

The effects of endoparasitism on the immune response to orally administered antigen in cats

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Abstract

The aim of the study was to assess whether infection with *Toxocara cati* (*T. cati*) facilitates the induction of immunoglobulin (Ig) E or other antibody responses to a specific antigen administered with food in kittens. Two groups of 10 cats each, either experimentally infected with *T. cati* or parasite-free, were dosed with human serum albumin (HSA) added daily to their food from day 7 to 28 inclusive. Levels of HSA-specific IgE, IgG, IgA and IgM were assessed in the serum by enzyme-linked immunosorbent assay (ELISA) in both groups of cats at weeks 0, 2, 4 and 8. Although weak, an IgE response was detected in most of the cats 1 week after exposure to HSA. However, HSA-specific IgG and IgA could only be detected from the third week after exposure to HSA. The group of parasitized cats had significantly higher levels of HSA-specific antibodies of the IgG and IgA at weeks 4 and 8 ($p < 0.05$ by Mann–Whitney) and IgE isotypes at weeks 2 and 4 ($p < 0.05$ by analysis of variance (ANOVA)) than did the group of parasite-free cats. Specific IgM antibody was not detected in the sera of any of the 20 cats. These findings are supportive of a role of *T. cati* infection in enhancing the IgE response to orally administered antigens, and hence possibly, in genetically susceptible individuals, in the development of food hypersensitivity.

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1. Introduction

The prevalence of food hypersensitivity appears to increase in parallel to other atopic diseases in man and it has been reported to affect 3–8% of the population younger than 3 years (Bock, 1987; Host and Halken, 1990; Sampson, 1999). There is insufficient evidence to accurately assess the prevalence of food allergy in cats and in dogs. However, the likely prevalence of food allergy in dogs has been estimated to be 7.6%

Abbreviations: ANOVA, analysis of variance; EPSC, end point of the standard curve; HAS, human serum albumin; RAU, relative antibody units; *T. cati*, *Toxocara cati*

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with 32.7% of those dogs showing signs compatible with a diagnosis of atopic dermatitis (Chesney, 2002), while in cats, it has been reported to range from 0 to 23% of allergic skin diseases (Foster and O'Dair, 1993; O'Dair et al., 1996; Prost, 1996).

Endoparasitic infections may predispose to the development of an IgE response to antigens administered parenterally at the same time (Turner et al., 1979). However, it is not known whether this is also the case when antigens are administered orally. In addition, it has been shown that genetically predisposed atopic individuals seem to be more prone to develop atopy/asthma following the additional allergenic stimulation provided by parasite infection (Moqbel and Pritchard, 1990). It is thus possible that endoparasitism may have a role in the development of food hypersensitivity in predisposed individuals.

A colony of dogs that spontaneously develop hypersensitivity to food ingredients and show increase in allergen-specific IgE concentrations as well as clinical signs of pruritus, otitis externa and colitis in response to oral challenge has been reported (Jackson et al., 2003). Also, models of food allergy have been successfully developed in dogs following sensitisation to food antigens by oral and/or parenteral administration (Guilford and Badcoe, 1992; Ermel et al., 1997; Teuber et al., 2002). In the first model, normal pups sensitised to codfish antigen by oral and parenteral administration developed high levels of serum food-specific IgE (Guilford and Badcoe, 1992). The second model was developed in dogs from an atopic colony. The dogs showed an IgE response to food antigens after being injected with three different food allergens and one pollen, and concomitantly immunised with a live attenuated canine distemper/adenovirus type 2/parainfluenza virus vaccine (Ermel et al., 1997). In the third model, dogs obtained from the same atopic colony were successfully sensitised to either peanut or tree nut and to soy and either wheat or barley. These dogs were also concomitantly immunised with modified live virus vaccines (Teuber et al., 2002). Following oral challenge with the relevant food antigen, some of the dogs developed transient pruritus (Guilford and Badcoe, 1992), vomiting (Guilford and Badcoe, 1992; Ermel et al., 1997; Teuber et al., 2002) and/or diarrhea (Guilford and Badcoe, 1992; Ermel et al., 1997) and even anaphylaxis (Teuber et al., 2002).

The use of animal models is of value in assessing different factors that may contribute to positive serology to food allergens, such as parasitism. Although the presence of food-specific IgE is not necessarily associated with the development of clinical signs, it is of interest to ask whether endoparasitism in animals orally sensitised to a food allergen could lead to the development of clinical disease.

