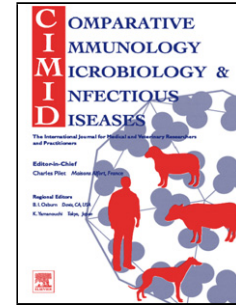


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Serological survey of *Toxoplasma gondii* in captive nonhuman primates in zoos in Spain

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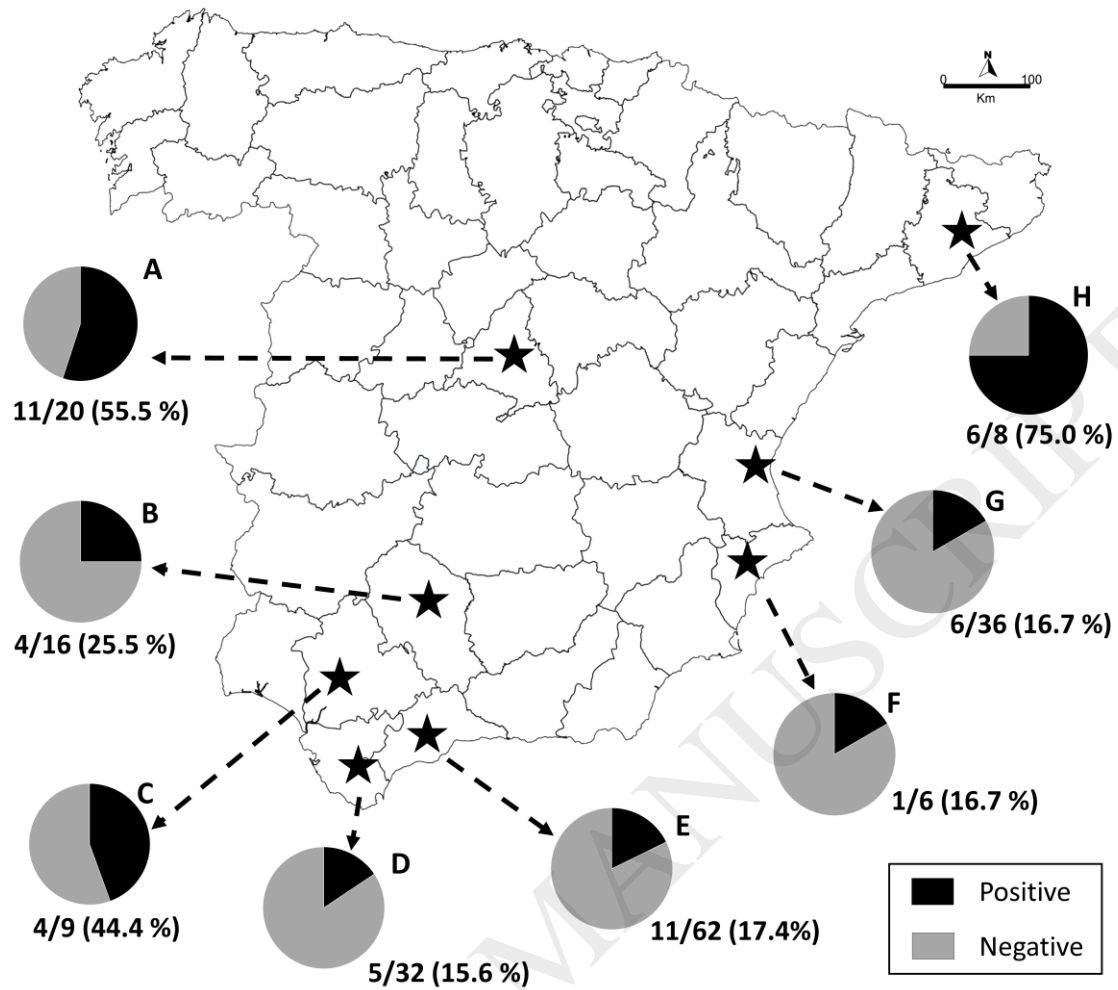
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Graphical abstract



Highlights

- We evince a widespread circulation of *T. gondii* in nonhuman primates in zoos in Spain
- Family Hominidae and sex were risk factors associated with seropositivity to *T. gondii*
- Results are of conservation concern, as several non-human primate species are highly susceptible to *T. gondii*

