Review Article

Individual Subject Meta-Analysis of Parameters for Giardia duodenalis Shedding in Animal Experimental Models

A. D. Adell,1,2 W. A. Miller,1,3 D. J. Harvey,4 E. Van Wormer,3 S. Wuertz,5,6,7 and P. A. Conrad1,3

1 Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, One Shields Avenue, University of California, Davis, CA 95616, USA
2 Escuela de Medicina Veterinaria, Facultad de Ecologia y Recursos Naturales, Universidad Andres Bello, Republica 440, 8370251 Santiago, Chile
3 One Health Institute, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
4 Department of Public Health Sciences, School of Medicine, University of California, Davis, CA 95616, USA
5 Department of Civil & Environmental Engineering, College of Engineering, University of California, Davis, CA 95616, USA
6 Singapore Centre on Environmental Life Sciences Engineering (SCELSE), School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551
7 School of Civil and Environmental Engineering, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551

Correspondence should be addressed to W. A. Miller; wasmith@ucdavis.edu

Received 17 January 2014; Accepted 4 March 2014; Published 1 April 2014

Academic Editor: Fabio Ribeiro Braga

Copyright © 2014 A. D. Adell et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Giardia duodenalis is a zoonotic protozoan parasite with public health importance worldwide. While articles about animal model infectivity have been published for G. duodenalis, the studies have used diverse protocols and parameters to evaluate the infectivity of this protozoan parasite. Hence, the objectives of this study were to (1) conduct a meta-analysis of published literature for cyst shedding and diarrhea outcomes in animal models and (2) develop recommendations to help standardize experimental dose response studies. Results showed that, for the outcome of cyst shedding in faeces, the covariates of infective stage (cyst versus trophozoite), Giardia dose, and the interactions between doses and infective stage, as well as dose and species of experimental host, were all significant ($P$ value $\leq 0.05$). This study suggests inoculation of the experimental host with cysts rather than trophozoites and administration of higher doses of Giardia will most likely result in cyst shedding. Based on the results of this meta-analysis, the infective stage (cyst versus trophozoite), parasite dose, and the interactions between dose and infective stage, as well as dose and species of experimental host, should be considered when designing experimental dose response studies that will assist in the study of zoonotic neglected tropical diseases globally.

1. Introduction

Giardia duodenalis is a zoonotic waterborne pathogen known to be released into the environment through human and animal faeces [1]. Infection and gastrointestinal disease in humans are caused by ingestion of small doses (>10 cysts) of Giardia cysts [2] directly from faeces or indirectly from contaminated water and food products [1, 3]. Giardia is considered the most common cause of protozoan diarrhea in developing countries and worldwide [1]. It has been estimated that in Africa, Asia, and Latin America about 200 million people have symptomatic giardiasis with some 500000 new cases being reported each year [4]. The number of reported cases in the US was 18,913 in 2008 [5]. In 2004, Giardia was included in the World Health Organization’s Neglected Disease Initiative for its link with poverty [6]. G. duodenalis, also called G. intestinalis and G. lamblia, is the only species found in humans [1, 3] and will be referred to as G. duodenalis throughout this paper.

Animal and human dose-response models as well as in vitro cell culture have been widely used to evaluate Giardia spp. infectivity. At present, human infectivity studies are not
considered to be practical because of the ethical concerns involved. Therefore, very few experiments have involved the attempted infection of humans with *Giardia* isolates [2, 7]. Instead, experimental animal models have been frequently used to estimate whether *G. duodenalis* is strictly host specific or zoonotic [1] and to provide information used for human health risk assessments. In vitro cell culture has been proposed as a potential alternative to animal assays because cultivation has the advantage of being cheaper and less time consuming and does not raise the ethical concerns associated with using animal models [8]. However, experimental animal model studies are often preferred when performing risk assessment, as they provide the most realistic scenario of what is happening with the pathogen once it enters the host. For this reason, this meta-analysis focuses on the diverse range of experimental animal model studies available in the peer-reviewed literature.

Numerous cross-transmission experiments involving the infection with isolates of *Giardia* in a variety of animal species have been published. Each of the experimental studies used different parameters such as animal species, age, cysts or trophozoites, dose, and detection methods to quantify cysts in faeces and evaluate the infectivity of *G. duodenalis* as well as outcomes ranging from laboratory detection of cyst shedding to clinical symptoms. Using diverse variables and protocols can be problematic in experimental studies because the variations in protocols between studies can affect the outcome of the experiments and thus their comparability [1, 9]. Consequently, a standardization of the experimental dose-response studies is required.

Hence the main objective of the present study was to develop recommendations for the standardization of animal dose-response experiments by conducting a meta-analysis of individual subjects and exploring study design characteristics that cause heterogeneity between included studies. The hypothesis tested in this meta-analysis was that selected experimental factors of interest are associated with increased *G. duodenalis* cyst shedding and diarrhoea. The results of this meta-analysis will be useful for more standardized and comparable studies of *Giardia* infectivity, so that in the future there may be fewer animal species and resources used to provide dose-response information more quickly and efficiently.

### 2. Methods

#### 2.1. Literature Search Strategy and Selection of Studies.

The search of published animal and/or human dose-response studies in all languages was performed using the electronic databases PubMed and Web of Science from March to December, 2010. The process is described in Figure 1, with search criteria consisting of the following algorithms: (i) protozoan infectious doses for humans, (ii) *Giardia* and dose response, (iii) *Giardia* and infectivity, (iv) *Giardia* and meta-analysis, (v) giardiasis and experimental and model, and (vi) experimental and infection and *Giardia*. Unpublished studies (grey literature) were not included in this meta-analysis.

The articles identified by means of these search criteria were subject to a further selection process consisting of the removal of the complete study or individuals within the articles that met any of the exclusion criteria (Table 1). One reviewer examined the titles and abstracts of all the articles found using the search criteria mentioned above. The full article for each of the relevant studies was then assessed by the same reviewer and a second reviewer was consulted as needed. Only those articles that met at least one of the search criteria and none of the exclusion criteria were included in the data analysis. Furthermore, the reference lists of all included articles were searched for further possible papers, but no additions were identified. Ethical approval was not required for this meta-analysis.

