Animal Behaviour 149 (2019) 97–106

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Faecal avoidance differs between the sexes but not with nematode infection risk in mandrills

Clémence Poirotte [a, b, *, 1], Cécile Sarabian [c, *, 1], Barthélemy Ngoubangoye [d], Andrew J. J. MacIntosh [c, 2], Marie Charpentier [a, 2]

[a] Institut des Sciences de L'Evolution de Montpellier (ISEM), UMR 5554, Montpellier, France
[b] Behavioral Ecology and Sociobiology Unit, German Primate Center, Göttingen, Germany
[c] Primate Research Institute, Kyoto University, Inuyama, Japan
[d] Centre de Primatologie, Centre International de Recherches Médicales de Franceville, Franceville, Gabon

ARTICLE INFO

Article history:
Received 26 March 2018
Initial acceptance 17 May 2018
Final acceptance 20 December 2018

MS. number: 18-00224R

Keywords:
antiparasitic behaviour
faecal avoidance
gastrointestinal parasites
Mandrillus sphinx

Animals have evolved a wide range of behaviours that act as barriers to decrease the risk of parasite infection. Faecal avoidance may, for example, limit contact with orofaecally transmitted parasites, such as gastrointestinal nematodes. When present in faeces, however, nematode eggs need to mature before reaching their infective stage. If strategies have evolved in hosts to specifically avoid nematodes, old faeces with infective larvae should elicit stronger avoidance behaviour than fresh faeces that contain noninfective stages. Here, we carried out two experiments to test the hypothesis that mandrills, *Mandrillus sphinx*, an Old-World primate, exhibit specific behavioural strategies to avoid nematode infection. Our results show that individuals did not avoid faeces in a nonfeeding context but did avoid eating food items contaminated with faecal material, females more so than males. However, neither the presence of nematodes nor the age of faeces influenced the level of avoidance observed, suggesting that mandrills avoid faecal material in general rather than nematodes specifically when foraging.

The 'behavioural immune system' (Schaller, 2006) consists of a suite of mechanisms providing a first line of defence against parasites and pathogens before the intervention of the physiological immune system. It allows individuals to detect cues in the environment indicating the presence of contaminants which may trigger emotional responses (i.e. disgust). This process facilitates functional behavioural reactions (i.e. avoidance) that decrease parasite infection risk, such as avoiding contagious conspecifics or contaminated substrates (Curtis, 2014; Hart, 1990). Avoiding faeces is a common strategy that several animal species may use to decrease exposure to gastrointestinal parasites, which are present in faeces and are typically transmitted via ingestion or skin penetration (Anderson, 2000; Goater, Goater, & Esch, 2014). Faecal avoidance has mainly been demonstrated in foraging contexts in grazing ungulates (for a review see Coulson, Cripps, Garnick, Bristow, & Beveridge, 2018) but similar studies are less common for other taxa, with a few notable exceptions (Garnick, Elgar, Beveridge, & Coulson, 2010; Sarabian & MacIntosh, 2015).

Despite the obvious benefits, faecal avoidance also entails costs. Indeed, avoiding areas contaminated with faecal material may decrease the amount of available food resources (Hutchings, Kyriazakis, Papachristou, Gordon, & Jackson, 2000). We therefore expect faecal avoidance to be influenced by food availability and its nutritive value. Accordingly, several species of ruminants were found to avoid feeding in contaminated areas when faced with the choice of a faeces-contaminated versus uncontaminated sward of identical nutritional value (Fleurance, Duncan, Fritz, Cabaret, & Gordon, 2005; Hutchings et al., 2000). These same animals, however, selected the contaminated sward if enriched in nitrogen or proteins. In addition, we also expect animals to modulate their feeding behaviour depending on the quantity of infectious agents present in faeces, although the importance of this trade-off in mediating behaviour has rarely been investigated.

Nematodes are ubiquitous gastrointestinal parasites. They may impact the survival and reproduction of a large range of host species, sometimes causing dramatic declines in wild animal

* Correspondence: C. Poirotte, Behavioral Ecology and Sociobiology Unit, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany; C. Sarabian, Primate Research Institute, Kyoto University, 41-2 Kanrin, Inuyama 484-8506, Japan.
E-mail addresses: c.poirotte@gmail.com (C. Poirotte), sarabiancecile@gmail.com (C. Sarabian).
[1] These authors contributed equally to this work.
[2] These authors contributed equally to this work.

https://doi.org/10.1016/j.anbehav.2019.01.013
0003-3472/

populations (Albon et al., 2002; Gulland, 1995; Hanssen, Folstad, Erikstad, & Oksanen, 2003; Hillegass, Waterman, & Roth, 2010; Pedersen & Davies, 2009). Gastrointestinal nematodes may therefore represent a major selection pressure favouring the evolution of mechanisms to detect and avoid infection. However, the risk of getting infected with nematodes varies according to the age of faeces because nematode eggs and larvae typically require time to develop before reaching their respective infective stages, for example infective third stage (L_3) larvae or embryonated eggs (Anderson, 2000). This developmental process varies with environmental conditions (temperature and humidity) and may last from a few days to a few weeks depending on the species (Neveu-Lemaire, 1942; Stromberg, 1997). Consequently, we expect nematode infection risk, and therefore faecal avoidance, to be high when faecal material has been in the environment for a certain time following defecation by infected hosts.

In this study, we aimed to determine whether mandrills, *Mandrillus sphinx*, exhibit faecal avoidance in both nonfeeding and feeding contexts, and whether the presence of nematodes at different developmental stages impacts their behaviour. In both contexts, we predicted that subjects would avoid faeces-contaminated areas (nonfeeding context) or food (feeding context), with stronger avoidance responses elicited in a feeding context since the latter involves ingestion, a major pathway to infection (e.g. orofaecal route; Antonovics et al., 2017). Furthermore, if mandrills can detect the presence of nematodes, we predicted that subjects would avoid faeces from parasitized individuals more strongly than those from nonparasitized individuals. Finally, we predicted stronger avoidance responses towards aged faeces compared to fresh faeces if mandrills can assess and respond specifically to nematode infection risk.