Abstract

Toxoplasma gondii is a widely distributed zoonotic protozoan parasite, which can affect most warm-blooded species. Some species of non-human primates (NHPs) are highly susceptible to *T. gondii* infection. The aim of the study was to determine the seroprevalence and risk factors associated with *T. gondii* infection in NHPs housed in zoos in Spain. Sera from 189 NHPs belonging to 33 species were collected in eight zoos. Additionally, 10 of the 189 animals were longitudinally sampled. Anti-*T. gondii* antibodies were detected in 48 NHPs (25.4%; **confidence interval of 95%** (CI_{95%}): 19.2-31.6) using a modified agglutination test (MAT; **cut-off = 25**). Seropositive animals had titers of **25** (6.3%), **50** (8.3%), **100** (8.3%) and **≥500** (68.8%). Seropositivity was detected in 15 of the 33 species (45.5%). Of the 10 NHPs sampled more than once, two animals (one Barbary macaque [*Macaca sylvanus*] and one common chimpanzee [*Pan troglodytes*]) seroconverted along the study period, while one seropositive chimpanzee increased antibody titers over time. The **Hominidae** family (OR=5.9; CI_{95%}: 2.7-12.8) and sex (females) (OR=2.1; CI_{95%}: 1.1-4.1) were risk factors potentially associated with **seropositivity to *T. gondii***. Our results evince a widespread circulation of *T. gondii* in NHPs in zoos in Spain, which may be of conservation concern. Control measures should be implemented to minimize the risk of exposure of these species to *T. gondii*.

Keywords: *Toxoplasma gondii*; zoo; primates; conservation; seroprevalence; risk factors.

1. Introduction

Toxoplasma gondii is an intracellular protozoan which has an indirect life cycle with a wide range of warm-blooded animals as intermediate hosts and felids as definitive hosts [1,2]. This zoonotic parasite can cause severe neuromuscular diseases in immunocompromised humans, abortion in pregnant women, and even death [2]. Even though *T. gondii* infections are usually **subclinical**, toxoplasmosis in nonhuman primates (NHPs) is a conservation concern as high susceptibility and mortality has been reported in captive New World monkey and lemur species in different countries. Worldwide reports of toxoplasmosis, ***T. gondii* infection or seropositivity to *T. gondii*** in primates up to 2009 were summarized previously [2]. Since then, there have been additional reports of fatal toxoplasmosis in New World primates confirmed by isolation and genetic characterization of viable *T. gondii* strains [3,4]. Although Old World primates are considered resistant to *T. gondii* infection, they have been experimentally infected to simulate pathogenesis of toxoplasmosis in humans. As an example, Schoondermark-Van de Ven et al. [5,6] used rhesus macaque (*Macaca mulatta*) to study pathogenesis of congenital toxoplasmosis because of their hemochorial placenta as in humans. Likewise, Holland et al. [7] studied ocular toxoplasmosis in the crab-eating macaque (*Macaca fascicularis*) by directly inoculating the macula of one eye to compare lesions in the uninoculated eye.

In Spain, fatal acute toxoplasmosis cases have previously been reported in NHPs housed in zoos [8,9], but little is known about the prevalence of antibodies to *T. gondii* in these species. The aim of the present study was to determine the seroprevalence and risk factors associated with *T. gondii* infection in NHPs in zoos in Spain.

2. Material and methods

2.1. Study design and sampling

A total of 203 sera from 189 NHPs belonging to 33 different species were obtained in eight different zoos in Spain between 2002 and 2018 (Table 1). The study adhered to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, text with **European Economic Area** (EEA) relevance for ethical treatment of Non-Human Primates. Most of the samples included in this study came from the serum banks of the sampled zoos. Sera were also opportunistically collected from specimens subjected to health programs, medical checkups or surgical interventions during the study period. Epidemiological information including species, sampling date, age, sex, **Hominidae** family and zoo, was gathered for each animal whenever possible. Additionally, 10 out of 189 animals were longitudinally sampled (Table 1).

2.2. Laboratory analysis

Sera were examined to detect anti-*T. gondii* antibodies using the modified agglutination test (MAT) as previously described [10]. Serum was tested at 1:25, 1:50, 1:100 and 1:500 dilutions. Sera with titers ≥ 25 (**positive at 1:25 or higher dilution**) were considered positive. The MAT, originally developed to detect *T. gondii* antibodies in humans, is applicable to all species of animals. It is highly specific and sensitive [2].

