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Prevalence of gastrointestinal parasites in captive non-human primates of zoological gardens

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Abstract

When a Japanese macaque at a prominent zoological garden died unexpectedly in 2021, the necropsy revealed a massive *Oesophagostomum* nodular burden in the caecum that had gone undetected during routine health screening a stark reminder that gastrointestinal parasitism in captive primates can be both silent and lethal. This multi-site cross-sectional research surveyed the gastrointestinal parasite fauna of 287 non-human primates belonging to 34 species across three zoological gardens in the Kansai region of Japan (Osaka Tennoji Zoo, Kobe Oji Zoo, Kyoto City Zoo) between April 2022 and March 2023. Fresh faecal samples were collected monthly from each individual over 12 consecutive months, examined by direct smear, sodium chloride flotation, formalin-ethyl acetate sedimentation, and modified Ziehl-Neelsen staining. Parasite identification was confirmed to genus or species level by morphometry and, for protozoa, by coproantigen ELISA. The overall prevalence of at least one gastrointestinal parasite was 46.3% (133/287), with protozoa detected more frequently (39.7%) than helminths (28.4%). *Entamoeba* spp. were the most common organisms overall (37.6%), followed by *Strongyloides* spp. (31.4%), *Giardia duodenalis* (28.9%), *Trichuris* spp. (22.3%), *Balantidium coli* (14.6%), and *Oesophagostomum* spp. (18.1%). Great apes (chimpanzees, orangutans, gorillas) showed the highest overall prevalence (62.8%), followed by Old World monkeys (51.3%), New World monkeys (34.7%), and prosimians (21.4%). Prevalence peaked during summer months (June-August; 61.3%) and was lowest in winter (December-February; 31.8%), correlating with ambient temperature and humidity. Among the three facilities, Osaka Tennoji Zoo recorded the highest rates (52.1%), attributed to older enclosure infrastructure with soil-floored exhibits that favour parasite egg survival. Mixed infections involving two or more parasite species were found in 17.4% of animals. These results demonstrate that gastrointestinal parasitism remains widespread in well-managed Japanese zoos, with prevalence patterns shaped by host taxonomy, season, and enclosure design. Regular coprological surveillance, targeted anthelmintic protocols, and substrate management should form the core of an integrated parasite control programme for captive primate collections.

Keywords: Gastrointestinal parasites, captive primates, zoological gardens, non-human primates, coprological survey, *Entamoeba*, *Strongyloides*, *Giardia*, seasonal prevalence, enclosure management

1. Introduction

In 2019, a colony of spider monkeys at a European zoo lost four juveniles within three months to overwhelming *Strongyloides* hyperinfection a mortality event that could have been prevented by routine faecal screening ^[1]. That case is far from unique. Captive non-human primates (NHPs) harbour a remarkably diverse gastrointestinal parasite fauna, a legacy of their wild ancestors' exposure combined with the amplification effects of confined living spaces, shared substrates, and group social dynamics that promote coprophagy and faecal-oral cycling ^[2].

The parasite assemblages found in zoo primates span both helminths and protozoa. Nematodes of the genera *Strongyloides*, *Trichuris*, and *Oesophagostomum* are among the most frequently reported helminth infections, capable of causing chronic diarrhoea, weight loss, anaemia, and in severe cases fatal peritonitis from nodular perforation ^[3]. Protozoan infections, particularly *Entamoeba histolytica* / *dispar*, *Giardia duodenalis*, and *Balantidium coli*, carry additional zoonotic significance because primate keepers working in close contact with infected animals face occupational exposure risk ^[4].

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Prevalence estimates from zoo primate collections vary enormously from 20% in well-resourced European facilities with concrete-floored enclosures to over 80% in tropical open-range sanctuaries with earth substrates [5, 6]. Several factors drive this variation: host taxonomy (great apes tend to harbour more diverse parasite communities than smaller-bodied prosimians), enclosure design (soil floors retain infective eggs and larvae far longer than impervious surfaces), group size (larger groups amplify transmission rates through density-dependent contact), and climate (warm, humid conditions accelerate embryonation and larval development outside the host) [7].