Decisions about whether to exploit a contaminated food resource may also depend on the forager's attributes. For example, in some primates (olive baboons, *Papio anubis:* Müller-Graf, Collins, Packer, & Woolhouse, 1997; Japanese macaques, *Macaca fuscata*: Sarabian & MacIntosh, 2015) females avoid faeces-contaminated substrates to a greater degree than males. Similarly, women exhibit greater disgust than men towards animals associated with the spread of infectious diseases, such as cockroaches (Olatunji, Sawchuk, Arrindell, & Lohr, 2005; Prokop & Fančovičová, 2010). However, in other primate species (chimpanzees, *Pan troglodytes*: Sarabian, Ngoubangoye, & MacIntosh, 2017; bonobos, *Pan paniscus*: Sarabian, Belais, & MacIntosh, 2018), males and females exhibit similar responses to contaminated food. Here, we predicted that male mandrills would be more risk prone regarding their feeding decisions, that is, tolerate a higher risk of infection than females to acquire food, as observed in other Papionini species (Müller-Graf et al., 1997; Sarabian & MacIntosh, 2015).

METHODS

Study Groups

We studied two semifree-ranging groups of mandrills living in forested enclosures of 6.5 ha (89 individuals) and 3.5 ha (57 individuals) at the 'Centre International de Recherches Médicales de Franceville' (CIRMF) in southern Gabon. These animals forage freely but are supplemented twice a day with fruits, vegetables and monkey chow, which are deposited in an enclosed 'provisioning area'. This area is used to isolate animals for protocols such as health checks and behavioural tests. This provisioning area is coupled with a 'test area' where we performed all behavioural tests analysed in this study.

Ethical Note

Experimental procedures were approved by the CENAREST Institute (permit number: AR0042/17/MESRS/CENAREST/CG/CST/CSAR) and the Animal Welfare and Animal Care Committee of the Kyoto University Primate Research Institute (no. 2016-138). Research permissions were granted by the CIRMF.

Experimental Design

Experiment 1: nonfeeding context

To study whether mandrills avoid faeces in the absence of food and whether the presence of nematodes influences this behaviour, we compared subjects' responses to three different bamboo sticks, rubbed with 'nematode-positive' faecal samples (i.e. nematode eggs present), 'nematode-negative' faecal samples (i.e. nematode eggs absent) and with a common plant used as a control.

Faecal sample collection. We used faecal samples routinely collected from another social group of free-ranging mandrills inhabiting the Lékédi park, a private park in southern Gabon (Peignot et al., 2008, Mandrillus Project: www.projetmandrillus.com). Since 2012, faecal samples have been opportunistically collected for qualitative identification of gastrointestinal parasites (see Poirotte et al., 2016). In addition, 1 g of these faecal samples was stored at −20 °C for future use. Frozen faecal samples collected from the same donor no more than 3.5 months apart were paired when one sample contained at least one nematode taxon (nematode positive) and one sample did not contain any nematode taxon (nematode negative). In total, we obtained 20 pairs of faecal samples collected between February and September 2014 (mean time spent frozen ± SD = 3.3 ± 0.4 months) from 14 donors (nine females aged 13.5 ± 3.8 years and five males aged 8.3 ± 4.1 years). Note that we did not use faecal samples collected from sexually receptive females to minimize bias due to potential chemosignals of their reproductive status.

Nematode-positive faecal samples may have contained as many as four nematode taxa (Poirotte et al., 2016): *Strongyloides* spp., *Trichostrongylus* spp. and *Necator americanus*/*Oesophagostomum* spp. complex (indistinguishable at the egg stage using standard microscopy). Nematode-negative samples did not contain any observable nematode eggs, but this does not necessarily mean that donors were not parasitized because eggs may be intermittently present in faeces. However, the presence of parasites within the faeces presented to our test subjects, and not the parasite status of the donor, was the variable of interest here.

Behavioural tests. In September 2014, we conducted 33 behavioural tests on 16 subjects from CIRMF (four females aged 13.2 ± 3.5 years; 12 males aged 11.2 ± 5.7 years; mean number of tests per subject ± SD = 2 ± 0.7; Appendix Table A1). Faecal samples of one pair (ca. 1 g of nematode-positive and 1 g of nematode-negative faecal samples) were rubbed on two bamboo sticks (ca. 30 cm long) attached along the fence of the test area, 4 m apart. These samples were thawed 20 min before use. In addition, a central control stick, placed in the middle of the two 'faecal sticks', was rubbed with a common herbaceous plant (one species) that did not belong to the mandrill's diet (see Appendix). We checked that mandrills did not prefer certain zones of the test area by presenting three sticks in a similar setting, all rubbed with the control plant, during preliminary tests (see Appendix).

Each tested subject was first isolated in the provisioning area and was able to see the experimental set-up. The test started once the subject entered the test area and lasted for 10 min. During this period, we recorded the time spent in proximity (less than 1 m) to

each stick. At the end of the test, the subject was released into the provisioning area. The test area was cleaned before the next test, performed with a new pair of sticks rubbed with faeces. Each subject participated in no more than three tests in a row. Faecal samples were stored in an icebox between tests and we alternated their position on the sticks between tests. To avoid multiple freeze–thaw cycles of the samples, each faeces pair was thawed only once and used one to three time(s) in the same half-day period.

Experiment 2: feeding context

To study whether mandrills avoid faeces in a feeding context and whether the presence of nematodes influences this behaviour, we compared subjects' responses to three different food items, contaminated with 'nematode-positive' faeces (i.e. eggs and/or larvae present), 'nematode-negative' faeces (no eggs, no larvae) and noncontaminated. In addition, to investigate whether the presence of different developmental stages of nematodes influenced behavioural responses, we presented subjects with the same faeces at three different times: on the first day (D1), after 1 week (D7) and after 2 weeks (D12) following defecation (see Fig. 1).

Faecal sample collection. On a single day in November and another day in December 2015, we dewormed two individuals from the two social groups with albendazole (15 mg/kg) mixed with milk powder and water. One week later, we isolated each dewormed individual along with an untreated individual from the same group. Individuals were separated from each other (in the test and provisioning areas) and held for approximately 12 h. We then collected and mixed all faeces from each donor. Faeces collected from a dewormed donor were paired with faeces collected from the untreated donor from the same group. We thus obtained four faeces pairs (two per month, one from each group) in total, collected from six donors (one female aged 11.3 years; five males aged 10.9 ± 2.3 years; two donors were collected twice; Appendix Table A2). We partitioned the mixture of faeces obtained from each donor into two parts, which were used either for behavioural tests or for the coproscopic analyses that were conducted during the 2 weeks after collection. We maintained faecal samples in plastic boxes at ambient temperature during the test period (2 weeks) to allow nematode eggs to develop into infective stages. The bottom of the boxes was covered with washed, fresh leaves to minimize drying of faeces.

