscopic techniques represent useful tools for epidemiological surveillance in jackal populations, provided that adequate sample sizes are used and prevalence estimates are adjusted for test sensitivity and specificity.

Keywords: Canidae, sensitivity and specificity, Mini-FLOTAC®, Flotation technique, wildlife monitoring, copromicroscopy

1. Introduction

The golden jackal (*Canis aureus* Linnaeus, 1758) is a medium-sized wild canid known for its remarkable adaptability, granted by a generalist diet and ecological flexibility (Lange et al., 2021). Since the 1980s, the species has undergone a notable range expansion, initially triggered by a substantial population increase in the Dalmatian and Balkan regions, probably due to change in land use, climate change, and the absence of larger competitors (Frangini et al., 2025). This expansion led to a westward dispersal, with individuals reaching the Istrian Peninsula and eventually northeastern Italy (Lapini et al., 2009). Initially mistaken for foxes, golden jackals successfully established stable populations in Friuli Venezia Giulia (FVG) and, from there, progressively spread throughout the Italian Peninsula (Lapini et al., 2009). To date, the species has been recorded in at least ten Italian regions, including FVG, Veneto, Trentino-Alto Adige, Lombardy, Piemonte, Valle d'Aosta, Emilia-Romagna, Toscana, Marche, and Lazio (Bacci and Lunghi, 2022; Di Francesco et al., 2023; Fortebraccio and Aleotti, 2022; Frangini et al., 2025; Lapini et al., 2021). FVG is considered the core area of the Italian population, hosting the highest number of breeding groups (Lapini et al., 2021) and acting as a crucial genetic corridor connecting Italian populations with their Balkan source populations (Fabbri et al., 2014).

With their high degree of flexibility in their diet and behavior, golden jackals are well adapted to human-dominated landscapes, where they can exploit food sources provided indirectly by people, including carrions and, occasionally, viscera of livestock (Ćirović et al., 2016; Lange et al., 2021). This adaptability often brings them in close proximity with humans and domestic animals, exposing them to a wide range of pathogens, some of which are relevant to both veterinary and public health (Gherman and Mihalca, 2017). Owing to these ecological traits, along with their genetic closeness to domestic dogs, golden jackals are recognized as reservoirs or carriers for several parasites and are often described as "bridge hosts" for emerging parasites in Europe, due to their capacity to introduce parasites into new areas and facilitate transmission to sympatric domestic animals (Frey et al., 2022; Gavrilović et al., 2019; Miljević et al., 2021; Orioles et al., 2024; Veronesi et al., 2023). As the species continues to expand in Europe, reports of helminthic infections in golden jackals are steadily increasing, frequently highlighting the presence of zoonotic taxa. Regarding

nematodes, *Toxocara canis* and *Ancylostoma caninum,* capable of causing ocular and cutaneous *larva migrans* in humans, respectively, are among the most detected (Ćirović et al., 2015; Miljević et al., 2021; Takács et al., 2014). Concerning cestodes, golden jackals host several *Taenia* species, as well as *Echinococcus granulosus sensu lato* and *E. multilocularis* (Bandelj et al., 2022; Ćirović et al., 2015; Frey et al., 2022; Miljević et al., 2021; Széll et al., 2013; Takács et al., 2014), the etiological agents of cystic and alveolar echinococcosis, respectively, both of major public health concern (Alvi and Alsayeqh, 2022). Notably, in areas where *E. multilocularis* is already highly prevalent due to the presence of the red fox (*Vulpes vulpes*) as the primary definitive host, such as Switzerland, the establishment of golden jackal populations may introduce an additional definitive host, potentially amplifying transmission dynamics and increasing environmental egg contamination (Frey et al., 2022; Széll et al., 2013). More recently, for the first time in Europe, Croatian researchers have found in jackals the zoonotic pseudophyllidean cestode *Spirometra mansoni* (Šikić et al., 2025) that causes a foodborne zoonosis known as sparganosis. The overlap between expanding jackal populations and areas with high densities of livestock and domestic dogs therefore creates conditions favorable for multi-host parasite transmission cycles, with potential spillover to humans.