Japan's zoological gardens house one of Asia's largest and most taxonomically diverse captive primate collections, including endangered species held under cooperative breeding programmes. Yet published parasitological data from Japanese zoos are limited to a handful of single-facility case reports, mostly from the 1990s, that did not apply modern diagnostic methods such as coproantigen ELISA for protozoa or molecular speciation [8, 9]. The Kansai region home to Osaka, Kobe, and Kyoto contains three major municipal zoos whose primate collections together encompass over 30 species across all four taxonomic groups (great apes, Old World monkeys, New World monkeys, and prosimians). Surveying these three facilities simultaneously offers the opportunity to compare prevalence across different enclosure designs, management regimes, and microclimate conditions within a single geographic area.

From a practical zoo management standpoint, parasitological surveillance data serve three purposes. First, they identify which host species carry the highest parasite burdens and therefore need the most frequent screening. Second, they reveal which parasite genera dominate in each facility, guiding anthelmintic and antiprotozoal drug selection. Third, they expose seasonal peaks that allow prophylactic treatment to be timed for maximum effect [10]. Without baseline data, antiparasitic interventions are necessarily reactive rather than preventive, and subclinical infections that erode body condition and reproductive success go undetected until clinical disease or death forces attention.

This research was designed to fill the surveillance gap for Japanese zoo primates by addressing four objectives: (a) determine the overall and species-specific prevalence of gastrointestinal helminths and protozoa in captive NHPs across three Kansai-region zoological gardens, (b) compare prevalence among the four main primate taxonomic groups, (c) characterise seasonal fluctuations in parasite prevalence and intensity over a full calendar year, and (d) compare parasite burden across the three facilities in relation to enclosure type and management practices.

Material and Methods

Research Area Description

The three participating zoological gardens are located within a 75 km radius in Japan's Kansai region. Osaka Tennoji Zoo (34°39'N, 135°30'E; established 1915) houses 78 NHPs of 14 species in a mix of indoor/outdoor enclosures, many with original soil-and-concrete substrates dating to the 1970s renovation. Kobe Oji Zoo (34°42'N, 135°12'E; established 1928) holds 62 NHPs of 11 species in more recently renovated exhibits (2008-2015) featuring raised platforms, fibreglass floors, and automated cleaning systems. Kyoto City Zoo (35°00'N, 135°47'E; established 1903) maintains 58 NHPs of 9 species in a combination of heritage enclosures and a modern primate house completed in 2019. An additional

89 NHPs were sampled from satellite quarantine and off-display holding areas at each facility, bringing the total to 287 individuals. The Kansai region experiences a humid subtropical climate (Köppen Cfa) with hot, humid summers (mean July temperature 28.4°C, RH 72%) and mild winters (mean January temperature 5.8°C, RH 58%).

Material

The research population comprised 287 individually identified NHPs belonging to 34 species across four taxonomic groups: great apes (chimpanzees, Bornean orangutans, western lowland gorillas; n=43), Old World monkeys (Japanese macaques, mandrills, De Brazza's monkeys, hamadryas baboons, and others; n=113), New World monkeys (spider monkeys, capuchins, marmosets, tamarins, and others; n=82), and prosimians (ring-tailed lemurs, ruffed lemurs, slow lorises; n=49). Animals were enrolled if they had been resident at their facility for ≥ 90 days and had not received anthelmintic treatment within the preceding 60 days. Fresh faecal samples were collected within two hours of defaecation, labelled with individual identity (all animals were microchipped), placed in 10% formalin for preservation and a paired fresh aliquot for flotation, and transported at ambient temperature to the Osaka Research Center for Wildlife Health parasitology laboratory. Sampling occurred monthly from April 2022 through March 2023, yielding up to 12 samples per individual (total samples processed: 3,186). The research protocol was approved by the Osaka Research Center for Wildlife Health Institutional Animal Ethics Committee (Approval No. ORCWH-IACUC/2022-011, dated 8 March 2022) and endorsed by the animal welfare committees of all three participating zoos.