The aims of this study were to assess whether helminth infection with *Toxocara cati* (*T. cati*) facilitates the induction of IgE or other antibody responses in kittens to a specific antigen administered with food, and also whether clinical signs develop. Human serum albumin (HSA) was chosen as the antigen because none of the cats would have been previously exposed to this protein.

2. Materials and methods

2.1. Animals

Ten domestic short hair cats (six males and four females) aged approximately 4 months were obtained from Hill Grove Family Farm Ltd. (Oxford, UK). All cats were parasite-free and came from a colony that has been parasite-free for many generations. They were maintained at the University of Edinburgh.

Ten additional male domestic short hair parasite-free kittens, aged 7–10 weeks, were obtained from the Central Animal Laboratory of the University of Nijmegen, The Netherlands, for infestation with *T. cati* in Belgium. They were maintained at the University of Liège.

None of the cats were vaccinated.

After nursing, kittens were weaned and fed Whiskas[®] chicken and beef for kittens (Waltham Centre for Pet Nutrition, Leicestershire, UK). They were fed once a day and water was available ad libitum.

2.2. Infection of cats with *T. cati*

The 10 kittens aged between 7 and 10 weeks were experimentally infected with *T. cati*. Infective second stage larvae were provided by Dr. Epe from the Institute for Parasitology, der Tierärztliche Hochschule Hann-

over (Hannover, Germany). A single dose of approximately 250 embryonated *T. cati* eggs suspended in 1 ml of water was administered orally to each cat. Faeces were examined for the appearance of ova 2 months later. The parasitised cats were euthanised at the end of the study with 5 ml of sodium pentobarbital and the gastrointestinal tract opened to assess the worm burden.

2.3. Antigen employed

Fraction V of HSA, 96–99% pure (Sigma, Poole, UK), was used for oral sensitisation of the cats and for the detection by ELISA of HSA-specific IgE, IgG, IgA and IgM.

2.4. Sensitisation of cats

At the time of oral sensitisation, the parasite-free cats and the parasitised cats were age matched and were approximately 4-month-old. Each cat was dosed with 100 mg of HSA added to the food daily from days 7 to 28 inclusive.

2.5. Blood samples

Blood was obtained by jugular venepuncture at weeks 0, 2, 4 and 8 (bleedings 1–4). After clot retraction, serum was harvested by centrifugation and stored at -20°C .

2.6. Antisera employed

A polyclonal rabbit anti-feline IgE, which was prepared as described earlier, was used (Gilbert and Halliwell, 1998a). The antiserum neutralised Prausnitz–Küstner (PK) reactivity in feline sera and failed to detect any IgE by ELISA in sera containing both IgE and IgG antibodies after the sera were heated at 56°C for 4 h (Gilbert and Halliwell, 1998a).

Alkaline phosphatase-conjugated goat anti-feline IgG, IgA and IgM (heavy chain-specific) were obtained from Bethyl Laboratories Inc. (Montlucon, France).

Unconjugated goat anti-feline IgM was purchased from Nordic Immunology (Tilburg, The Netherlands).

Mouse monoclonal alkaline phosphatase-conjugated anti-rabbit and anti-goat immunoglobulins were purchased from Sigma.

2.7. ELISA for HSA-specific antibodies

2.7.1. Procedure for the detection of HSA-specific IgE

HSA-specific IgE in feline sera was assessed by indirect ELISA, as described in a previous study on feline IgE (Gilbert and Halliwell, 1998b) with some modifications. Plates were coated with 0.5% HSA and blocked with donkey serum. Donkey serum also substituted HSA in the washing buffer and the ELISA buffer. Serum samples were diluted in ELISA buffer at an initial dilution of 1:2. A standard serum with a high level of IgE antibody was included in each plate, and the OD was read when the standard reached an OD of 0.56. Preimmunisation bleedings were used as the negative controls and were included in each assay.