Effective surveillance of zoonotic parasites at the wildlife-livestock-human interface requires monitoring methods that are both epidemiologically representative and logistically feasible at a population scale. In FVG, wildlife health monitoring currently relies primarily on necropsies of animals opportunistically found dead in the environment, most often due to human-related causes. While necropsies provide valuable epidemiological insights, they are time-consuming and subject to sampling bias, as carcass sampling tends to overrepresent individuals found near roads: typically, young animals attempting to disperse by crossing landscapes fragmented by roads and anthropogenic barriers. In contrast, individuals living in remote or less accessible and more natural areas, and those dying from non-anthropogenic causes, often go undetected.

Fecal samples collected in the field offer a valuable, non-invasive alternative for studying wildlife, enabling data collection without the need to capture or directly observe the animals. Such samples can support a wide range of analyses, including investigations of gastrointestinal parasitic infections through coprodiagnostic techniques as the commonly used copromicroscopy, which requires relatively low time and financial investment. If validated, such technique would allow broader and more representative epidemiological surveys, enabling the estimation of true parasite prevalence across jackal populations and informing risk assessments at the wildlife-domestic animal interface. However, its diagnostic performance has never been assessed in the golden jackal.

In light of the golden jackal ongoing expansion across Italy and other parts of Europe, and of its role as a reservoir and bridge host for zoonotic helminths, this study aimed at: (1) assessing the performance (i.e., sensitivity and specificity) of two commonly used copromicroscopic tests (flotation technique (FT), and Mini-FLOTAC® (MF)) using as reference the Scraping, Filtration and Counting Technique (SFCT), the current standard for helminth detection in carcasses; (2) evaluating the concordance between the two copromicroscopic tests, to determine whether their diagnostic performance is comparable when used for this species.

2. Material and Methods

2.1. *Collection, Processing, and Parasitological Examination*

Road-killed golden jackals were collected in the FVG region (Italy) between February 2009 and January 2025 as part of the regional wildlife health surveillance network. Carcasses were stored at –20 °C and subsequently necropsied at the University of Udine, Italy. For biosecurity purposes, the intestines were excised and frozen at –80 °C for 72 hours to inactivate *Echinococcus* spp. eggs (Eckert et al., 2001). Intestinal samples were examined within one year after collection either at the University of Udine (Department of Agricultural, Food, Environmental and Animal Sciences) or at the University of Padua (Department of Animal Medicine, Production and Health).

Before examination, intestines were thawed at room temperature and processed using SFCT (modified from Santa et al., 2018) for helminth retrieval and identification. When present, fecal samples were also collected directly from the rectum prior to dissection. Briefly, each intestine was cut open longitudinally and rinsed thoroughly with tap water. The rinse, along with material scraped from the intestinal mucosa, was collected into a beaker. This material was then passed through a series of sieves (1,000, 500, 250, and 75 µm mesh sizes). The retained material was examined under a stereomicroscope (Olympus-SZX12) to isolate and count helminths. Parasites were

morphologically identified, under a light microscope (Nikon Eclipse Si, Nikon Instruments), to the lowest possible taxonomic level using standard identification keys from the literature (Anderson et al., 2009; Hrckova et al., 2011; Loos-Frank, 2000; Scholz et al., 2024), and subsequently counted to quantify helminth burden. Whenever possible, sex of helminths was noted.

When fecal material was available in sufficient quantity (i.e., at least two grams), copromicroscopic analysis was performed using two methods: (1) the flotation technique (FT) (Taylor et al., 2016) and (2) the Mini-FLOTAC® technique (MF) (Cringoli et al., 2017), both employing a zinc sulfate ($ZnSO_4$) solution with a specific gravity (SG) of 1.350, targeting eggs of cestodes and nematodes (Maurelli et al., 2018). The MF method was applied starting in 2021. Two grams of feces were used for each method, and priority was given to MF, if fecal material was not enough for both analyses. Eggs were morphologically identified according to Taylor et al. (2016). For FT, samples were recorded as positive or negative according to the presence or absence of each helminth taxon. MF was used to count the numbers of EPGs (i.e., eggs per gram of feces): 5 was the analytic sensitivity value and the multiplication factor for counted eggs for each taxon (Cringoli et al., 2017).