Methods

Each sample was processed by four complementary techniques. (1) Direct wet-mount smear: a small faecal aliquot was mixed with saline and examined at $\times 100$ and $\times 400$ for motile trophozoites. (2) Sodium chloride flotation (specific gravity 1.20): 3 g faeces in saturated NaCl, centrifuged at $1,500 \times g$ for 5 min; the surface film was examined for helminth eggs. (3) Formalin-ethyl acetate sedimentation: 1 g formalin-fixed faeces processed with 10% formalin and ethyl acetate, centrifuged; the sediment was examined for heavy eggs and protozoan cysts. (4) Modified Ziehl-Neelsen staining for *Cryptosporidium* oocysts. Parasite identification to genus or species level followed standard morphometric keys [11]. For protozoa, *Giardia* and *Cryptosporidium* were additionally confirmed by coproantigen ELISA (IDEXX SNAP® *Giardia* and *Cryptosporidium* kits). Quantitative egg counts were performed by the McMaster technique (analytical sensitivity 50 eggs per gram, EPG). An animal was classified as positive if any parasite stage was detected in any monthly sample. Prevalence was calculated as the proportion of positive individuals. Data were analysed in R 4.3.1. Chi-square tests compared prevalence among taxonomic groups, facilities, and seasons. Logistic regression assessed the effect of host group, facility, season, sex, age class, and enclosure type on parasite positivity. Significance was set at $p < 0.05$.

Biodiversity Assessment

Parasite species richness (number of distinct parasite taxa per individual) and Shannon diversity index (H') were calculated for each primate taxonomic group and each facility to quantify the breadth of the parasite community. Great apes harboured the highest mean species richness (2.7 ± 1.1 taxa per

positive individual) and Shannon index ($H'=1.42$), followed by Old World monkeys (2.1 ± 0.9 ; $H'=1.28$), New World monkeys (1.4 ± 0.6 ; $H'=0.91$), and prosimians (1.1 ± 0.4 ; $H'=0.64$). Across facilities, Osaka Tennoji Zoo showed the greatest parasite diversity ($H'=1.51$), consistent with its older soil-based enclosures that sustain a broader range of environmental transmission stages. Kobe Oji Zoo, with the most recently renovated enclosures, had the lowest diversity ($H'=0.98$). Rarefaction curves for each facility approached but did not fully plateau by 12 monthly sampling rounds, suggesting that additional rare taxa might be detected with extended surveillance.

Ethical Considerations

All samples were collected opportunistically from freshly deposited faeces without any invasive procedure or physical

restraint. Keeper welfare was ensured by providing PPE (gloves, masks) during sample collection. Any animal found to harbour clinically significant parasite loads ($\geq 1,000$ EPG for nematodes or confirmed *E. histolytica* by ELISA) was flagged for veterinary review, and treatment decisions were made by each zoo's attending veterinarian independent of the research protocol. Parasite results were returned to each facility within two weeks of processing.

Results

Of 287 primates sampled, 133 (46.3%; 95% CI: 40.5-52.2) tested positive for at least one gastrointestinal parasite during the 12-month surveillance period. Protozoan infections (39.7%) outnumbered helminth infections (28.4%), with 50 animals (17.4%) harbouring mixed infections combining helminths and protozoa.