Coproscopic analyses. Following faecal collection, we first counted nematode eggs using the McMaster technique with a saturated sugar flotation solution (1900 g/litre; Sloss, Kemp, & Zajac, 1994). We then searched for nematode larvae using the Baermann method (Sloss et al., 1994). At D1, we confirmed that faecal samples collected from dewormed donors contained no or very few nematode eggs per gram (≤ 50; Appendix Table A2) and no larvae. By contrast, faecal samples collected from untreated donors contained numerous eggs per gram (> 1000; Appendix Table A2), although no larvae were present. We repeated these analyses at D7 and D12. At these dates, while dewormed faecal samples were still free of nematodes, faecal samples collected from untreated donors contained transmissible larvae from one to three nematode taxa (*Strongyloides* spp.: transmissible larvae observed at D7; *N. americanus* and *Oesophagostomum* spp.: transmissible larvae observed at D12; Appendix Table A2).

Behavioural tests. Following faeces collection, we performed 38 'series of tests' with 22 subjects (seven females aged 12.6 ± 3.5 years and 15 males aged 11.7 ± 5.7 years; Appendix Table A3;

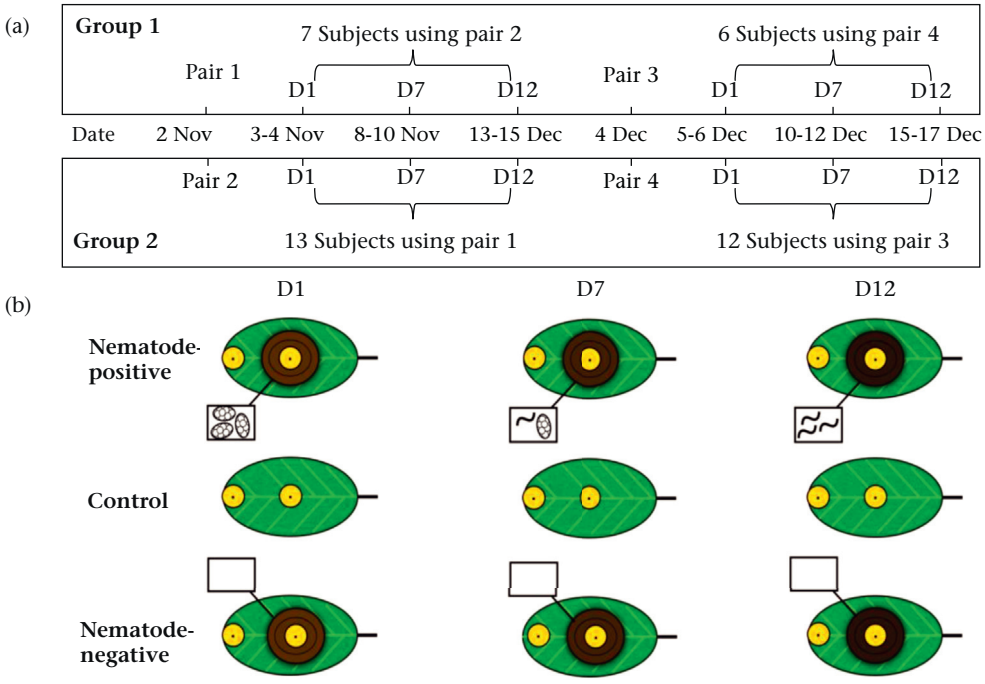

Figure 1. (a) Schedule of experiment 2 and (b) experimental protocol for one series of behavioural tests. In (a), 'pair 1–4' reflects the collection of faeces obtained from an untreated donor and a dewormed donor from the same group, the same day. We obtained in total four faeces pairs (two from each group). Each pair from one group was presented to subjects from the other group during behavioural tests that were conducted during the 2 weeks after collection. One full series of tests corresponds to three tests performed with the same subject and using the same faeces pair at three different times: on the first or second day (D1), 6–8 days (D7) and 11–13 days (D12) following defecation of donors. Faeces pairs 1, 2, 3 and 4 were used in 13, 7, 12 and 6 series of tests, respectively, representing a total of 19, 36, 16 and 36 tests because some series could include only one or two test(s). In (b), 'nematode-positive' reflects leaves containing faecal samples from untreated donors, 'control' represents leaves left uncontaminated and 'nematode-negative' reflects leaves containing faecal samples from dewormed donors; with the three types of leaf presented at D1, D7 and D12.

Fig. 1a). Each series comprised three behavioural tests performed with the same subject and the same faeces pair at D1, D7 and D12 (Fig. 1a and b). Because of time and field constraints across tests, D1 spanned the first and second days following defecation and faeces were therefore aged 1–2 day(s). Similarly, at D7 and D12, faecal samples were respectively aged 6–8 days and 11–13 days. In detail, in November, we performed 20 series of tests involving 20 subjects (one series per subject, i.e. 3 test days per subject). In December, we performed 18 series of tests involving 18 subjects, using 16 subjects already tested in November and two untested individuals (Appendix Table A3). Six series of tests were incomplete because we were unable to isolate the subject at each time stage (for five series: tests only occurred at D1 and D7; for one series: one test was performed at D1). In total, we performed 107 tests. Tested subjects and donors always came from different social groups to avoid possible confounding effects of familiarity.

Before each test, we aligned three large leaves of *Marantacea* sp. (ca. 20×10 cm) 2 m apart in the test area. This plant species does not belong to the mandrill's diet, although it is commonly found in its habitat. We positioned either a nematode-positive or a nematode-negative faecal sample of one pair at the centre of each peripheral 'faecal' leaf (hereafter, 'nematode-positive leaf' and 'nematode-negative leaf'). Faecal samples were of similar size and resembled a natural faecal bolus (ca. 5 cm in diameter). We left the middle leaf uncontaminated as a control (hereafter, 'control leaf'). We then positioned two identical food items (banana pieces) on each of the three leaves (six food items in total), approximately 7–8 cm apart. On the nematode-positive and nematode-negative leaves, we positioned one food item atop the faecal sample ('centre' position), and one food item 5 cm from the faecal sample ('side' position). All food items on faecal leaves were considered 'faeces-contaminated food items', but the central and side positions corresponded to high and low contamination levels, respectively. Note that these two levels of contamination referred to faecal contamination and not to nematode contamination. We also positioned food items at the centre and on the side of the control leaf (hereafter, 'noncontaminated food items') to serve as controls for high and low food contamination, respectively (Fig. 1b).