2.2. *Data analyses*

Helminths identified through SFCT were grouped according to the taxonomic resolution achieved by the copromicroscopic tests, excluding taxa which were never recorded in any of the fecal samples. For parasite taxa detected by SFCT, descriptive statistics [prevalence and 95% confidence intervals (CI) using Clopper-Pearson method, range, mean intensity and bootstrap bias-corrected and accelerated 95% confidence intervals (BCa 95% CI) with 2000 replications, and aggregation parameter *k*] were calculated using Quantitative Parasitology (Reiczigel et al., 2019).

To evaluate the diagnostic performances with respect to SFCT, the sensitivity and specificity of FT and MF were computed for each helminthic taxa using the Clopper-Pearson (exact) method for 95% CI implemented on EpiTools (https://epitools.ausvet.com.au/). For nematodes, sensitivity and specificity were also calculated considering only infestations at SFCT involving more than one adult specimen per species, and the presence of both sexes, as infections with single worm or single-sex individuals would not produce eggs and could not be detected by copromicroscopy (defined as “corrected”, Marchiori et al., 2023).

The influence of adult parasite intensity (defined as the total number of adult helminths recovered from a single host) estimated by SFCT on the qualitative outcome of copromicroscopic tests was assessed using three complementary statistical approaches, considering only individuals with at least one adult in the intestines (i.e., positive at SFCT) and only when the final subset of samples was larger than 15. First, adult parasite intensity was compared between copromicroscopy-positive and -negative hosts using the Mann–Whitney U test. Second, univariable logistic regression models were fitted to evaluate the association between adult parasite intensity and the log-odds (the logit of the probability) of copromicroscopic positivity, for each taxon and each method, separately. For this analysis, adult parasite intensity values were $\log_{10}$-transformed [$\log_{10}(n + 1)$]. Finally, for the quantitative MF outcome, Spearman’s rank correlation coefficient was used to assess the relationship between adult parasite intensity and the MF egg count. A p-value <0.05 was considered statistically significant.

To assess the agreement between FT and MF and determine whether the two methods differ significantly in performance or provide comparable diagnostic results, concordance and Gwet’s γ(AC1) were computed (Gwet, 2008). For γ(AC1) index, confidence intervals were calculated using closed formula of variance estimates in Microsoft Excel, adopting a 95% confidence limit. Gwet’s γ(AC1) assumes values ranging from 0 (no agreement) and 1 (perfect agreement). Gwet’s γ(AC1) was preferred over Cohen’s Kappa or Scott’s π to avoid its paradoxical effects, as it is less influenced by prevalence and marginal probabilities (Giammarino et al., 2021; Wongpakaran et al., 2013).

All statistical analyses, except those already mentioned, were performed using Microsoft Excel (2025) and R ver. 026.01.0+392 (R Core Team, 2026).

2.3 *Data accessibility*

All data supporting this study are available as an open-access dataset at 10.17632/3j5pv9nvxh.1.

3. Results

3.1 *SFCT findings*

In total, 84 intestines were examined using SFCT, and fecal samples were collected from all of them. Of these, 79 were analyzed using the FT, while 50 of these were analyzed using the MF. Among these, 45 samples were analyzed using both FT and MF.

SFCT enabled the detection of seven nematode taxa (*T. canis, Trichuris vulpis, Uncinaria stenocephala, A. caninum, Molineus legerae, Physaloptera* sp., *Pterygodermatites affinis*), three cestodes (*Mesocestoides* spp., *Taenia* spp., *Dipylidium caninum*), and two trematodes (*Metagonimus spp.* and *Alaria alata*).