#### 2.2. Data Extraction.

Data regarding the following variables for each individual animal were extracted and recorded from each article: (i) *Giardia* species (*Giardia*), (ii) isolate analyzed, such as HP-10 (Isolate), (iii) assemblage of the *Giardia* sp. such as A (Assemble), (iv) original source of isolate (Isolate Source), (v) whether cysts or trophozoites were subject to passage before challenge (Passage), (vi) storage time of cysts or trophozoites (in weeks) prior to inoculation of experimental host (Storage Time), (vii) method used to confirm cyst or trophozoite viability prior to inoculation (Viability Method), (viii) animal species used as experimental host (Experimental Species), (ix) number of subjects per group inoculated (Number), (x) age of the experimental host (Age), (xi) whether the experimental host was subject to immunosuppression (Immunosuppression), (xii) method of immunosuppression of the experimental host (Immunosuppression Cause), (xiii) whether cysts or trophozoites were administered to the experimental host (Infective Stage), (xiv) cyst or trophozoite dose administered to the experimental host (*Giardia* Dose), (xv) administration route used to inoculate the cysts or trophozoite dose to the experimental host (Administration Route), (xvi) cyst detection method in faeces of experimental hosts after inoculation (Detection Method), and (xvii) the number of animals that shed cysts and/or presented with diarrhoea after inoculation.

The unit of analysis was the individual animal; thus, the value for each variable was collected for each animal and included in the analysis. If a variable was not reported at the animal level, the variable was left blank. Table 2 provides information regarding the number of subjects and missing values by variable and study. The primary outcomes of interest were the presence of cyst shedding and diarrhoea. An animal was considered to have shedding or diarrhoea when the condition of the animal was described with the words diarrhoea or cyst shedding in the study from which the information was extracted. All of the studies included in this analysis evaluated shedding over prolonged periods so even if shedding was intermittent, any report of cysts observed in the feces was considered as positive for shedding. For diarrhoea and cyst shedding, the classification of Yes/No was used. Initially, attempts were made to contact authors for clarification, but, due to lack of responses, this approach was not systematically implemented throughout the entire study.

#### 2.3. Classification of Variables.

The extracted variables were classified according to the information provided by the
1045 records were identified through database searching

The abstracts of 1045 studies were screened

974 records were removed

71 full-text articles were assessed for eligibility

41 records were excluded, with reasons provided in Table 1

30 studies were included in quantitative synthesis (meta-analysis) for the cyst shedding outcome

4 studies were included in quantitative synthesis (meta-analysis) for the diarrhea outcome

1432 individual participant data were included in the quantitative synthesis (meta-analysis) for the cyst shedding outcome (Table 2)

82 individual participant data were included in the quantitative synthesis (meta-analysis) for the diarrhea outcome (Table 2)

Table 1: Exclusion criteria for studies and number of articles excluded from the *Giardia duodenalis* meta-analysis.

| Exclusion criteria                                                                 | Articles excluded |
|-----------------------------------------------------------------------------------|-------------------|
| (1) *Giardia* different than *G. duodenalis*, *G. intestinalis*, or *G. lamblia* were used | 19                |
| (2) Infection was assessed only by histology on tissue sections                    | 6                 |
| (3) Number of animals with diarrhoea or shedding was not reported                  | 4                 |
| (4) Animal study groups were subject to treatments other than the single inoculation/single outcome design used in this meta-analysis | 3                 |
| (5) Infection was assessed by cyst shedding and histology together and it was not possible to distinguish how many animals were shedding | 4                 |
| (6) Experimental dose response was measured in humans only                         | 2                 |
| (7) Study was not an experimental dose response experiment                          | 1                 |
| (8) Cyst dose administered to the experimental host was not provided or a range was provided | 1                 |
| (9) Number of animals used was not provided                                       | 1                 |

selected studies for each outcome (Table 3). In the case of the variable “Isolate Source,” the classification “Other than Humans” was composed of ruminants (cattle and lambs), primates, rodents, and drinking water. For the variable “Passages,” the classification “Yes” was composed of in vivo (animal passage), in vitro (cultures), and both in vivo and in vitro passages, while the classification “No” corresponded to cysts that were not subject to any passage. For the “Storage Time” variable, the class “<1 week” included animals that were inoculated with cysts or trophozoites that were stored for less than one week; “≥1 week” included all those animals that were inoculated with cysts or trophozoites that were stored for one week or more. Treating the variable “Storage Time” as a continuous variable, with or without log transformation, did not
improve the model fit. Regarding the variable “Experimental Species,” the category “Other Animals” included dogs, cats, and cattle, while the class “Other Rodents” comprised rats and hamsters. The variable “Age” was categorized following published criteria (Table 4), which were consistent with the criteria used in the studies included in this meta-analysis. The classification of age was dependent on the animal model as animal species mature at different rates. For instance, 2-month-old kittens are in a different maturity stage than 2-month-old gerbils and thus might have different susceptibilities to protozoan infection. When ranges of age were reported, the mean of the range was used in the meta-analysis. The classification “Young” in the “Age” variable for the shedding outcome consisted of newborn and weanling animals, while the classification “Adult” included only adult animals. For the diarrhoea outcome, no adult animals with diarrhoea were reported. Therefore, the “Age” variable for the diarrhoea outcome was composed of “Newborn” and “Weanling.” For the variable “Administration Route,” “Gastric Intubation” included the terms gastric intubation, intragastric route, and stomach tube, while “Other Than Gastric Intubation” included the terms orally, intraesophageal route, and nasogastric. The variable “Detection Method” was only included in the cyst shedding outcome and included three classes: “Hemocytometer,” “Microscopy,” and “Flotation Techniques.” “Microscopy” included indirect fluorescent antibody (IFA), fluorescent microscopy, and Nomarski interference contrast (NIC) microscopy. “Flotation Technique” included parasite concentration methods such as zinc sulfate flotation and sucrose gradient flotation. The variable “Giardia Dose” was a continuous variable. As the values in the “Giardia Dose” variable ranged from 4 to 10⁷ cysts or trophozoites, the data for this variable were log transformed to make the distribution of the data more normal. The mean of the whole dose series (mean log scale = 4.12) was subtracted from every dose value to center the data. This enabled interpretation of the main effect in the presence of an interaction term at a meaningful value (the mean) [10]. For instance, in the multivariable model, the main effect of “Infective Stage” in the model that also includes the interaction “Giardia Dose by Infective Stage” corresponded to a difference between cysts and trophozoites at a mean dose. If the mean of the whole dose series was not subtracted, this comparison would have been the difference between cysts and trophozoites at a log dose of zero (dose of 1).