2.3. Statistical analysis

Seroprevalence was estimated from the percentage of positive samples to the total number of samples tested, with the exact binomial confidence intervals of 95% (CI_{95%}) [11]. Associations between prevalence of anti-*T. gondii* antibodies and

explanatory variables were analyzed using the Pearson's chi-square test or Fisher's exact test when there were less than six observations per category. Variables with P -values <0.15 in the bivariate analysis were included for further analysis. Collinearity between pairs of variables was tested by Cramer's V coefficient. Finally, a generalized estimating equation (GEE) **multivariable** model was carried out. The number of seropositive animals was assumed to follow a binomial distribution and "zoo" was included as a random effect. Values with $P<0.05$ were considered statistically significant. SPSS 25.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analyses.

3. Results

Anti-*T. gondii* antibodies (MAT ≥ 25) were detected in 48 of the 189 NHPs tested (25.4%; CI_{95%}: 19.2-31.6). Titers of **25, 50, 100 and ≥ 500** were found in three (6.3%), four (8.3%), eight (16.7%) and 33 (68.8%) seropositive animals, respectively. Seropositivity was detected in 15 of the 33 host species (45.5%), with values that ranged between 14.3% in mandrills (*Mandrillus sphinx*) and 100% in lar gibbons (*Hylobates lar*), Bornean gibbons (*Hylobates muelleri*), Japanese macaques (*Macaca fuscata*) and white-headed lemurs (*Eulemur albifrons*) (Table 1). The distribution of *T. gondii* seropositivity by sex, age and **Hominidae** family is shown in Table 2. Seropositive animals were detected in all the zoos analyzed, with within-zoo seroprevalences ranging from 15.6% to 75.0% (Figure 1). The **GEE multivariable** analysis showed that the **Hominidae** family and sex were risk factors potentially associated with *T. gondii* infection. Significantly higher seroprevalence was detected in hominids (57.5%; OR=5.9; $P<0.001$; CI_{95%}: 2.7-12.8) compared to the rest of primate

families (16.8%) and seroprevalence was significantly higher in females (34.8%; OR=2.1; $P=0.03$; CI_{95%}: 1.1-4.1) than in males (18.9%) (Table 2).

Of the 10 NHPs sampled more than once, seven were always seronegative; one common chimpanzee (*Pan troglodytes*) showed seropositivity in all four samplings performed since 2007 until 2015, with an increase in antibody titers after the second sampling in 2009. Two animals (one Barbary macaque [*Macaca sylvanus*] and one chimpanzee) seroconverted during the study period (Table 3).

4. Discussion

The results obtained in the present study evince a wide exposure to *T. gondii* in captive NHPs in Spain. The overall seroprevalence detected (25.4%; 48/189) is consistent with previous studies carried out in other countries such as Chile (22.5%; 4/17), the Czech Republic (27.7%; 13/47), China (31.2%; 5/16) and Portugal (33.3%; 7/21) [12-15]. **Apparently** higher seroprevalences were found in captive NHPs in Italy (50.0%; 2/4), Brazil (49.2%; 88/179 - 55.0%; 11/20) and Mexico (66.6%; 4/6) [16-19]. In contrast, **apparently** lower prevalences of antibodies were observed in the United States of America (8.2%; 6/73) [20]. In an earlier study in Spain, *T. gondii* antibodies were not found in NHPs, however only two animals were sampled [21]. Differences between studies should be carefully interpreted given the diversity of species analyzed, the number of animals tested and the diagnostic methods employed.

The high percentage (68.8%) of seropositive animals with titers ≥ 500 as well as the detection of anti-*T. gondii* antibodies in all the sampled zoos, indicate an active and widespread circulation of *T. gondii* in NHPs in Spain. Interestingly, seroconversions were detected in one common chimpanzee and one Barbary macaque longitudinally sampled from the same zoo, which indicates exposure to *T. gondii* in these animals in

2014 and 2016, respectively. Moreover, a titer of **25** was found in one chimpanzee sampled in 2007, while titers \geq **500** were detected in the same animal in the three subsequent samplings in 2009, 2011 and 2015. This finding suggests *T. gondii* re-exposure during the sampling period and/or long-lasting humoral immune response in NHPs.