Copromicroscopic tests allowed the detection of eggs attributable to *T. canis, T. vulpis*, strongylids, *Mesocestoides* spp., and *Taenia* spp.. No eggs of *Physaloptera* sp., *P. affinis*, and *D. caninum* were detected through copromicroscopic examination in any sample. Trematode eggs were not targeted in this study and were therefore not recovered.

For data analyses, helminths were grouped based on the taxonomic resolution achievable by the copromicroscopic methods, as follows: (1) *T. canis*, (2) *T. vulpis*, (3) strongylids (including *U. stenocephala*, *A. caninum*, and *M. legerae* identified through SFCT), (4) *Mesocestoides* spp., and (5) *Taenia* spp. (Table 1).

Table 1. Adult burden and aggregation patterns (prevalence and 95% CI, range, mean intensity and BCa 95% CI, and aggregation parameter *k*) for parasite taxa detected by SFCT (n= 84).

Taxon	Prevalence (%) (95% CI)	Range	Mean Intensity (BCa 95% CI)	k
Toxocara canis	14.3 (7.6-23.6)	0-8	2.7 (1.6-4.4)	0.0921
Trichuris vulpis	8.3 (3.4-16.4)	0-19	5.1 (2.1-12.0)	0.0329
Strongylids	28.6 (19.2-39.5)	0-452	40.7 (18.2-106.0)	0.0647
• ***Uncinaria stenocephala***	27.4 (18.2-38.2)	0-207	30.6 (17.0-59.3)	0.0667
• ***Ancylostoma caninum***	1.2 (0.0-6.5)	0-11	11.0 *	NA *
• ***Molineus legerae***	4.8 (1.3-11.7)	0-245	65.7 (1.5-188)	0.0082
Physaloptera sp.	1.2 (0.0-6.5)	0-1	1.0 *	NA*

Pterygodermatites affinis	6.0 (2.0-13.3)	0-8	3.4 (1.4-6.2)	0.0301
***Mesocestoides* spp.**	10.7 (5.0-19.4)	0-126	30.8 (3.9-74.8)	0.0225
***Taenia* spp.**	39.3 (28.8-50.5)	0-13	3.2 (2.3-4.7)	0.2799
Dipylidium caninum	1.2 (0.0-6.5)	0-1	1.0 *	NA*
Alaria alata	3.6 (0.7-10.1)	0-44	20.0 (4.0-33.3)	0.0082
Metagonimus spp.	6.0 (2.0-13.3)	0-146	55.6 (10.2-112.0)	0.0108

* BCa 95% CI and k could not be computed as only one sample was positive.

3.2 *Copromicroscopy performances*

Regarding the performance of FT compared to the SFCT, sensitivity for nematode detection was generally low to moderate, ranging from 33.3% for *T. vulpis* to 56.5% for strongylids, while specificity was consistently high across all taxa (85.7-100%) (Table 2). Corrected values slightly improved sensitivity for *T. canis* and strongylids, but had a little effect on *T. vulpis*. MF showed similarly low to moderate sensitivity for nematodes, with a notable exception for *T. vulpis*, where corrected values reached 100%, albeit with a wide 95% CI. Specificity for MF was also high across all nematode taxa. For cestodes, both FT and MF showed low sensitivity for *Taenia* spp. and *Mesocestoides* spp., while specificity remained high for both methods. Overall, sensitivity estimates should be interpreted with caution, particularly for taxa with low SFCT prevalence (namely *T. canis*, *T. vulpis,* and *Mesocestoides* spp.), given the small number of positive individuals in both FT and MF subsets, which results in wide confidence intervals and substantial uncertainty.

Table 2. Sensitivity and specificity for nematodes (*T. canis*, *T. vulpis*, and strongylids) and cestodes (*Mesocestoides* spp. and *Taenia* spp.). Sensitivity and specificity values and their 95% CI calculated using Clopper-Pearson (exact) method are reported for all cases, as well as after excluding infections involving only a single worm or single-sex individuals, which do not produce eggs (Corrected).