2.4. Statistical Analyses. In this meta-analysis, the statistical analysis and publication bias assessment were done as described in Adell et al. [11]. Final models were selected based on inclusion of the four key variables “Experimental Species,” “Age,” “Giardia Dose,” and “Infective Stage,” allowing for as many interaction terms as possible and having a combination of variables that generated narrower confidence intervals or more precision. The fit of the model was assessed by the ratio of the generalized chi-square statistic and its degrees of freedom (generalized chi-sq/df), which could not exceed a value of 1, and residual plots. The statistical software JMP 9 (SAS 2010) and SAS 9.3 (SAS 2011) were used to perform all the analyses.

3. Results

3.1. Search Strategy and Selection of Studies. As shown in Figure 1, the initial search identified 1045 potentially relevant studies. Subsequent to reviewing the abstracts, 71 studies were considered for further screening, of which 41 were not eligible since at least one of the exclusion criteria (Table 1) was met. After examination of the full text, 30 studies were included for the cyst shedding outcome while 4 were incorporated for the diarrhoea outcome. The studies included in this meta-analysis were published between 1978 and 2010 and are shown in Table 2. The total number of individuals included in this meta-analysis was 1432 individuals for the shedding outcome and 82 individuals for the diarrhoea outcome.

3.2. Analysis for the Cyst Shedding Outcome. The bivariate analysis showed that the covariates “Passage,” “Experimental Species,” “Age,” “Infective Stage,” and “Giardia Dose” were associated with cyst shedding in faeces (P value ≤ 0.2) (Table 5). These variables were then incorporated in the multivariable analysis which provided one model that best fulfilled the selection criteria. The final multivariable model (Table 6) shows that the variables “Infective Stage” and “Giardia Dose” and the interactions “Giardia Dose by Experimental Species” and “Giardia Dose by Infective Stage” have at least one category with a statistically significant difference (P value ≤ 0.05) from the reference category (Table 6). The variables “Experimental Species,” “Age,” and “Administration Route” were not significant, but they were incorporated into the model to control for confounding and effect modification. “Administration route” and “Age” were identified as a potential confounder (an epidemiologic term specifically describing a variable associated with a change in the coefficient estimate of at least one variable when placed in the model) of the relationship between “Giardia dose” and cyst shedding and “Experimental Species” and cysts shedding, respectively. “Experimental species” was part of the interactions “Giardia Dose by Experimental Species.”

The multivariable model indicated that inoculating cysts into the experimental host had 5.02 times higher odds of cyst shedding than inoculating trophozoites at the mean log dose of 0.0538 (P value ≤ 0.001; CI: 2.63, 9.56). For each 1 unit of change in the log of trophozoite dose administered to the reference experimental host “Gerbil,” the odds of cyst shedding increased 2.67 times (P value < 0.0001; CI: 1.81, 3.94), whilst the odds ratio corresponding to an increase of one in log dose in cysts was 0.57 times that in trophozoites (P value = 0.002; CI: 0.41, 0.81).

The model also indicated that, for each unit of increase in the log dose administered to “Mice,” the odds of cyst shedding increased 4.36 times compared to “Gerbils” (P value: 0.004; CI: 1.59, 11.93). Whereas, for each unit increase in the log dose administered to “Other Rodents,” the odds of having cyst shedding were reduced 0.09 times compared to “Gerbils” (P value: 0.02; CI: 0.01, 0.72). Figure 2(a) shows the range of doses compared to the odds of detecting shedding by experimental species, illustrating how the choice of the experimental animal species does not have an effect until beyond a log scale 6, with mice only having an increase in
**Table 2:** Studies included for *Giardia duodenalis* meta-analysis of diarrhoea (D) and shedding (S) outcomes (chronological order).