2.7.2. Procedure for the detection of HSA-specific IgG, IgA and IgM

Sera were assayed for HSA-specific IgG, IgA and IgM by direct ELISA, as performed in a previous clinical study for the detection of *Dermatophagoides farinae*-specific IgG (Gilbert and Halliwell, 1998b). In addition, sera were also assayed for HSA-specific IgM by indirect ELISA as performed for HSA-specific IgE. Serum samples were initially assessed at a dilution of 1/10 in ELISA buffer including donkey serum. Incubation time for the test sera were reduced from 2 to 1.5 h and for the conjugate from 2 to 1 h. Polyclonal alkaline phosphatase-conjugated anti-feline IgG and IgA were used at 1/500 and 1/150, respectively, whereas alkaline phosphatase-conjugated anti-feline IgM was used both at 1/500 and 1/1000. The polyclonal unconjugated anti-feline IgM was assessed at 1/100, 1/1000 and 1/5000 dilutions.

A standard curve was established with a minimum of four points for the detection of IgG and IgA. Results for these isotypes were recorded as relative antibody units (RAU) by reference to a standard which was given an arbitrary RAU value of 1000 when undiluted. If the result did not fall in the sensitive part of the standard curve, the assay was repeated at a lower or higher dilution as appropriate. A serum sample whose RAU at 1/2 fell below the end point of the standard curve (EPSC) was deemed to be negative. For the purpose of statistical analyses, these sera were assigned a value half way between the RAU of the EPSC and zero, which was 3.906.

2.8. Statistical analysis

Results were analysed using a statistical computer package (MINITAB release 11.0 Minitab Inc., State College, PA, USA). The Mann–Whitney test was used to compare the HSA-specific IgA and IgG responses between different groups of cats and the levels of HSA-specific IgE were analysed by analysis of variance (ANOVA). Results were considered significant at $p < 0.05$.

Correlation between the levels of IgG and IgA at weeks 4 and 8 in the parasite-free and parasitised cats were calculated using Spearman's rank correlation.

3. Results

3.1. Infection with *T. cati*

T. cati eggs were found in the faeces of the 10 cats, 8–10 weeks after being orally dosed with embryonated eggs. Adult *T. cati* were found in the digestive tract in all the 10 cats at termination of the study, which was 4 months after infection.

3.2. Immune response to HSA

3.2.1. HSA-specific IgE response

Positive OD were obtained in the presensitisation bleedings and were assumed to be the result of non-specific binding. In general, these data were only slightly positive. However, an OD > 0.1 was seen in serum from 15 cats and > 0.2 in one of them. There was no significant difference between the OD values found in the presensitisation bleedings in the parasitised and parasite-free cats.

Although weak, an HSA-specific IgE response was detectable in most of the parasitised cats 1 week after exposure to HSA (week 2) and rose to a maximum 3 weeks after exposure to HSA (week 4, Fig. 1). Levels of IgE at week 8 decreased in 15 cats out of the 20 (Fig. 1). The lowest levels of HAS-specific IgE were found in the parasite-free cats with two of them (both females) showing essentially undetectable responses.

The group of cats infected with *T. cati* had significantly higher levels of HSA-specific IgE at weeks 2 and 4, but not at week 8, than did the group of parasite-free cats ($p < 0.05$ by ANOVA). However,

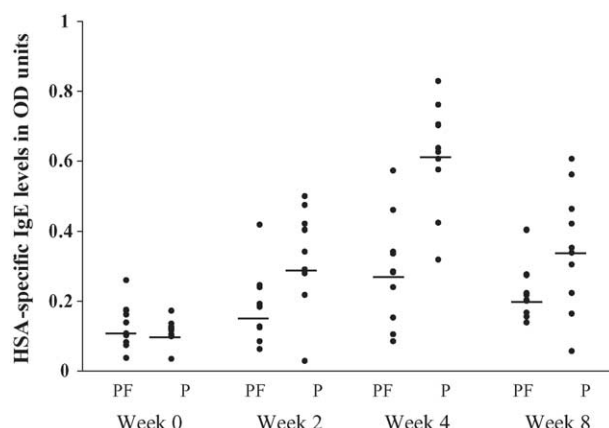


Fig. 1. Levels of HSA-specific IgE in OD units in parasitized (P) and non-parasite-free (PF) cats in the four bleedings. Bar = median.

the most significant differences between the two groups were found 3 weeks after exposure to HSA (week 4, $p < 0.001$) (Table 1(a); Fig. 1).