Parasitic taxa	**Tests**	**Sensitivity % (95% CI) [Corrected]**	**Specificity % (95% CI) [Corrected]**
Toxocara canis	SFCT *vs* FT (*n*= 79)	50.0 (21.1-78.9)	100 (94.6-100)
		[75.0 (19.4-99.4)]	[96.0 (88.8-99.2)]

	SFCT *vs* MF (*n*= 50)	28.6 (3.7-71.0)	100 (91.8-100)
		[28.6 (3.7-71.0)]	[100 (91.8-100)]
Trichuris vulpis	SFCT *vs* FT (*n*= 79)	33.3 (4.3-77.7)	97.3 (90.5-99.7)
		[33.3 (0.8-90.6)]	[96.0 (88.9-99.2)]
	SFCT *vs* MF (*n*= 50)	33.3 (0.8-90.6)	93.6 (82.5-98.7)
		[100 (2.5-100)]	[93.9 (83.1-98.7)]
Strongylids	SFCT *vs* FT (*n*= 79)	56.5 (34.5-76.8)	85.7 (73.8-93.6)
		[64.7 (38.3-85.8)]	[83.9 (72.3-92.0)]
	SFCT *vs* MF (*n*= 50)	52.9 (27.8-77.0)	87.9 (71.8-96.6)
		[58.3 (27.7-84.8)]	[84.2 (68.8-94.0)]
***Mesocestoides* spp.**	SFCT *vs* FT (*n*= 79)	11.1 (0.3-48.3)	100 (94.9-100)
	SFCT *vs* MF (*n*= 50)	16.7 (0.4-64.1)	100 (92.0-100)
***Taenia* spp.**	SFCT *vs* FT (*n*= 79)	18.2 (7.0-35.5)	97.8 (88.5-99.9)
	SFCT *vs* MF (*n*= 50)	29.4 (10.3-56.0)	93.9 (79.8-99.3)

Given the variability observed in the diagnostic performance of FT and MF among parasite taxa, we further investigated whether copromicroscopic positivity was influenced by adult parasite intensity. Adult parasite burdens showed an aggregated distribution across all taxa (Table 1). Strongylids exhibited the highest intensities among nematodes, while *Mesocestoides* spp. reached higher values compared to *Taenia* spp.. Other taxa displayed comparatively low intensities, not exceeding 19 specimens per host (Table 1).

Higher parasite intensities were not consistently associated with a greater probability of copromicroscopic detection. For most taxa, at least one individual within the first quartile of SFCT-positive animals (ranked by intensity) yielded a negative copromicroscopic result. An exception was strongylids, which were detected by FT in all seven individuals with the highest burdens (33-452 specimens). Conversely, the two individuals harboring the highest *Mesocestoides* spp. burdens (126 and 122 specimens) tested negative by both FT and MF.

Formal statistical analyses were restricted to strongylids and *Taenia* spp., as only these taxa had subsets of positive animals larger than 15 samples. The Mann–Whitney test revealed a significant positive trend between adult strongylid burden and FT positivity (p = 0.037), which was further supported by logistic regression (p = 0.050), suggesting a tendency toward increased detection at higher adult parasite intensities. No significant associations were observed for *Taenia* spp. under either method, nor for strongylids detected by MF. Similarly, Spearman's rank correlations between adult worm counts and quantitative MF egg counts were generally low and non-significant across all taxa, indicating a weak relationship between adult burden and copromicroscopic egg counts.

3.3 *Agreement assessment*

The concordance values between the two copromicroscopic tests (FT and MF) were high, ranging from 87% (strongylids) to 100% (*T. canis* and *Mesocestoides* spp.). $\gamma(AC_1)$ provided a high level of agreement, in line with concordance values. A perfect agreement was observed for *T. canis* and *Mesocestoides* spp. (1.00), whereas substantial agreement was found for *T. vulpis*, strongylids and *Taenia* spp. (0.95, 0.77 and 0.89, respectively) (Table 3).