| Reference | Year | Outcome | IS² | P² | ST³ | ES³ | A⁴ | IS⁶ | GD⁷ | AR⁸ | DM⁹ |
|-----------|------|---------|-----|----|-----|-----|----|-----|-----|-----|-----|
| [23]      | 1978 | S¹⁰     | 20  | 20 | 20  | 20  | 20 | 20  | 20  | 20  | 20  |
| [24]      | 1979 | S¹⁰     | 47  | 47 | NR²⁰ | 47  | 47 | 47  | 47  | 47  | 47  |
| [17]      | 1982 | S¹⁰     | 11  | 11 | 11  | 11  | 11 | 11  | 11  | 11  | 11  |
| [19]      | 1982 | S¹⁰     | 150 | 150| 150 | 150 | 150| 150 | 150 | 150 | 150 |
| [25]      | 1984 | S¹⁰     | 49  | 49 | NR²⁰ | 49  | 49 | 49  | 49  | 49  | 49  |
| [26]      | 1985 | S¹⁰     | 25  | 25 | 14  | 25  | 25 | 25  | 25  | 25  | 25  |
| [18]      | 1986 | S¹⁰     | 60  | 60 | NR²⁰ | 60  | 60 | 60  | 60  | 60  | 60  |
| [27]      | 1988 | S¹⁰     | 204 | 204| 204 | 204 | 204| 204 | 204 | 204 | 204 |
| [28]      | 1989 | S¹⁰     | 10  | 10 | NR²⁰ | 10  | 10 | 10  | 10  | 10  | 10  |
| [29]      | 1990 | S¹⁰     | 75  | 75 | NR²⁰ | 75  | 75 | 75  | 75  | 75  | 75  |
| [30]      | 1991 | S¹⁰     | 109 | 109| 109 | 109 | 109| 109 | 109 | 109 | 109 |
| [31]      | 1992 | S¹⁰     | 14  | 14 | 14  | 14  | 14 | 14  | 14  | 14  | 14  |
| [32]      | 1993 | S¹⁰     | 62  | 62 | NR²⁰ | 62  | 62 | 62  | 62  | 62  | 62  |
| [33]      | 1994 | S¹⁰     | 4   | 16 | NR²⁰ | 16  | 16 | 16  | 16  | 16  | 16  |
| [34]      | 1996 | S¹⁰     | 10  | 10 | 10  | 10  | 10 | 10  | 10  | 10  | 10  |
| [35]      | 1997 | S¹⁰     | 94  | 94 | NR²⁰ | 94  | 94 | 94  | 94  | 94  | 94  |
| [36]      | 2002 | S¹⁰     | 56  | 56 | NR²⁰ | 56  | 56 | 56  | 56  | 56  | 56  |
| [37]      | 2005 | S¹⁰     | 6   | 6  | NR²⁰ | 6   | 6  | 6   | 6   | 6   | 6   |
| [38]      | 2006 | S¹⁰     | 97  | 97 | 97  | 97  | 97 | 97  | 97  | 97  | 97  |
| [39]      | 2007 | S¹⁰     | 6   | 6  | NR²⁰ | 6   | 6  | 6   | 6   | 6   | 6   |
| [40]      | 2007 | S¹⁰     | 12  | 12 | 12  | 12  | 12 | 12  | 12  | 12  | 12  |
| [41]      | 2008 | S¹⁰     | 60  | 60 | 60  | 60  | 60 | 60  | 60  | 60  | 60  |
| [42]      | 2010 | S¹⁰     | 40  | 40 | NR²⁰ | 40  | 40 | 40  | 40  | 40  | 40  |
| [43]      | 2010 | S¹⁰     | 33  | 33 | 33  | 33  | 33 | 33  | 33  | 33  | 33  |
| [44]      | 1995 | S and D¹² | 30³⁰ | 28³⁰ | 70³⁰ | 28³⁰ | 70³⁰ | 28³⁰ | 70³⁰ | 28³⁰ | 70³⁰ |
| [45]      | 1995 | S and D¹² | 52³⁰ | 30³⁰ | 52³⁰ | 30³⁰ | 52³⁰ | 30³⁰ | 52³⁰ | 30³⁰ | 52³⁰ |
| [46]      | 1997 | S and D¹² | 10¹⁶ | 10¹⁶ | NR²⁰ | 10¹⁶ | 10¹⁶ | 10¹⁶ | 10¹⁶ | 10¹⁶ | 10¹⁶ |
| [47]      | 2010 | S and D¹² | 14¹⁶ | 14¹⁶ | NR²⁰ | 14¹⁶ | 14¹⁶ | 14¹⁶ | 14¹⁶ | 14¹⁶ | 14¹⁶ |

¹Isolate Source, ²Passage, ³Storage Time (weeks), ⁴Experimental Species, ⁵Age, ⁶Infective Stage, ⁷*Giardia* Dose, ⁸Administration Route, ⁹Detection Method, ¹⁰Shedding outcome, ¹¹Information was not reported in study; thus no individual data were included, ¹²Shedding and Diarrhoea outcome, ¹³Number of individuals for shedding outcome, ¹⁴Number of individuals for diarrhoea outcome, ¹⁵Does not apply, ¹⁶Number of individuals for both outcomes.

**Figure 2:** Range of *Giardia* dosages captured in the articles included in this meta-analysis by the odds of the cyst shedding outcome for the categories “Age” and “Experimental Species.” (a) Range of *Giardia* dosages (log scale) by odds of cyst shedding by Experimental Species. (b) Range of *Giardia* dosages (log scale) by odds of cyst shedding by Age.
Table 3: Classification of categorical variables for both *Giardia duodenalis* cyst shedding and diarrhoea outcomes.

| Variable          | Shedding outcome | Diarrhoea outcome |
|-------------------|------------------|-------------------|
|                   | Classification of variables | Number of studies | Number of individuals |
|                   | A                | 3                 | 148                |
|                   | B                | 1                 | 49                 |
|                   | E                | 1                 | 6                  |
|                   | A and E          | 1                 | 14                 |
| Assemblage        |                  |                   |                    |
| Isolate Source    | Humans           | 19                | 1128               |
|                   | Other Than Humans| 4                 | 86                 |
|                   |                  |                    |                    |
|                   | Humans           | 1                 | 28                 |
|                   | Other Than Humans| 3                 | 54                 |
| Passage           | No               | 17                | 755                |
|                   | Yes              | 16                | 667                |
|                   |                  |                    |                    |
|                   | No               | 2                 | 24                 |
|                   | Yes              | 2                 | 58                 |
| Storage Time      | <1 week          | 10                | 420                |
|                   | ≥1 week          | 3                 | 183                |
|                   |                  |                    |                    |
|                   | <1 week          | 1                 | 8                  |
| Experimental Species | Mice            | 7                 | 180                |
|                   | Other Animals    | 8                 | 87                 |
|                   | Other Rodents    | 4                 | 202                |
|                   | Gerbils          | 15                | 963                |
| Age               | Adult            | 12                | 458                |
|                   | Young            | 18                | 828                |
|                   |                  |                    |                    |
|                   | Newborn          | 2                 | 24                 |
|                   | Weanling         | 2                 | 58                 |
| Infective Stage   | Cysts            | 20                | 1021               |
|                   | Trophozoites     | 18                | 411                |
|                   |                  |                    |                    |
|                   | Cysts            | 3                 | 64                 |
|                   | Trophozoites     | 2                 | 18                 |
| Administration Route | Gastric Intubation | 6                  | 233               |
|                   | Other Than Gastric Intubation | 23 | 1153 |
|                   |                  |                    |                    |
|                   | Gastric Intubation | 2              | 50                 |
|                   | Other Than Gastric Intubation | 2 | 24 |
| Detection Method  | Hemocytometer    | 15                | 749                |
|                   | Microscopy       | 3                 | 53                 |
|                   | Flotation Technique | 9          | 523                |
|                   |                  |                    |                    |
|                   |                  |                    |                    |

Categories not listed indicate that there were no animals from those categories in the studies.