As before, each subject was first isolated in the provisioning area and was able to see the experimental set-up. The test started once the subject entered the test area and lasted for 3 min, because subjects generally fed (or not) at the very beginning of the tests. We recorded two behavioural responses: (1) whether subjects consumed each of the six food items ('feeding decision'; binary variable); and if they did (2) the latency to feed ('feeding latency'; s, continuous variable). At the end of the test, the subject was released into the enclosure. The test area was cleaned before the next test, performed with another subject using new food items deposited on the same faecal samples.

Statistical Analyses

Experiment 1: nonfeeding context

We tested whether subjects spent equal time in proximity to each bamboo stick using a nonparametric, one-way ANOVA following rank transformation of the data (time spent near each stick).

Experiment 2: feeding context

In a first set of analyses, we investigated whether male and female subjects avoided faeces-contaminated food items, irrespective of the level of faecal contamination and irrespective of whether the faeces contained nematodes or not. Using generalized linear mixed models (GLMMs), we first analysed subjects' feeding decisions towards each of the six food items (model 1 with a binomial distribution, with a logit link function; $N = 642$, corresponding to the number of food items presented throughout the 107 tests) as a function of faecal contamination on food items, subjects' sex and their interaction. We compared the relative fits of the model with and without this interaction using likelihood ratio tests (LRTs). We then studied the feeding latency towards each food item consumed in males (model 2 with a negative binomial distribution for overdispersed count data and a log link function; $N = 214$, corresponding to the number of food items eaten by males throughout the 107 tests) as a function of faecal contamination on food items. Here, we included only males because females almost never fed on faeces-contaminated food items (see Results). In models 1 and 2, we included three random effects: the identity of the faeces pair and the identity of the test (to account for the nonindependence of feeding responses towards the six food items within a test) nested within subject identity.

In a second set of GLMMs, we investigated whether males modulated faecal avoidance behaviour according to the level of faecal contamination and the presence of infective or noninfective stages of nematodes within faeces. As previously, we examined male feeding decisions (model 3, with a binomial distribution and logit link function; $N = 498$, corresponding to the number of food items presented to males throughout the 107 tests) and feeding latency (model 4, with a negative binomial distribution for overdispersed count data and a log link function; $N = 214$, corresponding to the number of food items eaten by males throughout the 107 tests) as a function of the type of leaf (three categories: nematode-positive, nematode-negative and control, i.e. no faeces), the age of faeces (days, continuous variable), the position of food items (two categories: centre versus side of the leaf), and an interaction between type of leaf and age of faeces as well as between type of leaf and position of food items. As above, the significance of these interactions was assessed using LRTs. We kept interactions in final models only when they outperformed models without the interaction term. We considered the same random effects as above.

All data were analysed using R v. 3.3.3 (R Core Team, 2016). GLMMs were fitted using the package lme4 (for models 1 and 3; Bates et al., 2017) and glmmADMB (for models 2 and 4; Skaug, Fournier, Magnusson, & Nielsen, 2016), using maximum likelihood estimations. We used the package lmtest (Hothorn et al., 2017) to conduct all LRTs. All raw data used in these analyses and the video of our experimental design are provided in the Supplementary Material.

RESULTS

Experiment 1: Nonfeeding Context

Mandrills did not spend equal time in proximity to each bamboo stick ($F_{2,30} = 7.01$, $P < 0.01$). While they spent similar amounts of time near sticks rubbed with nematode-positive and nematode-negative faecal samples ($F_{2,10} = 0.42$, $P = 0.67$), they spent significantly more time near each of them than near the control stick ($P < 0.01$ in both cases).

Experiment 2: Feeding Context

The interaction between the presence or absence of faecal contamination of food items and the subjects' sex significantly influenced observed feeding decisions (model 1, Appendix Table A4). Both sexes fed less on faeces-contaminated than noncontaminated food items (model 1, Table 1, Fig. 2, Appendix Table A5). Faecal avoidance was, however, significantly higher in

Table 1
Summary of the models investigating faecal avoidance

Statistical model	Predictors (reference level)	Estimate	SEM	z	$P(>z)$
Model 1, N=642	Faecal contamination * Sex				
Feeding decision	**C * male (NC, female)**	2.36	0.81	2.93	**0.003**
~ Faecal contamination * Subject's sex	Type of leaf				
+ (Test ID|Subject ID)	**C (NC, female)**	−4.11	0.77	−5.33	**<0.001**
+ (1|Faeces pair ID)	**C (NC, male)**	−1.75	0.27	−6.37	**<0.001**
	Sex				
	Male (NC, female)	0.58	1.07	0.54	0.59
	Male (C, female)	2.94	1.21	2.43	**0.015**
Model 2, N=214	Faecal contamination				
Feeding latency	**C (NC)**	0.94	0.14	6.98	**<0.001**
~ Faecal contamination					
+ (Test ID |Subject ID)					
+ (1|Faeces pair ID)					

Model 1 includes data obtained from both sexes, while model 2 includes only data obtained from males. Equation and sample size are given for each model. The reference level is indicated for each categorical level in parentheses (sex: male/female; faecal contamination: faeces-contaminated 'C'/noncontaminated 'NC'). For model 1, we ran the model three times, varying the reference level of only one variable at each run to display the parameter estimates for all combinations of sex and faecal contamination. We present the result of comparisons between faeces-contaminated and noncontaminated food items within sexes, and between sexes within faeces-contaminated and noncontaminated food items. Significant test statistics are highlighted in bold ($P < 0.05$).