Table 3. Agreement [concordance and Gwet's γ(AC1) Index] between FT and MF for the detection of all helminths considered.

Parasitic taxa	**Concordance rate (%)**	**$\gamma(AC_1)$ Index (95% CI)**
Toxocara canis	100	1.00 (0.88-1.00)
Trichuris vulpis	96	0.95 (0.83-1.00)
Strongylids	87	0.77 (0.66-0.89)
***Mesocestoides* spp.**	100	1.00 (0.88-1.00)
***Taenia* spp.**	91	0.89 (0.77-1.00)

4. Discussion

This study aimed to evaluate the performance of two copromicroscopic techniques, the flotation technique and Mini-FLOTAC®, in detecting the presence and estimating the burden of helminth species in a golden jackal population, to offer recommendation for the most suitable technique to be applied for monitoring purposes. This represents the first investigation into the diagnostic reliability of copromicroscopy in this range-expanding wild canid species. Previous studies have shown that diagnostic performance cannot be readily extrapolated across parasite species, even within the same taxonomic order; therefore, copromicroscopic methods require validation for each target parasite and host species (Becker et al., 2016).

Our results indicate that copromicroscopy tended to detect helminth species occurring at high prevalence values at SFCT, while taxa recorded only sporadically were generally missed (two nematodes, *P. affinis* and *Physaloptera* spp., and the cestode *D. caninum*).

The helminth community detected in this study through SFCT reflects a diverse assemblage typical of wild canids, partially overlapping with that reported in the red fox and the wolf (*Canis lupus*) in Italy. All nematode and cestode taxa, along with *A. alata*, have been previously documented in Italian red foxes and wolves, although prevalence values varied among regions (Cafiero et al., 2025; de Macedo et al., 2019; Ferraro et al., 2026; Fiocchi et al., 2016; Magi et al., 2009; Perrucci et al., 2023), confirming the potential for parasite sharing across sympatric canid hosts. Notably, *Metagonimus spp.*, fishborne zoonotic heterophyid trematode, has only been reported in the same jackal population in FVG (Lapini et al., 2009; Scholz et al., 2024), representing, to our knowledge, the only records of these fishborne trematodes in wild canids in Italy. The progressive westward expansion of the golden jackal into Italy thus appears to be accompanied by the introduction of a diverse helminth fauna, some components of which represent new records for wild canids in the region, while others, although previously known in sympatric canids, may find in the jackal an additional host capable of amplifying transmission at the wildlife-domestic interface.

Overall, the sensitivity of copromicroscopy was low; however, zoonotic and veterinary-relevant parasites were detected and, for certain taxa, sensitivity reached levels above 50%, especially when considering corrected values (i.e., excluding infections involving only single worms or individuals of a single sex). All taxa showed high specificity, in most cases higher than 90%, confirming that the observation of the eggs in the feces is most likely associated with the presence of the adult parasites.