Interestingly, antibodies against *T. gondii* were not found in New World monkey species analyzed (**Callitrichidae** and **Cebidae** families) (Table 1). The absence of seropositivity in this group of species may reflect a limited contact with the parasite or a high susceptibility to *T. gondii* infection. In this context, numerous outbreaks of fatal toxoplasmosis have previously been described in New World monkeys [2-4]. New World monkeys can die of peracute toxoplasmosis without prior clinical signs. Unfortunately, information about mortality or clinical signs associated with *T. gondii* infection could not be obtained during the study period. In agreement with our results, antibodies against *T. gondii* were not found in 126 golden-headed lion tamarins (*Leontopithecus chrysomelas*) in Brazil [22]. Similarly, De Camps et al. [20] failed to detect seropositivity in 36 New World monkeys of 10 different species in the United States of America. In contrast, significantly higher seropositivity was observed in New World (52.2%; 84/161) than Old World (22.2%; 4/18) monkeys in Brazil [19].

Pregnancy outcomes including spontaneous abortion, stillbirth, and congenital disorders in live born infants have been widely reported in humans as a result of *T. gondii* infection [23]. In accordance, reproductive disorders and neonatal mortality associated with *T. gondii* could also be of importance for NHPs. In our study, the risk for being seropositive for *T. gondii* was significantly higher in females than in males. The high exposure to *T. gondii* detected in females may implicate a higher risk of congenital toxoplasmosis in NHPs. Our finding contrasts with those observed by

Minervino et al. [18] who suggested that both captive females and males NHPs are equally exposed to this parasite. Further studies are required to assess the relationship between sex and the risk of *T. gondii* infection in these species.

Significantly higher seroprevalence was found in the Family **Hominidae** compared to the other primate families tested. The high longevity of hominids, 40 to 50 years on average in common chimpanzees, western gorillas (*Gorilla gorilla*) and Bornean orangutans (*Pongo pygmaeus*) [24], implies a higher probability of exposure to *T. gondii* throughout their lives. In addition, because the volume of food ingested is directly related with the size of the animal, the likelihood of infection by consumption of contaminated food or drinks with sporulated oocysts or ingestion of tissue cysts from raw meat, could also be proportionally increased.

5. Conclusions

The high seroprevalence detected in the present study indicates that *T. gondii* is widespread in captive NHPs in zoos in Spain. The results are of conservation concern, as several NHPs species are highly susceptible to *T. gondii* infection. Control measures, including restriction of access for feral cats, efficient rodent control programs, proper washing of fruit and vegetables and freezing meat before feeding primate species, should be properly implemented in zoos to minimize the risk of exposure of NHPs to *T. gondii*.

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Figure captions

Figure 1. Geographic distribution of the zoos (A-H) sampled in Spain. Pie charts indicate the number of seropositive (black) and seronegative (grey) to *T. gondii* in nonhuman primates.