Table 4: Criteria used to determine age categories for experimental species (alphabetical order).

| Animal species | Newborn | Criteria used | Weanling | Adult |
|----------------|---------|---------------|----------|-------|
| Cattle         | <21 weeks | 21–64 weeks [50] |          | ≥65 weeks [51] |
| Cats           | <8 weeks  | 8–25 weeks [52]  |          | ≥26 weeks [51] |
| Dogs           | <8 weeks  | 8–29 weeks [53]  |          | ≥30 weeks [51] |
| Gerbils        | <4 weeks  | 4–7 weeks [54]   |          | ≥8 weeks [55,56] |
| Hamsters       | <3 weeks  | 3 weeks [57]     |          | >3 weeks |
| Mice           | <3 weeks  | 3 weeks [58]     |          | >3 weeks [51] |
| Rabbits        | <4 weeks  | 4 weeks–17 weeks [59, 60] |          | ≥18 weeks [51] |
| Rat            | 3 weeks   | 3–7 weeks [61]   |          | ≥8 weeks [51] |

Weanling was merged with the newborn category to create the classification “Young” in the “Age” variable for the cyst shedding outcome.

odds. When “Mice” was removed from the analysis, the odds of shedding cysts were similar for all animals until log 7 after which the odds of shedding were greater in “Gerbils” as opposed to “Other animals.” Figure 2(b) shows the ranges of *Giardia* doses compared to the odds of detecting shedding and how choosing young experimental animals had a higher impact on shedding in response to different doses being investigated compared to adult experimental animal.

The fit of the model was assessed by the ratio value for generalized chi-sq/df value and residual plots. The generalized chi-sq/df for the multivariable model was 0.66, indicating that the model had good fit. The residual plots (not shown), in which the residual values were plotted against the linear predictor, showed that two observations in each model had large residuals (≥15) and were possible outliers from the cluster of observations. Removing the individuals that had large residuals did not change the significance of the other variables of the model; thus, they were kept in the model.

In this meta-analysis, we found evidence of possible publication bias for the cyst shedding outcome. The funnel plot showed a higher number of studies over the mean value (Figure 3). The Egger regression test suggested a significant
Table 5: Bivariate analysis results for risk factors associated with Giardia cyst shedding outcomes.

| Variable                  | P-value | Statistical association between variable and cyst shedding (Yes/No) |
|---------------------------|---------|---------------------------------------------------------------|
| Passage                   | <0.0001 | Yes                                                          |
| Storage Time              | 0.9269  | No                                                           |
| Experimental Species      | <0.0001 | Yes                                                          |
| Age                       | 0.0023  | Yes                                                          |
| Infective Stage           | <0.0001 | Yes                                                          |
| Giardia Dose              | 0.0061  | Yes                                                          |

1 Data sparseness prevented analytic calculations for Isolate Source, Administration Route and Detection Method.
2 Significant level ≤ 0.2.
3 Mantel-Haenzel bivariate analyses analysis taking into account the correlation between studies.
4 GLIMMIX bivariate analyses.

As the data for the diarrhoea outcome were sparse, the analysis done for the shedding outcome could not be performed for the diarrhoea outcome. Therefore, the bivariate analysis was done considering the data as a pool across studies to estimate associations rather than accounting for the correlation between studies as done for the shedding outcome. The bivariate analysis showed that, for the outcome of diarrhoea in exposed animals, the covariates “Isolate Source” and “Experimental Species” were significant (P value ≤ 0.1) (Table 7). Due to the smaller number of studies reporting on the outcome of diarrhoea and variability in the reporting of these potential covariates across studies, it was not possible to create a multivariable model for this outcome. The publication bias for diarrhoea outcome was not assessed due to the low number of studies (4 studies) included in this meta-analysis.

4. Discussion

This meta-analysis evaluated the effects of multiple experimental covariates on cyst shedding or diarrhoea as indicators of G. duodenalis infection. The results obtained in this meta-analysis identified covariates that potentially cause heterogeneity between the outcomes of the dose results experiments and suggest that administering cysts or trophozoites to experimental hosts and the dose administered can all significantly impact the incidence of cyst shedding.

Based on the results of this meta-analysis, it would be appropriate to make the following recommendations for future dose-response experiments on G. duodenalis when assessing infection by means of the presence of cyst shedding in experimentally infected animals to make the studies more comparable and increase likelihood of cyst shedding: (i) use cysts to infect the experimental animals rather than trophozoites, (ii) consider the infective stage used (cysts versus trophozoites) and the Giardia dose administered together, (iii) consider the animal species used as an experimental host and dose together, and (iv) taking this into account, consider using mice as experimental hosts rather than gerbils, rats, hamsters, dogs, cats, and cattle. These parameters should be considered when designing experimental dose-response studies, as once the designs of the dose-response studies are more standardized, they will provide better information and more comparable results for more accurate risk assessments that consider infection as the outcome.

In the case of assessing infection by means of the presence of diarrhoea in experimentally infected animals, more experimental studies in animal models should be conducted, as not enough studies have been reported to obtain estimates of the effect of different experimental parameters on diarrhoea in individual animals. It should be noted that while the diarrhoea outcome is of clinical relevance, the presence of asymptomatic infected individuals is a limitation of using diarrhoea to represent infectious status. Based on the bivariate analyses, it would be appropriate to consider and report the following in future dose-response experiments: (i) the assemblage being inoculated into the experimental host, (ii) original source of the cysts being inoculated, (iii) whether cysts or trophozoites were subject to any passage before inoculation into the experimental host or not, (iv) animal species used as experimental hosts, (v) age of the experimental host, and (vi) the administration route used to inoculate the cysts or trophozoites into the experimental host. These studies will provide better information and more comparable results for risk assessments that consider illness as an outcome.
Table 6: Multivariable generalized linear mixed model showing risk factor associations for *Giardia duodenalis* cyst shedding outcomes.