In general, the low sensitivity of copromicroscopy for the detection of intestinal helminths is not unexpected and has been reported in several host species (Byrne et al., 2018; Gassó et al., 2015; Karamon et al., 2018; Kolapo et al., 2021; Magi et al., 2015; Marchiori et al., 2023; Miljević et al., 2021). A qualitative comparison between intestinal scraping and coprological examination in jackals from Serbia was previously attempted by Miljević et al. (2021), who reported that copromicroscopy identified all nematode taxa detected at necropsy (*T. canis, Toxascaris leonina*, and *U. stenocephala*), while taeniid eggs were recovered at very low prevalence in fecal samples and *Mesocestoides* spp. was not detected. However, that study was limited to a qualitative comparison of prevalence estimates between the two methods, without formally quantifying diagnostic performance in terms of sensitivity and specificity, as done in the present study. Similar low sensitivity values have been reported in red foxes, where flotation technique using a Zinc chloride solution (SG 1.350) performed worse than in our study, with sensitivity values below 40% for ascarids (36.3%) and ancylostomatids (28.9%), and as low as 9.8% for Taeniidae (Marchiori et al., 2023). The particularly poor copromicroscopic detectability of Taeniidae across wild canids is further corroborated by Kolapo et al. (2021), who tested the sensitivity of fecal flotation relative to SFCT in wolves, coyotes (*Canis latrans*), and foxes in Canada, reporting values ranging from 0% in foxes to 25% in coyotes. For nematodes, the low sensitivity may be partly attributable to deep freezing, which can deform or destroy thin-walled eggs, such as those of Ancylostomatidae and *Pterygodermatites* spp., reducing their detectability during flotation (Marchiori et al., 2023). The intermittent shedding of proglottids and/or eggs, particularly in cestodes, as well as prepatent infection, are well-known causes of frequent false negatives in copromicroscopic examinations (Morelli et al., 2024). The detection of *Mesocestoides* eggs in this study was somewhat surprising, as their thin egg walls are prone to deformation from both freezing and the flotation solution. Additionally, the thick-walled parauterine organ of this cestode hinders egg release during sample preparation, contributing further to false negatives. In domestic species, direct observation of fresh proglottids in feces is considered a more sensitive method for detecting this parasite (Széll et al., 2015), but it is obviously inapplicable when collecting environmental fecal samples. Indeed, for epidemiological surveys on cestodes, molecular techniques targeting copro-DNA seem to be a more promising alternative (Kolapo et al., 2021; Ulziijargal et al., 2020).

The specificity of copromicroscopy in our study was constantly above 90% in all taxa, apart from strongylids, which showed values around 85%. In a study on badgers (*Meles meles*), copromicroscopy using a modified formol–ether concentration technique showed a specificity of 100% for the detection of *U. criniformis*, a common hookworm in this species, with no false positives detected in the sampled population (Byrne et al., 2018). This difference between that study and ours may be explained by methodological and sampling factors: in Byrne et al. (2018), the badgers were collected during a culling program for control of bovine tuberculosis and their gastrointestinal tracts were examined within 1-3 days after euthanasia, whereas our samples were collected opportunistically and not always under optimal conditions. Moreover, intestinal samples in our study were stored at −80 °C prior to analysis, a process that may have compromised the worm integrity and further reduced detection at SFCT. These reasons might explain why specificity of strongylids decreased when considering corrected values. Indeed, two specimens of *U. stenocephala* of a single sex were found in two intestines and were therefore considered negative in the calculation of the corrected values. However, the fecal samples tested positive for the presence of eggs, indicating that additional adult worms

were present in the gut but went undetected. Nevertheless, safety protocols (e.g., deep freezing) are unavoidable when dealing with such host species, and, realistically, the consequent bias must be acknowledged. Alternatively, false positive samples may be related to the presence of spurious parasites. High specificity for Taeniidae is nonetheless consistent with findings from Kolapo et al. (2021), who reported values ranging from 94% to 100% for fecal flotation in wild canids from Canada. Interestingly, in the same study, copro-real-time PCR showed markedly lower specificity, ranging from 63% to 86%, resulting in a higher proportion of false positives; the authors therefore suggested combining fecal flotation with copro-PCR to maximize diagnostic and epidemiological information.

Generally, animals with low adult parasite burdens frequently test negative at copromicroscopy. However, our analyses (i.e., Mann Whitney test and logistic regression) could not detect any significant influence of adult parasite intensity on the detection of infection with copromicroscopic techniques for most taxa, apart from strongylids analyzed by FT, for which significant differences were observed. This pattern was especially evident for *Mesocestoides* spp., where even hosts with high adult burdens were negative at both FT and MF. This suggests that factors other than parasite number, such as intermittent egg shedding or parasite biology, may strongly affect copromicroscopic detectability. Consistently, Becker et al. (2016), in their validation of the sedimentation–flotation method using a $ZnSO_4$ solution (SG 1.30), reported higher diagnostic reliability for gastrointestinal nematodes of herbivores, whereas ascarids, *T. vulpis*, *A. caninum*, and *U. stenocephala* showed markedly lower detectability.