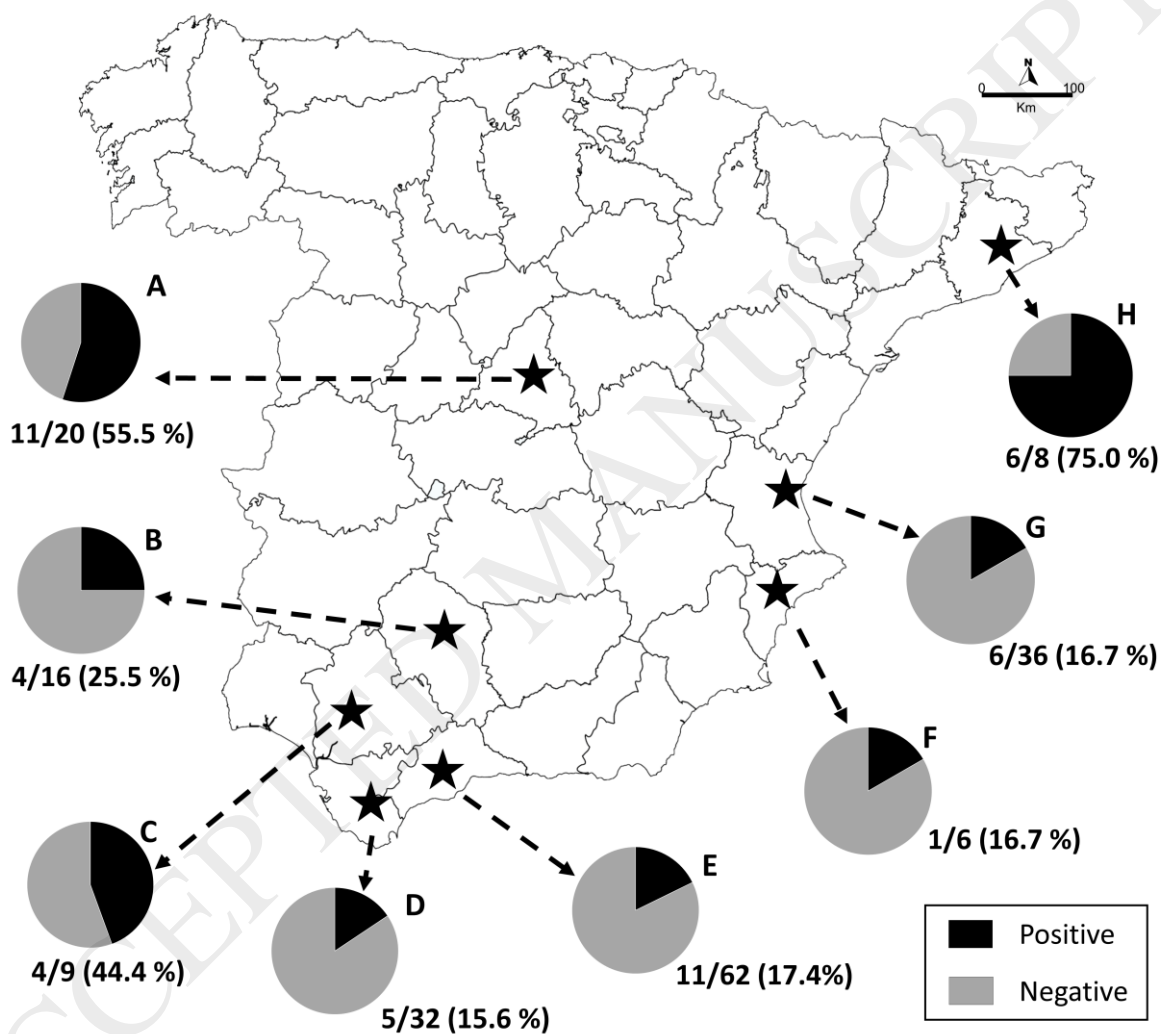


Table legends

Table 1. Seroprevalence and titers of *Toxoplasma gondii* by Modified Agglutination Test (MAT ≥ 25) in captive nonhuman primates from zoos in Spain.