3.2.2. HSA-specific IgG response

The cats began to develop an HSA-specific IgG response between the first and the third week following exposure to HSA (Fig. 2). However, one cat from the parasitised group and four cats from the parasite-free group (two males and two females) remained negative during the 2 months of the study. Four parasite-free cats had HSA-specific IgG only in the last bleeding (week 8) and only at very low levels. IgG antibody levels decreased in 8 cats out of the 15

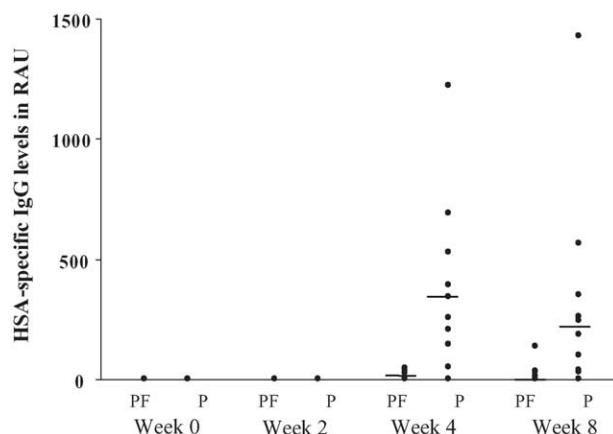


Fig. 2. Levels of HSA-specific IgG in RAU in parasitized (P) and parasite-free (PF) cats in the four bleedings. Bar = median.

Table 1
Median and range of HSA-specific IgE (a), IgG (b) and IgA (c)

	Week 2	Week 4	Week 8
(a) HSA-specific IgE			
P	0.315 (0.028–0.498)	0.63 (0.318–0.828)	0.344 (0.056–0.606)
PF	0.155* (0.061–0.418)	0.284* (0.084–0.571)	0.209 (0.137–0.404)
(b) HAS-specific IgG			
P		302 (4–1224)	219 (4–1432)
PF		4* (4–50)	16* (4–139)
(c) HAS-specific IgA			
P		463 (4–4497)	293 (4–1973)
PF		8* (4–59)	15* (4–35)

(P) Parasitised cats and (PF) parasite-free cats. (a) Results in OD units, (b) and (c) results in RAU.

* Test significant at $p < 0.05$. Differences in IgE levels were assessed for significance using ANOVA, and differences in IgG and IgA levels were assessed by Mann–Whitney test.

cats that developed an IgG response to HSA at week 8, which was 4 weeks after conclusion of the antigenic challenge. Interestingly, the parasite-free cats which had undetectable or only low levels of HSA-specific IgG also showed either a weak or undetectable IgE response. However, this was not the case in the parasitised cats with the exception of one cat that showed a low response for all isotypes including IgE.

Cats infected with *T. cati* had significantly higher levels of antigen-specific IgG antibody than did the parasite-free cats at weeks 4 and 8 ($p < 0.05$) (Table 1(b), Fig. 2).

3.2.3. HSA-specific IgA response

As was the case with HSA-specific IgG, HSA-specific IgA could only be detected from week 4 (Fig. 3). One parasite-free cat and one parasitised cat never developed an IgA response to HSA. Four parasite-free cats also showed only a weak IgA response in the last bleeding. The levels of allergen-specific IgA in the last bleeding declined in 8 out of the 20 cats.

The group of parasite-free cats developed only a weak IgA response to HSA and differences between the levels of HSA-specific IgA in both bleedings 3 and 4 (weeks 4 and 8) from parasitised and parasite-free cats were significant ($p < 0.05$) (Table 1(c), Fig. 3).

3.2.4. HSA-specific IgM response

HSA-specific IgM could not be detected in the sera of any of the 10 cats of either group at any dilution tested employing either the alkaline phosphatase-

conjugated or by an indirect ELISA employing unconjugated goat anti-feline IgM.

3.2.5. Correlation between the levels of HSA-specific IgG and IgA

The IgG and IgA responses at weeks 4 and 8 taken together, in the parasite-free and the parasitised cats were highly correlated ($r = 0.924$ and 0.619 , respectively, $p = 0.001$ and 0.004). When the correlations between the levels of IgG and IgA were assessed either at week 4 or week 8, they were significant in both parasite-free and parasitized cats at week 4 ($r = 0.964$ and 0.803 , $p < 0.001$ and 0.023 , respectively), but they were significant in the parasitized cats alone at week 8 ($r = 0.803$, $p < 0.005$).