| Variable                      | Categories          | Odds ratio estimate | 95% Wald confidence limits | P value |
|-------------------------------|---------------------|---------------------|----------------------------|---------|
| **Experimental Species**      |                     |                     |                            |         |
|                               | Gerbils2             |                     |                            |         |
|                               | Mice                 | 0.16                | (0.003, 8.87)              | 0.37    |
|                               | Other Animals        | 0.034               | (<0.001, 5.05)             | 0.18    |
|                               | Other Rodents        | 4.302               | (0.10, 183.05)             | 0.45    |
| **Age**                       |                     |                     |                            |         |
|                               | Young2               | 0.36                | (0.05, 2.43)               | 0.30    |
|                               | Adult               |                     |                            |         |
| **Infective Stage**           |                     |                     |                            |         |
|                               | Trophozoites2        |                     |                            |         |
|                               | Cysts                | 5.026               | (2.63, 9.56)               | <0.001 |
| **Giardia Dose**              | Not applied (continuous variable) | 2.677               | (1.81, 3.94)               | <0.001 |
| **Administration Route**      |                     |                     |                            |         |
|                               | Gastric Intubation1  |                     |                            |         |
|                               | Other Than Gastric Intubation | 10.39              | (0.19, 573.61)             | 0.25    |
|                               |                     |                     |                            |         |
| **Giardia Dose by Experimental Species** |                     |                     |                            |         |
|                               | Mice                 | 4.368               | (1.59, 11.93)              | 0.0042  |
|                               | Other Animals        | 2.123               | (0.12, 37.45)              | 0.61    |
|                               | Other Rodents        | 0.090               | (0.01, 0.72)               | 0.0212  |
| **Giardia Dose by Infective Stage** |                     |                     |                            |         |
|                               | Cysts                | 0.5721              | (0.41, 0.81)               | 0.0022  |

1Pseudo-AIC = 7013.49.
2Generalized chi-square statistic and its degrees of freedom (gener. Chi-sq/df) = 0.66.
3The odds ratio estimate corresponds to Mice versus Gerbils at the mean log dose.
4The odds ratio estimate corresponds to Other Animals versus Gerbils at the mean log dose.
5The odds ratio estimate corresponds to Other Rodents versus Gerbils at the mean log dose.
6The odds ratio estimate corresponds to Cysts versus Trophozoites at the mean log dose.
7The odds ratio estimate corresponds to an increase of one in log dose in Gerbils with Trophozoites.
8The odds ratio estimate corresponds to an increase of one in log dose in Mice is 4.36 times that in Gerbils.
9The odds ratio estimate corresponds to an increase of one in log dose in Other Animals is 2.12 times that in Gerbils.
10The odds ratio estimate corresponds to an increase of one in log dose in Other Rodents is 0.09 times that in Gerbils.
11The odds ratio estimate corresponds to an increase of one in log dose in Cysts is 0.57 times that in Trophozoites.
12Risk factors with statistically significant results.

Table 7: Bivariate analysis results for risk factors associated with diarrhoea outcome after *Giardia* infection.

| Variable1 | P value | Statistical association between variable and presence of diarrhoea (Yes/No)2 |
|-----------|---------|--------------------------------------------------------------------------|
| Isolate Source | <0.00011 | Yes                                                                       |
| Passage    | 0.352   | No                                                                        |
| Experimental Species | 0.073 | Yes                                                                       |
| Age        | 0.355   | No                                                                        |
| Infective Stage | 0.255 | No                                                                        |
| Giardia Dose | 0.255 | No                                                                        |
| Administration Route | 0.325 | No                                                                        |

1Data sparseness prevented analytic calculations for Storage Time.
2Significant level ≤0.2.
3Mantel-Haenszel bivariate analyses not taking into account the correlation between studies.
4GLIMMIX bivariate analyses.

Interestingly, the significant “*Giardia Dose by Experimental Species*” interaction suggests that an increase of one in log of dose administered has a larger impact on cyst shedding in “Mice” than “Gerbils” as experimental hosts, indicating a larger difference between the experimental host-groups as the administered dose increases. But, an increase of one in log of dose administered had a protective impact on cyst shedding in “Other Rodents” compared to “Gerbils” as experimental hosts. Nevertheless, differences in cyst shedding among experimental animal hosts may depend on the dose of *Giardia* administered, and it is advisable to consider these two variables together when designing experimental studies. In addition, it is important to take into consideration that some species might vary in their susceptibilities to different *G. duodenalis* assemblages. For instance, dogs are the only species that have been reported to be susceptible to *G. duodenalis* assemblage C and D, cats to assemblage F, hoofed livestock to assemblage E, and rats to assemblage G [1, 3, 12], while a wide variety of animals, such as cattle, dogs, cats, rodents, and other wild animals and humans are susceptible to *G. duodenalis* assemblage A [1, 3, 12]. Thus, choosing to use mice, other rodents, or other animals as experimental hosts would be appropriate only for some study objectives, such as evaluating the infectivity of the different *G. duodenalis* assemblages by means of cyst shedding.

Pathogen shedding patterns for newborns, weanlings, and adults can be quite different across host species. For instance, it has been reported that weanling calves generally lack a strong specific humoral immune response to *G. duodenalis* infection, while newborns may be protected by the anti-*Giardia* activity of colostrums [15], indicating that weanlings may be more susceptible to *Giardia* infection than newborns or adults. Unfortunately, in this meta-analysis there were not enough studies to analyse newborn and weanling animals separately, thus both categories had to be merged and analyzed as “Young.” Based on the meta-analysis results, young animals were not statistically different from adult animals.
with regard to cyst shedding. This finding is in contrast to numerous studies that reported the prevalence and cyst excetration peaks in young animals [14–16]. However, our results from this meta-analysis are in concordance with the findings reported by Hewlett et al. [17], where young mongrel dogs were not found to be more susceptible than adults, and Woo and Paterson [18] where adult and young dogs and cats did not present with infection after being challenged with 300000 cysts. The data available for this meta-analysis were unfortunately inadequate to provide estimates to determine how the dose and the choice of the age of the experimental species impact the odds of cyst shedding.