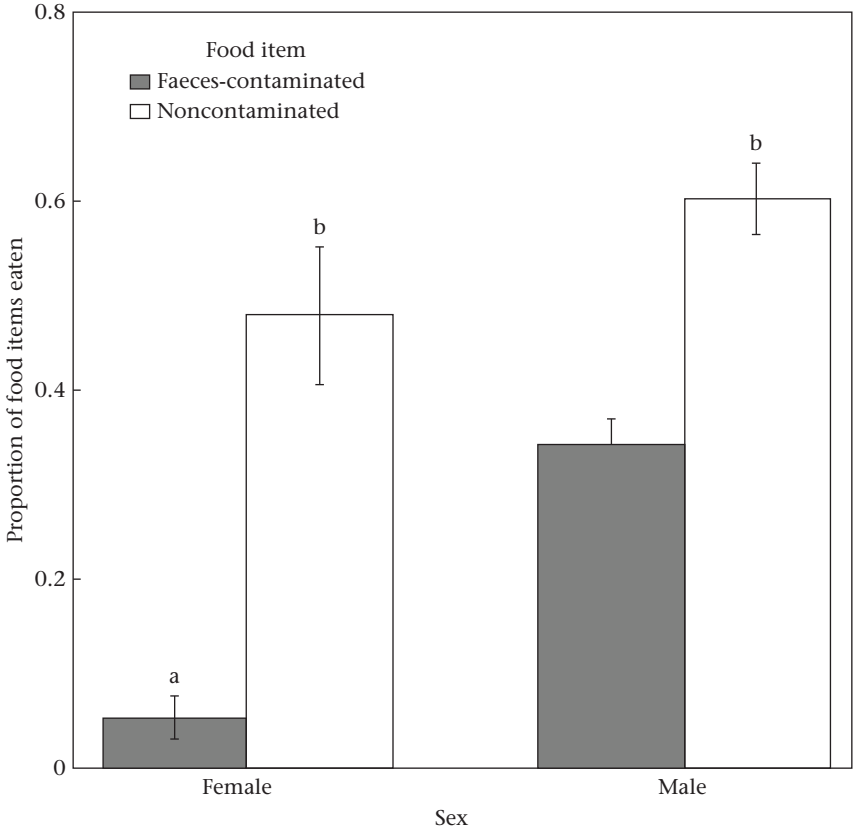

Figure 2. Feeding decisions of males and females according to faecal contamination ($N = 642$). During a given test, six food items were presented: four food items were positioned on two leaves with a faecal sample placed at the centre of the leaf ('faeces-contaminated food items') and two food items were positioned on one leaf without any faecal sample ('noncontaminated food items'). We performed 107 tests in total with 22 subjects. The proportions of the faeces-contaminated and noncontaminated food items eaten are represented with the associated error bars (standard errors of the proportion). Different letters above bars indicate significant differences ($P < 0.05$) according to the model (Table 1, model 1).

females than in males (model 1, Table 1, Fig. 2, Appendix Table A5): females and males consumed 5.2% and 34.3% of faeces-contaminated food items versus 47.9% and 60.2% of non-contaminated food items, respectively. When males consumed food items, feeding latency was significantly higher for faeces-contaminated than noncontaminated food items (model 2, Table 1; Appendix Table A5).

The interaction between type of leaf (nematode-positive, nematode-negative and control) and age of faeces did not significantly influence male feeding decisions (model 3, Appendix Table A4) or male feeding latency (model 4, Appendix Table A4), and was therefore excluded from both models. In addition, male responses towards food items did not vary across time (Table 2). By contrast, the interaction between type of leaf and position of food