Spearman's rank correlation between adult parasite intensity and quantitative MF results was generally not significant across all taxa. These findings support the view that fecal egg counts (FEC) obtained through copromicroscopy do not linearly reflect adult parasite burden, particularly under conditions of low burden and high parasite aggregation in hosts. Several biological factors may contribute to this limited correlation, including host immune response, parasite fecundity, female size, sex ratio, and temporal variation in egg shedding (Kuzmina et al., 2012; Taylor et al., 2016). Our results are consistent with current veterinary practice, in which FEC is considered primarily informative for strongyles of ruminants and horses, and is mainly used to identify individuals shedding high numbers of eggs for targeted anthelmintic treatment (Maurizio et al., 2021; Scala et al., 2020).

The comparison between the two copromicroscopic methods revealed very similar performance and a high level of agreement across all taxa. This suggests that the choice between them can be guided primarily by operational constraints or specific study objectives. Among the two, MF offers the additional advantage of providing quantitative data; however, as discussed above, the ecological interpretation of these quantitative outputs remains limited.

Copromicroscopic analysis can represent a useful tool for surveillance purposes, provided that its intrinsic limitations are acknowledged, particularly the likely underestimation of prevalence and the reduced detectability of certain parasite taxa. In contrast, for ecological investigations and comprehensive descriptions of helminth communities, more exhaustive approaches should be considered, foremost among them necropsy-based SFCT, which remains the reference method for accurately assessing parasite burden and community composition. The diagnostic performance estimates generated in this study provide a practical tool for improving helminth surveillance in golden jackal populations. By incorporating the sensitivity and specificity values into prevalence calculations, apparent prevalence derived from non-invasive fecal sampling can be adjusted to obtain more reliable estimates of true infection levels in wild populations. This is particularly relevant for species such as the golden jackal, where large-scale necropsy-based investigations are logistically and ethically constrained. Correcting for imperfect diagnostic performance reduces the risk of underestimating infection rates in low-prevalence cases and improves the interpretation of spatial and temporal trends. From a management perspective, more accurate prevalence estimates strengthen risk assessment for zoonotic helminths, support targeted control strategies at the wildlife-domestic animal interface, and enhance evidence-based decision-making within a One Health framework.

5. Conclusions

The present study investigated the use of the flotation technique and the Mini-FLOTAC, two copromicroscopic tests widely used in parasitology, assessing and comparing their diagnostic performances when applied to golden jackal. This wild species, and specifically its population that is expanding throughout the Italian territory, requires an intensive monitoring within the framework of a wildlife health surveillance program. Although species-level identification was possible only for certain parasitic taxa and both methods showed low sensitivity, their use in epidemiological surveys should not be disregarded. In fact, although at the individual level copromicroscopic results

were often unreliable, when applied at population level, these techniques provided a more informative and encouraging perspective. All parasitic taxa of major zoonotic and veterinary relevance were successfully detected. The low sensitivity of copromicroscopy inevitably leads to an underestimation of true prevalence for all parasite taxa; nevertheless, this bias can be consistently accounted for during data interpretation, and true prevalence estimated using adequate sensitivity and specificity estimates, as the ones we provided. Therefore, despite their limitations, copromicroscopic techniques can be effectively applied for surveillance purposes, provided that adequate sample sizes are used and results are interpreted with appropriate caution. Molecular diagnostics applied directly to fecal samples can enhance the detection of low-prevalence parasite species, including *E. multilocularis* (Knapp et al., 2014; Obber et al., 2022; Umhang et al., 2014). However, these approaches require higher costs and are more suitable for targeted surveillance of high-priority zoonotic taxa, such as Taeniidae, rather than for routine screening of the overall community of helminths, including nematodes, that would require even more complex and more expensive molecular tools (e.g., meta-barcoding; Miller et al., 2024).

Ethics Statement

This study did not involve live-animal handling. Golden jackal carcasses were retrieved in FVG as part of the regional wildlife health surveillance network (InfoFaunaFVG). For this reason, no ethical approval was required.

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