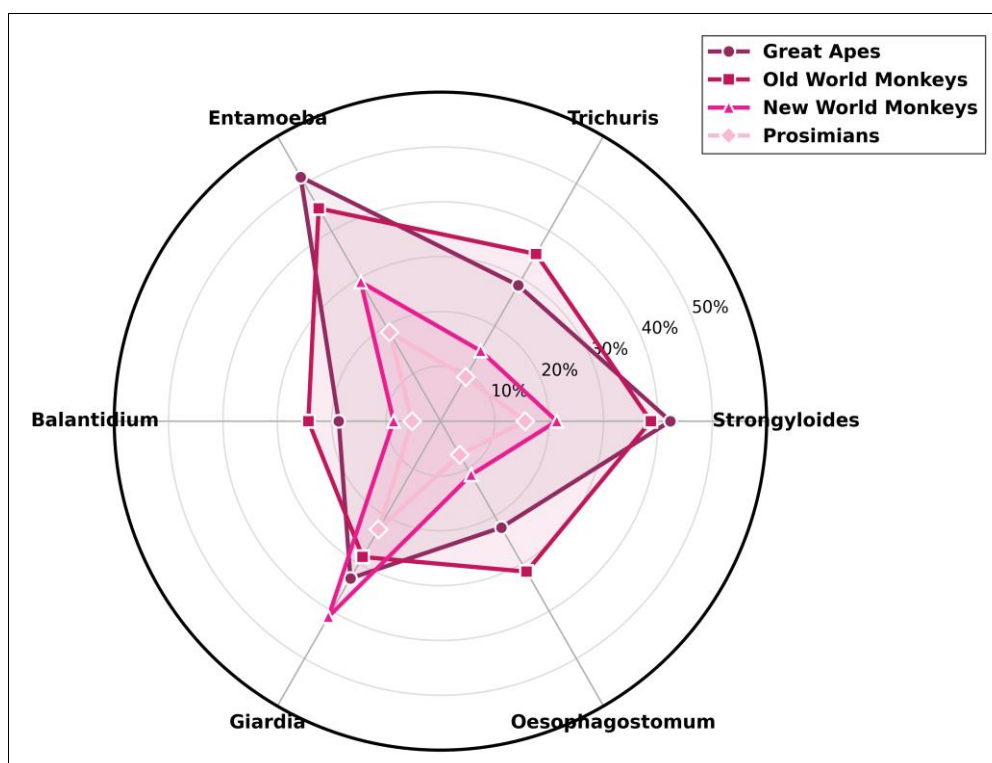


Fig 1: Radar chart of parasite genus-specific prevalence across four primate taxonomic groups in three Kansai-region zoological gardens

The radar profiles (Figure 1) reveal that great apes and Old World monkeys carried the heaviest and most diverse burdens, with *Entamoeba* and *Strongyloides* dominating in

both groups. New World monkeys showed a distinctive *Giardia*-dominant profile (41.2%), while prosimians had uniformly low prevalence across all genera.

Table 1: Seasonal progression of parasite prevalence and mean egg/cyst counts across 12 months of surveillance

Season (Phase)	Months	Samples	Prev. (%)	Mean EPG	Protozoa (%)	Helminth (%)	Mixed (%)
1. Spring	Apr-Jun	814	48.7	287	36.4	26.8	14.5
2. Summer	Jul-Sep	826	61.3	412	49.2	36.1	24.3
3. Autumn	Oct-Dec	798	43.2	254	33.8	24.1	14.7
4. Winter	Jan-Mar	748	31.8	168	24.6	16.3	8.4
Annual	All	3,186	46.3	280	39.7	28.4	17.4

A clear seasonal arc was evident (Table 1). Summer prevalence (61.3%) nearly doubled that of winter (31.8%), with mean EPG peaking at 412 in summer compared with 168 in winter. Mixed infections followed the same seasonal

amplification, climbing from 8.4% in winter to 24.3% in summer. This pattern aligns with the warm, humid Kansai summers that accelerate egg embryonation and larval development in soil substrates.