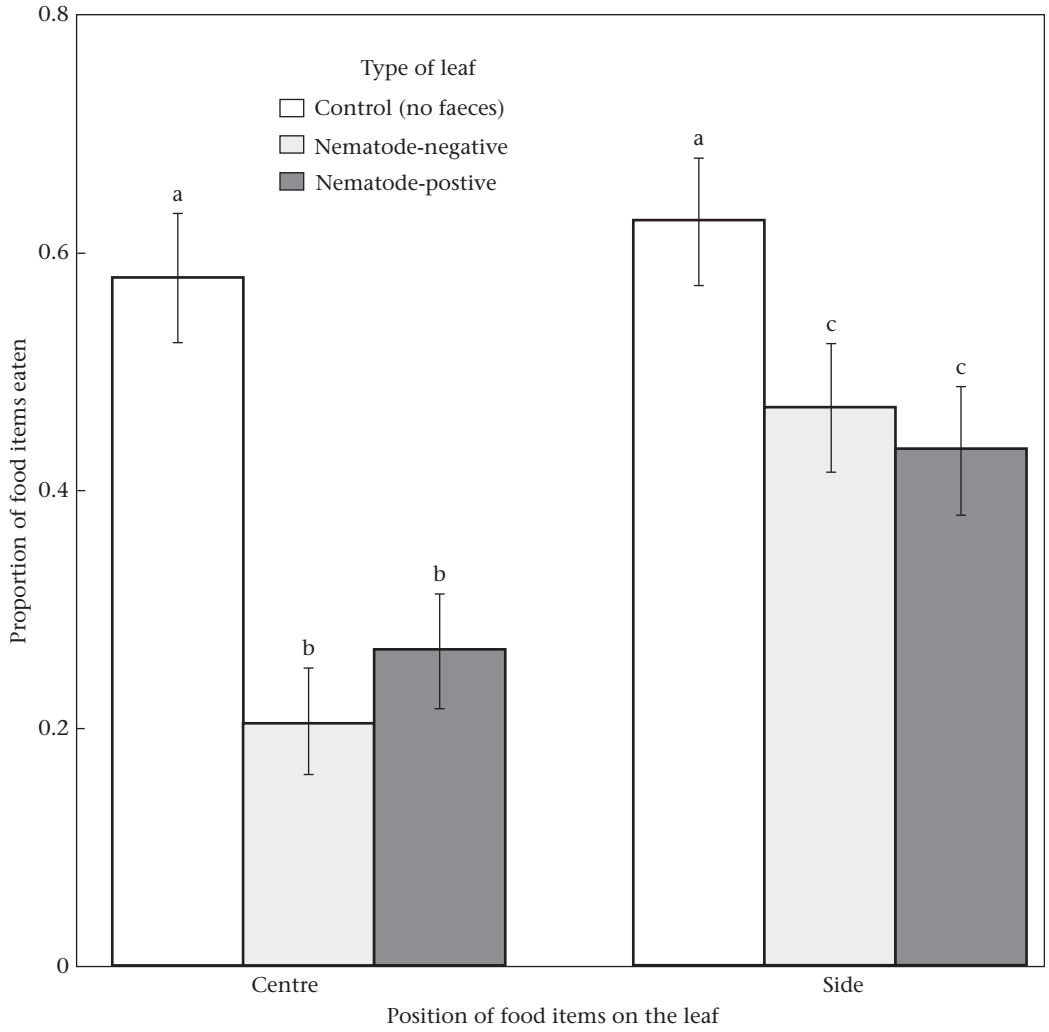

Figure 3. Feeding decisions of males according to faecal contamination and nematode infection risk ($N = 498$). During one test, six food items were positioned on three different leaves. Two food items were positioned on a 'control' leaf without any faecal sample, either at the centre or on the side of the leaf. Two other food items were positioned on a 'nematode-negative' leaf with a nematode-negative faecal sample on it, and two food items on a 'nematode-positive' leaf with a nematode-positive faecal sample on it, either on the faecal material itself ('centre' position, corresponding to high faecal contamination) or 5 cm from the faecal sample ('side' position, corresponding to low faecal contamination). The proportions of food items eaten on each leaf and in each position are shown, and error bars represent standard errors of the proportion. Different letters above bars indicate significant differences ($P < 0.05$) in feeding decision between types of faeces or between positions of food, according to the results of the models presented in Table 2.

items significantly influenced both male feeding decisions (model 3, Appendix Table A4) and male feeding latencies (model 4, Appendix Table A4). We found that males fed on faeces-contaminated food items less or with a longer latency than non-contaminated food items, for both levels of faecal contamination (Table 2, Fig. 3). However, male responses towards faeces-contaminated food items did not differ between food items located on 'nematode-positive' and 'nematode-negative' leaves (Table 2, Fig. 3). Finally, males modulated their avoidance with the level of faecal contamination: they fed on food items with high contamination less or with a longer latency than food items with low contamination (Table 2, Fig. 3). Indeed, males consumed 45.2% of high contamination level food versus 23.2% of low contamination level food, compared to 62.7% and 23.2% for the central and side control positions, respectively.

DISCUSSION

We showed that mandrills exhibited faecal avoidance in a feeding context but, contrary to our prediction, did not avoid faeces in a nonfeeding context. In addition, and again contrary to our predictions, mandrills did not differentiate between faeces containing nematode eggs and those without, nor did they discriminate between fresh and old faeces containing different nematode developmental stages. As we did predict, however, females exhibited greater avoidance of faeces-contaminated food items than did males.

In the nonfeeding context, subjects did not avoid faecal material and did not discriminate between faeces with or without nematodes. They even spent more time near each faecal sample compared to the control sample. Nematode infection risk was, however, minimal because this experiment did not involve ingestion behaviour and faeces contained only noninfective nematode eggs. Subjects therefore may have had little incentive to exhibit avoidance behaviour. By contrast, mandrills appeared attracted to faeces in this experiment, possibly because they were collected from unknown conspecifics and may have conveyed important olfactory cues about the donor's social and/or sexual status. A similar experiment recently showed that mandrills selectively avoid faeces containing gastrointestinal protozoa (Poirotte, Massol,

Table 2
Summary of the models investigating nematode avoidance in males

Statistical model	Predictors (reference level)	Estimate	SEM	z	P (> z)
Model 3, N=498	Type of leaf∗Position of food				
Feeding decision	**NN∗side (control, centre)**	1.56	0.60	2.57	**0.010**
~ Type of leaf∗Position of food + Age of faeces	NP∗side (control,centre)	0.82	0.59	1.40	0.16
+ (Test ID|Subject ID)	Type of leaf				
+ (1|Faeces pair ID)	**NP (control, centre)**	−2.20	0.44	−5.01	**<0.001**
	NN (control, centre)	−2.68	0.46	−5.83	**<0.001**
	NP (control, side)	−1.38	0.42	−3.25	**0.001**
	NN (control, side)	−1.13	0.42	−2.69	**0.007**
	NP (NN, centre)	0.49	0.44	1.11	0.27
	NP (NN,side)	−0.25	0.40	−0.62	0.54
	Position of food				
	Centre (side, control)	−0.36	0.42	−0.87	0.39
	Centre (side, NN)	−1.91	0.44	−4.35	**<0.001**
	Centre (side, NP)	−1.19	0.42	−2.84	**0.004**
	Age of faeces	−0.03	0.03	−0.76	0.45
Model 4, N=214	Type of leaf∗Position of food				
Feeding latency	**NN∗side (control, centre)**	−0.92	0.31	−2.93	**0.003**
~ Type of leaf∗Position of food + Age of faeces	**NP∗side (control, centre)**	−0.75	0.29	−2.56	**0.010**
+ (Test ID|Subejct ID)	Type of leaf				
+ (1|Faeces pair ID)	**NP (control, centre)**	1.28	0.23	5.58	**<0.001**
	NN (control, centre)	1.60	0.26	6.26	**<0.001**
	NP (control, side)	0.52	0.19	2.70	**0.007**
	NN (control, side)	0.68	0.19	3.53	**<0.001**
	NP (NN, centre)	−0.32	0.29	−1.12	0.26
	NP (NN,side)	−0.16	0.20	−0.78	0.44
	Position of food				
	Centre (side, control)	−0.10	0.17	−0.60	0.55
	Centre (side, NN)	0.81	0.26	3.13	**0.002**
	Centre (side, NP)	0.65	0.24	−2.73	**0.006**
	Age of faeces	0.02	0.01	1.46	0.14

Equation and sample size are given for each model. The reference level is indicated for each categorical level in parentheses (type of leaf: nematode-negative 'NN'/nematode-positive 'NP'/control; position of food: centre/side). We ran the model five times, varying the reference level of only one variable at each run to display the parameter estimates for all combinations of type of leaf and position of food. We present the result of comparisons between types of faeces within both positions of food, and between positions of food within both types of faeces. Significant test statistics are highlighted in bold ($P < 0.05$).

et al., 2017). In contrast to nematodes, protozoa in faeces are readily infectious, which might explain why the behavioural responses observed differed in relation to these different parasites. A specific olfactory detection mechanism may have evolved regarding protozoa and their avoidance, facilitating the reported social avoidance of contagious individuals (Poirotte, Massol, et al., 2017).