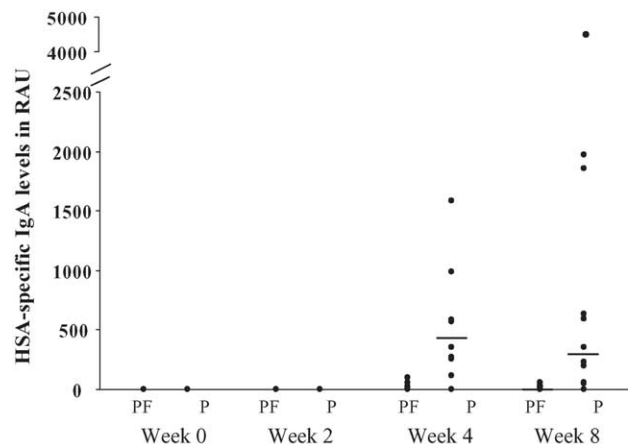


Fig. 3. Levels of HSA-specific IgA in RAU in parasitized (P) and parasite-free (PF) cats in the four bleedings. Bar = median.

4. Discussion

The oral administration of HSA daily for 3 weeks induced a significant antibody response involving IgG, IgA and IgE isotypes. This is in accordance with a recent study in which an antigen-specific IgE and IgG responses could be induced in the brown Norway rat model by daily oral administration of various protein extracts without adjuvant, including partly purified ovalbumin (Knippels and Penninks, 2003). In the present study, although weak, an IgE response was already detectable 1 week after oral exposure to HSA in the cats infected with *T. cati* and in some of the parasite-free cats, whereas the IgG and IgA responses were only detected later. However, firm conclusions regarding the kinetics of the different isotype responses cannot be made as the sensitivity of the ELISA for each isotype could have differed. It was notable that the IgE response was of shorter duration than that of the other isotypes.

As expected, almost all the cats developed a serum IgG and IgA response to the HSA. Food allergen-specific IgG and IgA are believed to reflect previous food exposure and not necessarily associated with adverse food reactions (Kletter et al., 1971; Johansson et al., 1984; Paganelli et al., 1984).

The cats showed no dermatological or gastrointestinal signs. However, the dose of HSA administered may not have been high enough or the HSA may not have been given for a sufficient long period of time to lead to the development of clinical signs. Challenge of the cats with HSA after the 3-week-sensitisation period could also have led to clinical signs. Recently anaphylactic reactions were induced by intravenous injections of ovalbumin in transgenic mice expressing T-cell receptor specific for a dominant T-cell determinant peptide of ovalbumin that had been fed 250 mg of ovalbumin daily for 5 weeks or more (Shida et al., 2000). However, the level of serum allergen-specific IgE was evident 2 weeks after being fed the ovalbumin (Shida et al., 2000). On the other hand, the cats in this study had no known genetic predisposition to allergy. The possible existence of IgE heterogeneity could be another explanation for the absence of clinical signs despite the presence of allergen-specific IgE (Lichtenstein, 1988; Jackson et al., 1996; MacDonald, 1996; Peng et al., 1997; Halliwell et al., 1998).

The inclusion of a group of parasitised cats not fed HSA was not considered necessary, as the bleeding

prior to oral sensitisation served as a control and showed that *T. cati* infection alone did not generate an antibody response to the HSA. The parasite-free cats showed much lower levels of food-specific antibodies than did the parasitised cats, with two of them apparently developing tolerance to the HSA. Therefore, the presence of *T. cati* seems to have prevented the development of oral tolerance in favour of an active immunity with activation of CD4+ T-cells leading to the induction of helper and memory cells. Interestingly, the cats which had a low IgE response to HSA had also low levels of other isotypes in their sera whereas the opposite was not always seen.

The four female cats were by random assignment all in the parasite-free group. All gave poor immune responses. When the females were excluded from the results, the level of HSA-specific IgE was still significantly different between the parasitised group and the parasite-free group at week 4 ($p = 0.004$) but not at week 2 ($p = 0.055$) when assessed by ANOVA. However, the difference between the levels of HSA-specific IgG and IgA remained significant between the two groups of cats at week 4 ($p = 0.006$ and 0.008 , respectively) and week 8 ($p = 0.023$ and 0.011 , respectively), when assessed by the Mann–Whitney. Although no sex-linked difference has ever been demonstrated in cats in respect of any immune response, and there is no sex-linked predilection for either atopic disease or food hypersensitivity in this species, the possibility of an effect of sex on the immune response must be considered.