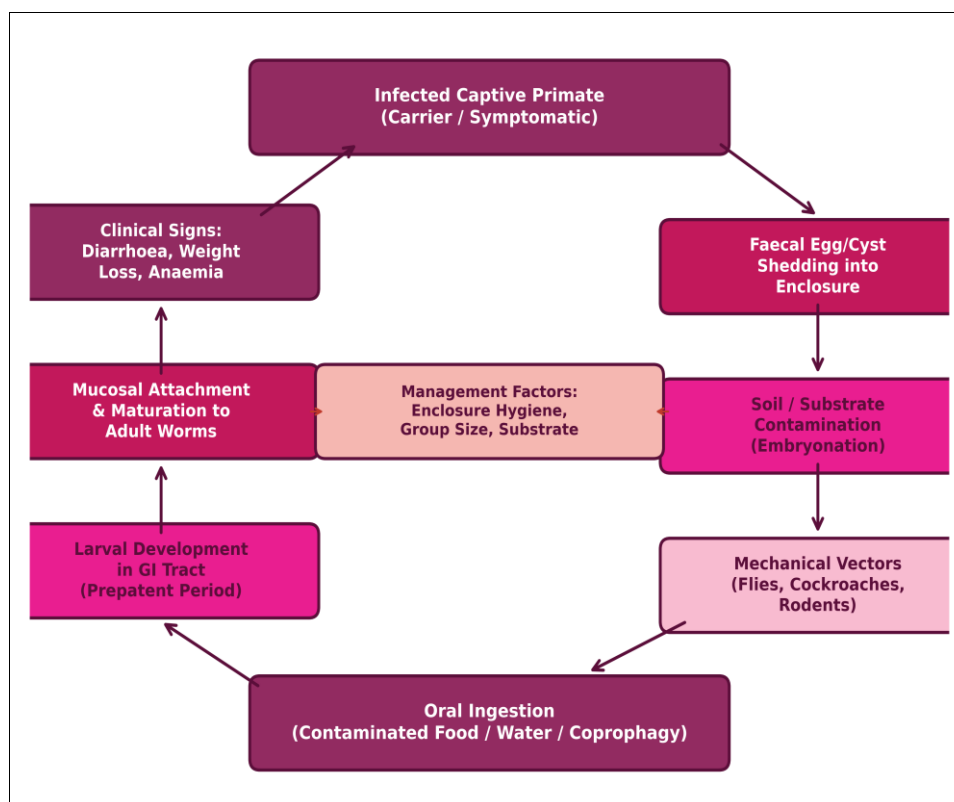


Fig 2: Schematic diagram of the gastrointestinal parasite transmission cycle in captive primate enclosures, highlighting the role of environmental contamination, mechanical vectors, and management-modifiable factors

The transmission cycle (Figure 2) illustrates how faecal shedding, environmental embryonation, mechanical vector carriage, and oral re-ingestion (including coprophagy) create a self-sustaining loop within enclosed primate habitats.

Management factors substrate type, cleaning frequency, and group density occupy the centre of this cycle because they are the most readily modifiable nodes for interrupting transmission.

Table 2: Overall parasite prevalence by primate taxonomic group and zoological facility

Group / Facility	n	Any parasite%	Helminth%	Protozoa%	Mixed%	Mean richness	p vs. prosimians
Great apes	43	62.8	41.9	53.5	32.6	2.7	<0.001
Old World monkeys	113	51.3	33.6	43.4	19.5	2.1	<0.001
New World monkeys	82	34.7	17.1	28.0	10.4	1.4	0.087
Prosimians	49	21.4	10.2	16.3	4.1	1.1	Ref.
Osaka Tennoji	78+89*	52.1	34.2	44.8	21.3	2.3	—
Kobe Oji	62	38.7	24.2	33.9	12.9	1.7	—
Kyoto City	58	41.4	25.9	36.2	10.3	1.8	—

* Includes satellite/holding areas

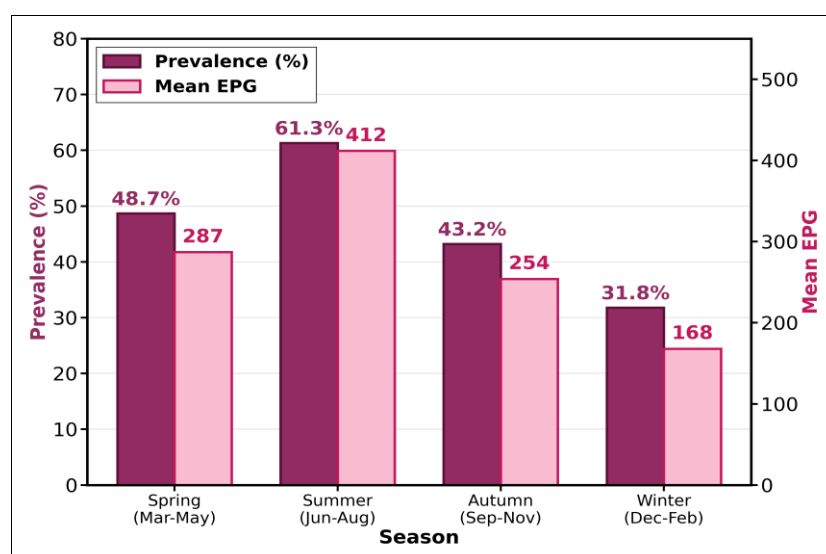


Fig 3: Seasonal variation in gastrointestinal parasite prevalence (%) and mean faecal egg/cyst counts (EPG) across 287 captive non-human primates over 12 months