In a feeding context, on the other hand, we found that mandrills fed on faeces-contaminated food items less or with a longer latency, as expected. However, subjects displayed similar levels of avoidance towards fresh and old faeces irrespective of whether it came from parasitized individuals (containing infective or noninfective stages of nematodes) or from dewormed, unparasitized individuals. Avoiding all types of faeces during foraging may allow mandrills to limit infection with all kinds of pathogens (e.g. protozoa, bacteria and viruses; Poirotte et al., 2016; Setchell et al., 2007; Zwick et al., 2002), increasing the benefits of nonspecific faecal avoidance. Moreover, infective mobile larvae might migrate out of the faeces and contaminate the surrounding area. Consequently, avoiding contaminated areas rather than particular faeces should represent a better strategy to reduce the risk of encountering infective stages of nematodes. Accordingly, mandrills use large home ranges, travel several kilometres a day (Brockmeyer et al., 2015), tend not to stay for several consecutive days in the same area and avoid reusing areas, including sleeping sites, that have been previously contaminated with nematodes (Poirotte, Benhamou et al., 2017). In line with our results, ungulates do not discriminate between faeces that do or do not contain nematodes (Brambilla, von Hardenberg, Kristo, Bassano, & Bogliani, 2013; Cooper, Gordon, & Pike, 2000; Hutchings, Kyriazakis, Gordon, & Coop, 1998). By contrast, the presence of infective stages in old faeces increases faecal avoidance while foraging in white-footed mice, *Peromyscus leucopus* (Logiudice, 2001) and while conspecific coprophagy is a common behaviour in canids, a recent study reported that dogs, *Canis lupus familiaris*, eat fresh rather than old faeces (Hart, Hart, Thigpen, Tran, & Bain, 2018). These divergent results may stem from the different costs and benefits of selective foraging strategies, which certainly vary across different host–parasite systems. Such balances may depend on the pathogenicity and the prevalence of the parasites, for example, or the ecology of the host.

Finally, we observed that females exhibited more avoidance than males: while females almost never ate faeces-contaminated food items (5.2%), males sometimes did (34.3%), especially food items with a low level of contamination (45.2%), probably resulting in higher infection risk. The fact that males generally maximize fitness by increasing mating opportunities, whereas females invest in longevity (Bateman's principle), could explain this sex bias: females invest more in immunity, including behavioural immunity, to maximize fitness gains (Rolff, 2002). Alternatively, or in concert, the extreme sexual size dimorphism in mandrills (Setchell, Lee, Wickings, & Dixson, 2001) might also influence caloric needs and force males to take more risks in their feeding decisions. This sexual bias in sensitivity to infection risk could impact disease epidemiology and dynamics, although in free-ranging mandrills males do not exhibit greater nematode species richness than females (Poirotte et al., 2016). Yet, in Japanese macaques, sex differences in avoidance behaviours result in higher nematode intensity in males than females (Sarabian & MacIntosh, 2015). More generally, such differences may contribute to the widely observed male-biased parasitism in vertebrates (Bundy, 1988; Goble & Konopka, 1973; Gregory, Keymer, & Harvey, 1996; Poulin, 1996).

Further investigations are now needed to evaluate and understand the causes of sex differences in the expression of hygienic behaviours, and the importance of these behaviours in mediating parasite distributions within host populations. The general parasite avoidance behaviour reported here, along with the specific strategy to avoid contagious parasites found in this study system (Poirotte, Massol, et al., 2017), suggest that contrasted parasite life history traits influence the costs and benefits of antiparasite strategies, and may have driven the evolution of different detection mechanisms and diverse behavioural counterstrategies.

Acknowledgments

We thank past and present field assistants of the Mandrillus Project and of the Centre International de Recherches Médicales de Franceville (CIRMF) for their help in data collection. We also thank Dr Angela Turner and two anonymous referees for their dedication to improving the quality of this paper. This study was supported by the 'Station d'Etudes en Ecologie Globale' (INEE-CNRS) and the 'Laboratoire International Associé' (CIRMF and INEE-CNRS) to M.J.E.C. and by the Leading Graduate Program in Primatology and Wildlife Science of Kyoto University as well as the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and the Japanese Society for the Promotion of Science (JSPS) to C.S. Some data used in this article were collected within the framework of the Mandrillus Project, funded by the 'Deutsche Forschungsgemeinschaft' (DFG, KA 1082-20-1; 2012–2015 to M.J.E.C.) and the 'Agence Nationale de la Recherche' (ANR SLEEP 17-CE02-0002, 2017–2020 to M.J.E.C.). This is a Mandrillus Project publication number 17 and ISEM 2019-007 SUD. C.P., C.S., A.M. and M.J.E.C. designed the study, analysed the data and wrote the article. C.P. and C.S. collected the data. B.N. helped with data collection.

Supplementary material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2019.01.013.

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Appendix

We conducted six preliminary tests during which we presented subjects with three bamboo sticks rubbed with a common herbaceous plant located in similar areas as those used in experiment 1. We tested whether subjects spent equal time in proximity to each stick using a nonparametric, one-way ANOVA following rank transformation of the data. We found that subjects spent the same amount of time near each stick ($F_{2,10} = 0.42$, $P = 0.67$).

Table A1
Details of subjects ($N = 16$) used during experiment 1

Subjects	Sex	Age	Group	No. of tests
PC	F	11.0	1	2
30	M	12.9	1	3
2D11	M	10.8	1	2
5I1	M	12.1	1	2
17F	F	20.1	2	3
6H	F	12.9	2	3
12M	F	18.0	2	2
5I3	M	7.4	2	1
17A6	M	16.1	2	2
17A10	M	10.7	2	3
16B	M	25.9	2	2
17B11	M	10.8	2	1
17D2A2	M	4.9	2	2
17F3	M	13.0	2	2
16I	M	16.0	2	1
PM	M	4.8	2	2

Sex (F/M: female/male) and age (years) at the time of the experiment (September 2014) are provided. Each subject performed one to three tests, involving one to three faeces pairs. The number of tests performed with each subject is indicated.