The multivariable model suggested that inoculating the experimental host with cysts would increase the likelihood of cyst shedding compared to inoculation with trophozoites. However, the model also indicated that an increase of one log in dose had less of an impact on cysts than it did on trophozoites. Therefore, differences by using cysts versus trophozoites for host infection depend on the Giardia dose used, and it is advisable to consider these two variables together when designing experimental studies. Results of the multivariable model also suggest that when a higher dose of trophozoites is administered to gerbils, the odds of cyst shedding increase. Numerous studies in which different doses of Giardia cysts or trophozoites have been administered to animal models have been published. However, whether the infectious dose may contribute to symptom variability is still unclear [1]. Studies have shown that the infectivity ratio is directly related with the number of cysts inoculated to the experimental host [19, 20]. Nevertheless, all of these reports were individual studies; therefore, there is the possibility that those results were influenced by the experimental design or other factors. Among the strengths of meta-analyses is that it provides an overall estimate of an association or effect based on a number of independent scientific studies and explores the variation in the observed effect across studies, thus obtaining a gain in statistical power to detect effects [21]. The result obtained in the multivariable model indicated that when a higher dose is administered, the odds of having cyst shedding increases.

Information for the variables “Assemblage,” “Isolate Source,” and “Storage Time” was scarce and thus the association between these variables and the shedding outcome could not be assessed. Passage of cysts or trophozoites used to infect experimental hosts was identified as a risk factor for cyst shedding in the bivariate analysis. However, information on this variable was not reported for all studies, which made it challenging to consider it jointly in a multivariable model. Once more experimental studies providing information regarding these variables are published, it would be beneficial to evaluate the association with the shedding outcome and incorporate them in the multivariable model “Experimental Species,” “Age,” “Infective Stage,” and “Giardia Dose” to ascertain the independent effect of these possible covariates on the presence of cyst shedding.

This meta-analysis showed evidence of possible publication bias for the cyst shedding outcome. This finding can be explained by the fact that smaller studies are more likely to be published if they have larger than average effects, which makes them more likely to meet the criterion for statistical significance [22]. The addition of studies by the trim and fill procedure improved the point estimate of the prevalence of cyst shedding of all the studies included in this meta-analysis. However, the adjusted estimate is similar to the original effect and thus indicates that the reported trends may be valid. Caution is advised when interpreting these results because there is evidence of high heterogeneity among the studies, thus precluding a full evaluation of publication. These findings suggest that it is important to publish those studies that have negative or nonsignificant results as well those that have significant or positive results in order to reduce or avoid the bias.

Based on the results of this meta-analysis, it is crucial that more experimental studies in animal models are conducted to assess infectivity of Giardia by means of cysts shedding and diarrhoea. These studies would provide useful data for risk assessments that consider either infection or illness as an outcome.
Acknowledgments

We would like to acknowledge the financial support received from the "Presidente de la República" Chilean scholarship, the Central Coast Long-term Environmental Assessment Network (CCLEAN) (Grant no. 06-076-553) from the California State Water Board to the City of Watsonville, and the National Science Foundation (NSF) Ecology of Infectious Disease Grant Program (Grant no. OCE-1065990). Thanks are due to Fernando O. Mardones and Francisco Puentes for comments and suggestions.

References

[1] H. D. Luján and S. Svärd, *Giardia: A Model Organism*, Springer, New York, NY, USA, 2011.

[2] R. C. Rendtorff, “The experimental transmission of human intestinal protozoan parasites: II. *Giardia lamblia* cysts gwen in capsules,” *American Journal of Epidemiology*, vol. 59, no. 2, pp. 209–222, 1954.

[3] G. Ortega-Pierrès, S. Cacciò, R. Fayer, T. G. Mank, H. V. Smith, and R. C. A. Thomson, *Giardia and Cryptosporidium from Molecules to Disease*, CAB International, London, UK, 2009.

[4] WHO, “Fighting disease, fostering development: the World Health report 1996,” Tech. Rep., World Health Organization, Geneva, Switzerland, 1996.

[5] CDC, “Preliminary Food Net data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009,” Morbidity and Mortality Weekly Report 59, Centers for Disease Control and Prevention, Washington, DC, USA, 2010.

[6] L. Savioli, H. Smith, and A. Thompson, “*Giardia* and *Cryptosporidium* join the ‘Neglected Diseases Initiative’,” *Trends in Parasitology*, vol. 22, no. 5, pp. 203–208, 2006.

[7] T. E. Nash, D. A. Herrington, G. A. Losonsky, and M. M. Levine, “Experimental human infections with *Giardia lamblia*,” *The Journal of Infectious Diseases*, vol. 156, no. 6, pp. 974–984, 1987.

[8] P. A. Rochelle, M. M. Marshall, J. R. Mead et al., “Comparison of in vitro cell culture and a mouse assay for measuring infectivity of *Cryptosporidium parvum*,” *Applied and Environmental Microbiology*, vol. 68, no. 8, pp. 3809–3817, 2002.

[9] R. Fayer and L. Xiao, *Cryptosporidium and Cryptosporidiosis*, Taylor & Francis, Boca Raton, FL, USA, 2nd edition, 2008.

[10] D. G. Kleinbaum, L. L. Kupper, A. Nizam, and K. E. Muller, *Applied Regression Analysis and Other Multivariable Methods*, Thomson Books/Cole, Belmont, Calif, USA, 4th edition, 2008.

[11] A. D. Adell, W. A. Miller, D. J. Harvey, E. Vanwormer, S. Wuertz, and P. A. Conrad, “Individual subject meta-analysis of parameters for *Cryptosporidium parvum* shedding and diarrhoea in animal experimental models,” *Epidemiology & Infection*, vol. 141, no. 8, pp. 1662–1678, 2013.

[12] R. C. A. Thompson and P. T. Monis, “Variation in *Giardia*: implications for taxonomy and epidemiology,” *Advances in Parasitology*, vol. 58, pp. 69–137, 2004.

[13] R. M. O'Handley, H. Ceri, C. Anette, and M. E. Olson, “Passive immunity and serological immune response in dairy calves associated with natural *Giardia duodenalis* infections,” *Veterinary Parasitology*, vol. 113, no. 2, pp. 89–98, 2003.

[14] B. J. Ralston, T. A. McAllister, and M. E. Olson, “Prevalence and infection pattern of naturally acquired giardiasis and cryptosporidiosis in range beef calves and their dams,” *Veterinary Parasitology*, vol. 114, no. 2, pp. 113–122, 2003.

[15] N. Itoh, N. Muraoaka, H. Sacki, M. Aoki, and T. Itagaki, “Prevalence of *Giardia intestinalis* infection in dogs of breeding kennels in Japan,” *The Journal of Veterinary Medical Science*, vol. 67, no. 7, pp. 717–718, 2005.