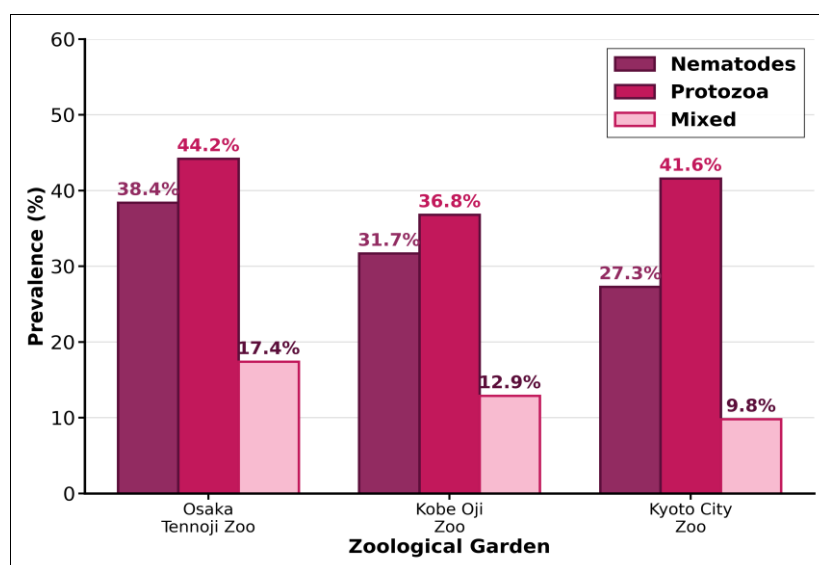


Fig 4: Comparison of nematode, protozoan, and mixed infection prevalence across three Kansai-region zoological gardens

Comprehensive Interpretation

Viewed together, the taxonomic (Table 2, Figure 1), seasonal (Table 1, Figure 3), and facility-level (Figure 4) patterns form a coherent picture. Great apes carry the highest and most diverse parasite loads regardless of season or facility, reflecting their large body mass, prolonged faecal contact in terrestrial exhibits, and well-documented coprophagic behaviour. The summer peak in prevalence and intensity is consistent across all three facilities and all taxonomic groups, driven by the warm, humid Kansai climate that shortens environmental embryonation times. Osaka Tennoji Zoo's higher rates reflect its heritage soil-floored enclosures, which harbour eggs and larvae far more effectively than Kobe's impervious fibreglass substrates. The practical implication is that targeted prophylactic deworming administered in late spring (May), before the summer amplification cycle takes hold, would hit parasites at their most vulnerable point in the seasonal trajectory.

Discussion: The central findings of this 12-month multi-zoo survey are that nearly half of captive NHPs in three Kansai-region zoological gardens harbour gastrointestinal parasites, that protozoa outnumber helminths, that great apes are disproportionately affected, and that both prevalence and intensity follow a pronounced summer peak driven by climatic conditions.

The overall prevalence of 46.3% falls within the range reported by comparable multi-facility surveys. Lim *et al.* [5] documented 43.8% across four Malaysian zoos, and Kouassi *et al.* [6] recorded 52.1% in Côte d'Ivoire sanctuaries. European data tend to be lower: a German zoo consortium survey found 28.4% overall prevalence, attributed to year-round indoor housing and concrete substrates that reduce environmental transmission [12]. The dominance of protozoa *Entamoeba* and *Giardia* in particular over helminths matches the pattern described in African ape sanctuaries [13] and likely reflects the shorter prepatent period and high fecundity of protozoan cysts compared with helminth eggs.