Table A2
Details of donors of faecal samples ($N = 6$) for each faeces pair and results of coproscopic analyses during experiment 2

Faeces pair	Donor (sex, age, group)	Donor status	Type/time of analyses					
			D1/MM	D1/B	D7/MM	D7/B	D12/MM	D12/B
1	33 (F, 11.3, 1)	Dewormed	0	0	0	0	0	0
	10F8 (M, 9.0, 1)	Parasitized	5050	0	3900	*Str.*	1100	*Oeso., Str.*
2	5I3 (M, 7.4, 2)	Dewormed	0	0	0	0	0	0
	17D6 (3, 13.0, 2)	Parasitized	3000	0	0	*Str.*	0	*Nec., Str.*
3	10F8 (M, 9.0, 1)	Dewormed	0	0	0	0	0	0
	2D9 (M, 9.0, 1)	Parasitized	1600	0	9	*Str.*	0	*Oeso., Str.*
4	17D6 (3, 13.0, 2)	Dewormed	50	0	0	0	0	0
	17D7 (M, 11.8, 2)	Parasitized	1600	0	500	*Str.*	300	*Nec., Oeso., Str.*

Sex (F/M: female/male), age (years) at the time of the experiment (October 2015), and group of donors are provided. Each faecal sample was analysed at three different times: D1, D7 and D12, which refer to the number of days following defecation. Two coproscopic analyses were performed each time: 'MM' refers to the quantitative McMaster technique, and the number below indicates the number of eggs per gram of faeces; 'B' refers to the qualitative Baermann technique to retrieve larvae; '0' indicates that no larvae was observed, while '*Nec.*', '*Str.*' and '*Oeso.*' refer to the presence of *Necator americanus*, *Oesophagostomum* spp. and *Strongyloides* spp., respectively.

Table A3
Details of subjects ($N = 22$) used in experiment 2

Subject	Sex	Age	Group	No. of tests/series and identity of the pair used in November	No. of tests/series and identity of the pair used in December
42	F	7.7	1	3 (pair 2)	3 (pair 4)
NB	F	12.7	1	2 (pair 2)	0
PC	F	11.0	1	2 (pair 2)	0
U2A2	F	10.0	1	0	3 (pair 4)
30	M	12.9	1	3 (pair 2)	3 (pair 4)
33A	M	7.1	1	3 (pair 2)	3 (pair 4)
2D9	M	13.0	1	3 (pair 2)	3 (pair 4)
10E7	M	9.8	1	3 (pair 2)	1 (pair 4)
17A7	F	14.1	2	2 (pair 1)	0
2D4	F	19.8	2	3 (pair 1)	0
6H	F	12.9	2	3 (pair 1)	3 (pair 3)
17A6	M	16.1	2	3 (pair 1)	3 (pair 3)
17A10	m	10.7	2	2 (pair 1)	3 (pair 3)
16B	m	26.9	2	3 (pair 1)	3 (pair 3)
17B11	m	10.8	2	3 (pair 1)	3 (pair 3)
17B7B	m	6.0	2	3 (pair 1)	3 (pair 3)
17B8A	m	7.2	2	3 (pair 1)	3 (pair 3)
17B9C	m	3.7	2	3 (pair 1)	3 (pair 3)
17D2A2	m	4.9	2	2 (pair 1)	3 (pair 3)
17F3	m	13.0	2	3 (pair 1)	3 (pair 3)
16I	m	16.0	2	3 (pair 1)	3 (pair 3)
5M	m	16.1	2	0	3 (pair 3)

Sex (F/M: female/male), age (years) at the time of the experiment (October 2015) and group of subjects are provided. For each series of tests in November and December, the number of tests performed per series and the identity of the faeces pair used are indicated. A full series comprised three tests performed with the same subject and the same faeces pair, at three different times: D1, D7 and D12, which refer to the number of days following defecation.

Table A4
Likelihood ratio tests comparing models with and without interactions

Model	Interaction tested	ΔLogLik	Δ*df*	X^2	P|>X^2|
Model 1, *N*=642 Feeding decision ~ Faecal contamination * Subject's sex + (Test ID|Subject ID) + (1|Faeces pair ID)	**Faecal contamination * Subject's sex**	9.01	1	11.01	**<0.001**
Model 3, N=498 Feeding decision ~ Type of leaf * Position of food + Age of faeces + (Test ID|Subject ID) + (1|Faeces pair)	Type of leaf * Age of faeces	1.64	2	1.36	0.51
	Type of leaf * Position of food	2.56	2	6.57	**0.038**
Model 4, N=214 Feeding decision ~ Type of leaf * Position of food + Age of faeces + (Test ID|Subject ID) + (1|Faeces pair ID)	Type of leaf * Age of faeces	0.64	2	1.27	0.53
	Type of leaf * Position of food	5.60	2	11.18	**0.004**

Model 1 includes data obtained from both sexes, while models 3 and 4 include only data obtained from males. Equations of the models retained for the analyses and sample sizes are given for each model. The interaction terms tested are indicated for each model. To assess the significance of an interaction, we compared the full model including the interaction with the model without the interaction. Bold text denotes cases for which we kept the interaction in the model (when $P < 0.10$). Significant statistical tests are highlighted in bold ($P < 0.05$).

Table A5
Feeding decisions according to subject's sex, level of faecal contamination and type of leaf

Sex	Faecal contamination	Type of leaf	Time of the test		
			D1	D7	D12
F	Low	Nematode-negative	0/9	0/9	1/6
F	Low	Nematode-positive	2/9	0/9	1/6
F	None (side of the leaf)	Control	4/9	4/9	4/6
M	Low	Nematode-negative	16/28	10/28	13/27
M	Low	Nematode-positive	13/28	12/28	11/27
M	None (side of the leaf)	Control	19/28	16/28	17/27
F	High	Nematode-negative	0/9	0/9	0/6
F	High	Nematode-positive	0/9	0/9	1/6
F	None (centre of the leaf)	Control	4/9	4/9	3/6
M	High	Nematode-negative	6/28	6/28	5/27
M	High	Nematode-positive	8/28	8/28	6/27
M	None (centre of the leaf)	Control	16/28	15/28	17/27

D1, D7, D12 refer to the number of days following defecation. 'Low' level of faecal contamination refers to food items 5 cm from the faecal samples, while 'high' level of faecal contamination refers to food items on faecal samples. Ratios indicate the number of tests during which subjects ate the food item on the total number of tests.