Cats infected with *T. cati* gave a much stronger antigen-specific-antibody response in the case of all three isotypes. Amongst the five cats which did not develop an IgG response to HSA, 4 were parasite-free. One of the two cats which did not give an IgA response and the two cats which responded weakly or not at all in respect of IgE were also parasite-free. The marked increase of HSA-specific IgE seen in the cats infected with *T. cati* was not unexpected since it is well known that the IgE antibody response to unrelated allergens is potentiated in individuals with helminth infections. The increase in total IgE has been reported to be largely a consequence of non-specific polyclonal activation of IgE B cells (Jarrett and Miller, 1982). A Th2 dominated response exhibiting a characteristic pattern of lymphokine production, versus a Th1 response producing IFN- γ , may be responsible for the

elevated levels of IgE seen in helminth infections (Romagnani, 1990; King et al., 1992). A preponderance of Th2 cells has been reported in human patients with IgE antibody against various helminths, including *T. canis* (Romagnani, 1990).

The two groups of cats were from different sources, and hence from different genetic backgrounds. There is clearly a possibility that this, rather than the fact that they were parasitised, could have been responsible for the markedly differing IgE response between the two groups. However, neither colony was inbred and so, this is unlikely. Also, the fact that both IgG and IgA followed the same trend at least eliminates the possibility of a genetically determined difference in isotype-specific immune response. As conditions of maintenance were very similar, and the diet identical, it is highly unlikely that the fact that the two groups were housed in different locations would have affected the results.

There are conflicting hypotheses on the relationship between the non-specific polyclonal potentiation of IgE induced by helminth infection and allergic conditions (Moqbel and Pritchard, 1990). Genetically predisposed atopic individuals seem to be more prone to develop atopy/asthma following the additional allergenic stimulation provided by parasite infection (Moqbel and Pritchard, 1990). The infection of cats with *T. cati* could therefore have a role in the development of food hypersensitivity in predisposed cats by the potentiation of the IgE response to orally administered antigen. However, it is also reported that the high total serum IgE levels seen in parasitised populations could have a protective role in atopic individuals (Lynch, 1992; Lynch et al., 1993, 1999).

HSA-specific IgG was also found to be increased in the parasitized cats. Like the IgE response, the IgG4 response in man has been shown to depend on Th2 cells and their cytokine profile (Spiegelberg et al., 1991), and helminth infection results in heightened parasite-specific and non-specific IgG4 antibody levels (IgG1 in the mouse) (Zakroff et al., 1989). In the present study, all subclasses of IgG were presumably detected by the ELISA, and therefore, the subclass specificity of the response is unknown. The increase of HSA-specific IgG in parasitized cats may also reflect increased antigen exposure due to increased mucosal permeability caused by the helminth infection. A recent study measured food allergen-specific IgG in normal dogs, dogs with atopic

dermatitis and dogs with gastrointestinal disease and found that the group of dogs with gastrointestinal disease had generally more IgG to food allergens than the two other groups (Foster et al., 2003).

The level of allergen-specific IgA was shown to be significantly increased in the cats sensitised to HSA which were infected with *T. cati*. IgA producing cells have also been shown to be upregulated by IL-5 and IL-6 (McGhee et al., 1989). Therefore, the increase in the levels of HSA-specific IgA found in the parasitised cats is not surprising. However, the level of total IgA was not assessed in these cats.

Although ELISA were performed with two different polyclonal anti-feline IgM reagents, a HSA-specific IgM response could not be detected. This is in accord with a recent study in which allergen-specific serum IgE, IgG and IgA responses, but not an IgM response, could be induced in transgenic mice expressing TCR specific for a dominant T-cell determinant peptide of ovalbumin, following oral administration of ovalbumin daily for 8 weeks (Shida et al., 2000).

In conclusion, the oral administration of HSA to parasite and parasite-free kittens for 3 weeks induced antigen-specific serum IgE, IgG and IgA responses, although weak in the group of parasite-free cats. The infection of the cats with *T. cati* significantly enhanced the production of allergen-specific IgE (weeks 2 and 4), IgG and IgA (weeks 4 and 8) isotypes. These findings are supportive of a possible role of endoparasitism in enhancing the immune response to orally administered antigen, and hence possibly, in genetically susceptible individuals, in the development of food hypersensitivity.

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