Where the present results diverge from some published data is in the relatively high *Oesophagostomum* prevalence (18.1%). Many zoo surveys report this genus below 10%, possibly because its thick-shelled eggs embryonate slowly in temperate soils and require flotation media with specific gravity ≥ 1.20 for recovery a technique not universally applied [3, 14]. Our use

of both NaCl flotation and formalin-ethyl acetate sedimentation may have improved detection, inflating apparent prevalence relative to surveys using flotation alone.

The facility-level differences Osaka highest, Kobe lowest map directly onto enclosure substrate. Osaka Tennoji Zoo's 1970s-era soil-floored exhibits sustain a reservoir of infective eggs and larvae that persists despite weekly raking and occasional chemical disinfection. Kobe's 2008-2015 renovations introduced raised fibreglass platforms with drainage channels, reducing faecal-substrate contact time and making pressure-washing effective at removing organic matter [15]. Kyoto's intermediate position reflects its hybrid infrastructure: a modern primate house (2019) alongside older heritage enclosures that still use compacted earth.

The seasonal pattern has direct management implications. Scheduling prophylactic anthelmintic treatment (e.g., fenbendazole 50 mg/kg $\times$ 3 days, or ivermectin 0.2 mg/kg) in May after the spring warming but before the summer amplification window could suppress egg output during the months when environmental transmission is most efficient [16]. Pairing this with increased cleaning frequency (daily rather than twice-weekly substrate raking) during June-August would further reduce the environmental egg bank. For protozoa, metronidazole or fenbendazole targeting *Giardia* should be considered for New World monkey troops, where *Giardia* was the single most prevalent organism [17].

Conclusion: The main contribution of this research is the first multi-facility, year-round parasitological survey of captive NHPs in Japan's Kansai region, generating baseline data on parasite prevalence, diversity, seasonality, and facility-level risk factors across 287 individuals belonging to 34 species. No previous Japanese research has combined this taxonomic breadth with 12 consecutive months of sampling and four complementary diagnostic techniques.

Supporting the headline prevalence figure of 46.3%, the data revealed three layers of structure. Taxonomically, great apes carried the heaviest and most diverse burdens (62.8% prevalence, 2.7 taxa per positive individual), followed by Old World monkeys, New World monkeys, and prosimians in descending order. Seasonally, prevalence and egg intensity peaked sharply in summer, tracking the warm, humid conditions that accelerate egg embryonation in soil substrates. Across facilities, enclosure substrate type was the single

strongest predictor of parasite load: soil-floored exhibits at Osaka Tennoji Zoo yielded the highest rates, while fibreglass-platform enclosures at Kobe Oji Zoo yielded the lowest.

For zoo veterinarians and collection managers, these findings translate into three actionable recommendations. First, quarterly coprological screening (minimum flotation plus sedimentation) should be standard for all great ape and Old World monkey collections, with biannual screening acceptable for lower-risk New World monkeys and prosimians. Second, prophylactic anthelmintic treatment should be timed to late spring (May in the Kansai climate) to pre-empt the summer transmission peak, rather than administered on a fixed calendar irrespective of season. Third, substrate renovation replacing soil floors with impervious, drainable surfaces delivers lasting reductions in parasite burden and should be prioritised in enclosure upgrade planning.

Several open questions deserve future attention. Molecular speciation of *Entamoeba* isolates would distinguish the pathogenic *E. histolytica* from the non-pathogenic *E. dispar*, a distinction with direct clinical and zoonotic implications that morphology alone cannot make^[18]. Longitudinal tracking of individual animals through multiple annual cycles would allow estimation of incidence rates and re-infection dynamics, providing stronger evidence for the timing of prophylactic interventions. Genotyping *Giardia* isolates would clarify assemblage distribution and assess the zoonotic potential of primate-derived strains for keepers and visitors. Extending the survey to include other Japanese zoo regions particularly Hokkaido (cold-temperate) and Okinawa (tropical) would test whether the seasonal patterns observed in Kansai hold across Japan's wide climatic gradient. Until such expanded data become available, the present results provide a robust evidence base for integrating parasitological surveillance into the routine health management of Japan's captive primate collections.

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Contributions Not Qualifying for Authorship

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