SPECIES	RESULTS		TITER			
	% MAT positive	Number/overall	25	50	100	≥ 500
Family: Callitrichidae						
<i>Callithrix jacchus</i>	0.0	0/5	-	-	-	-
<i>Leontopithecus chrysomelas</i>	0.0	0/1	-	-	-	-
<i>Saguinus midas</i>	0.0	0/1	-	-	-	-
Sub-total	0.0	0/7	-	-	-	-
Family: Cebidae						
<i>Sapajus apella</i>	0.0	0/7	-	-	-	-
Sub-total	0.0	0/7	-	-	-	-
Family: Cercopithecidae						
<i>Cercocebus atys</i>	0.0	0/4	-	-	-	-
<i>Cercopithecus hamlyni</i>	0.0	0/2	-	-	-	-
<i>Cercopithecus mona</i>	50.0	1/2	-	-	-	100%
<i>Cercopithecus neglectus</i>	0.0	0/10	-	-	-	-
<i>Colobus guereza</i>	0.0	0/1	-	-	-	-
<i>Colobus guereza kikuyuensis</i>	22.2	2/9	-	-	-	100%
<i>Erithrocebus patas</i>	50.0	1/2	-	-	-	100%
<i>Lophocebus aterrimus</i>	0.0	0/3	-	-	-	-
<i>Macaca fuscata</i>	100	1/1	-	100%	-	-
<i>Macaca sylvanus</i>	38.5	5/13	-	40.0%	60.0%	-
<i>Mandrillus leucophaeus</i>	0.0	0/2	-	-	-	-
<i>Mandrillus sphinx</i>	14.3	1/7	-	-	-	100%
<i>Miopithecus ogouensis</i>	18.8	3/16	100%	-	-	-
<i>Papio anubis</i>	0.0	0/12	-	-	-	-
Sub-total	16.7	14/84	21.4%	21.4%	21.4%	35.7%
Family: Hominidae						
<i>Gorilla gorilla gorilla</i>	60.0	3/5	-	-	33.3%	66.6%
<i>Pan troglodytes</i>	62.5	15/24	6.6%	-	20.0%	73.3%
<i>Pongo pygmaeus</i>	45.5	5/11	-	-	20.0%	80.0%
Sub-total	57.5	23/40	4.3%	-	26.1%	69.6%
Family: Hylobatidae						
<i>Hylobates lar</i>	100	3/3	-	-	-	100%
<i>Hylobates muelleri</i>	100	1/1	-	-	-	100%
<i>Nomascus gabriellae</i>	33.3	1/3	-	-	-	100%
Sub-total	71.4	5/7	-	-	-	100%
Family: Lemuridae						
<i>Eulemur albifrons</i>	100	1/1	-	-	-	100%
<i>Eulemur fulvus</i>	0.0	0/2	-	-	-	-
<i>Eulemur macaco</i>	0.0	0/4	-	-	-	-
<i>Eulemur mongoz</i>	0.0	0/2	-	-	-	-
<i>Eulemur rubriventer</i>	0.0	0/4	-	-	-	-
<i>Lemur catta</i>	0.0	0/20	-	-	-	-
<i>Varecia rubra</i>	0.0	0/2	-	-	-	-
<i>Varecia variegata</i>	71.4	5/7	-	-	-	100%
Sub-total	14.3	6/42	-	-	-	100%
Family: Lorisidae						
<i>Nycticebus pygmaeus</i>	0.0	0/2	-	-	-	-
Sub-total	0.0	0/2	-	-	-	-
TOTAL	25.4	48/189	8.3%	6.3%	16.7%	68.8%

Table 2. Explanatory variables included in the bivariate analysis of seroprevalence of *T. gondii* in captive nonhuman primates in zoos in Spain.

Variable	Positive	Tested ^a	Frequency (%)	<i>P</i> -value ^b
Age				
Adult	27	110	24.5	0.248
Juvenile	7	40	17.5	
Sex				
Male	17	90	18.9	0.012
Female	31	89	34.8	
Family Hominidae				
Yes	23	40	57.5	<0.001
No	25	149	16.8	

^a Missing values omitted. ^b Results of the bivariate analysis

Table 3. Nonhuman primates longitudinally sampled for presence of *Toxoplasma*

I D	Species / Zoo	2007	20 08	2009	201 0	2011	201 2	201 3	2014	2015	2016
1	Common chimpanzee / C	-	-	-	-	-	-	-	-	▲	▲ ▲
2	Common chimpanzee /C	-	-	-	-	-	-	-	▲ (100) ▲	-	-
3	Barbary macaque / C	-	-	-	-	-	-	-	-	▲	▲ (50)
4	Mangabey / C	▲	-	-	▲	-	-	-	-	-	-
5	Brazza's monkey / G	▲	-	-	▲	-	-	-	-	-	-
6	Brazza's monkey / G	-	-	-	-	▲ ▲	-	-	-	-	-
7	Mongoose lémur / G	-	▲	-	-	-	-	▲	-	▲	-

gondii antibodies in zoos in Spain. Black and grey triangles indicate seropositive and seronegative samples in the animals, respectively. In seropositive samples, antibody titers against *T. gondii* by Modified Agglutination Test are shown in brackets.

8	Red-bellied lémur / G	-	-	-	-	▲ ▲	-	-	-	-	-
9	Common chimpanzee / G	▲ (25)	-	▲ (≥500)	-	▲ (≥500)	-	-	-	▲ (≥500)	-
10	Common chimpanzee / G	-	-	-	-	-	-	▲	-	▲	-

ACCEPTED MANUSCRIPT