[16] M. C. Gates and T. J. Nolan, “Endoparasite prevalence and recurrence across different age groups of dogs and cats,” *Veterinary Parasitology*, vol. 166, no. 1–2, pp. 153–158, 2009.

[17] E. L. Hewlett, J. S. Andrews Jr., J. Ruffier, and F. W. Schaefer III, “Experimental infection of mongrel dogs with *Giardia lamblia* cysts and cultured trophozoites,” *The Journal of Infectious Diseases*, vol. 145, no. 1, pp. 89–93, 1982.

[18] P. T. Woo and W. B. Paterson, “*Giardia lamblia* in children in day-care centres in southern Ontario, Canada, and susceptibility of animals to *G. lamblia*,” *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 80, no. 1, pp. 56–59, 1986.

[19] J. C. Craft, “Experimental infection with *Giardia lamblia* in rats,” *The Journal of Infectious Diseases*, vol. 145, no. 4, pp. 495–498, 1982.

[20] F. W. Schaefer III, C. H. Johnson, C. H. Hsu, and E. W. Rice, “Determination of *Giardia lamblia* cyst infective dose for the Mongolian gerbil (*Meriones unguiculatus*),” *Applied and Environmental Microbiology*, vol. 57, no. 8, pp. 2408–2409, 1991.

[21] I. Dohoo, W. Martin, and S. Henrik, *Veterinary Epidemiologic Research*, Ver, Charlottetown, Canada, 2nd edition, 2010.

[22] H. R. Rothstein, A. J. Sutton, and M. Borenstein, *Publication Bias in Meta-Analysis: Prevention, Assessment and Adjustments*, John Wiley & Sons, New York, NY, USA, 2005.

[23] D. R. Grant and P. T. Woo, “Comparative studies of *Giardia* spp. in small mammals in southern Ontario. II. Host specificity and infectivity of stored cysts,” *Canadian Journal of Zoology*, vol. 56, no. 6, pp. 1360–1366, 1978.

[24] V. K. Vinayak, G. L. Sharma, and S. R. Naik, “Experimental *Giardia lamblia* infection in Swiss mice—a preliminary report,” *Indian Journal of Medical Research*, vol. 70, no. 2, pp. 195–198, 1979.

[25] J. Dickerson, G. S. Visvesvara, and G. Healy, “Infectivity of trophozoites and cysts of *Giardia-lamblia* to mongolian gerbils (*Meriones unguiculatus*),” *The Journal of Protozoology*, vol. 31, no. 4, p. A19, 1984.

[26] C. E. Kirkpatrick and G. A. Green IV, *Susceptibility of domestic cats to infections with *Giardia lamblia* cysts and trophozoites from human sources*, *Journal of Clinical Microbiology*, vol. 21, no. 5, pp. 678–680, 1985.

[27] G. S. Visvesvara, J. W. Dickerson, and G. R. Healy, “Variable infectivity of human-derived *Giardia lamblia* cysts for Mongolian gerbils (*Meriones unguiculatus*),” *Journal of Clinical Microbiology*, vol. 26, no. 5, pp. 837–841, 1988.

[28] H. H. Hamadto, S. M. Rashad, A. M. el-Ridiet al., “Effect of diet on experimental giardiasis,” *Journal of the Egyptian Society of Parasitology*, vol. 19, no. 2, pp. 573–581, 1989.

[29] S. A. Romia, A. A. Abou-Zakham, T. Gamil, and E. S. el-Khouly, “Virulence of *Giardia lamblia* isolates to laboratory mice,” *Journal of the Egyptian Society of Parasitology*, vol. 20, no. 2, pp. 633–638, 1990.

[30] J. A. Reynolds, R. C. Thompson, and B. P. Meloni, “In vivo efficacy of albendazole against *Giardia duodenalis* in mice,” *Parasitology Research*, vol. 77, no. 4, pp. 325–328, 1991.
[32] M. R. Torres, M. E. Silva, E. C. Vieira et al., “Intra gastric infection of conventional and germfree mice with Giardia lamblia,” Brazilian Journal of Medical and Biological Research, vol. 25, no. 4, pp. 349–352, 1992.

[33] G. R. Finch, E. K. Black, C. W. Labatiuk, L. Gyurek, and M. Belosevic, “Comparison of Giardia muris and Giardia muris cyst inactivation by ozone,” Applied and Environmental Microbiology, vol. 59, no. II, pp. 3674–3680, 1993.

[34] J. D. Campbell and G. M. Faubert, “Comparative studies on Giardia lamblia encystation in vitro and in vivo,” The Journal of Parasitology, vol. 80, no. 1, pp. 36–44, 1994.

[35] M. E. Olson, D. W. Morck, and H. Ceri, “The efficacy of a Giardia lamblia vaccine in kittens,” Canadian Journal of Veterinary Research, vol. 60, no. 4, pp. 249–256, 1996.

[36] R. Arguello-Garcia and M. G. Ortega-Pierres, “Experimental infection with Giardia duodenalis: analysis of humoral immune response in experimentally infected gerbils (Meriones unguiculatus),” Archives of Medical Research, vol. 28, no. 2, pp. 171–178, 1997.

[37] K. G. Linden, G.-A. Shin, G. Faubert, W. Cairns, and M. D. Sobsey, “UV disinfection of Giardia lamblia cysts in water,” Environmental Science & Technology, vol. 36, no. 11, pp. 2519–2522, 2002.

[38] A. Arévalo, S. Duque, and R. S. Nicholls, “Experimental infection of the gerbil (Meriones unguiculatus) by Colombian isolates of Giardia duodenalis,” Biomédica, vol. 25, no. 3, pp. 305–314, 2005.

[39] T. Geurden, E. Claerebout, L. Dursin et al., “The effect of a fenbendazole treatment on cyst excretion and weight gain in calves experimentally infected with Giardia duodenalis,” Veterinary Parasitology, vol. 169, no. 1-2, pp. 18–23, 2010.

[40] C. E. Story, R. J. Rasby, R. T. Clark, and C. T. Milton, “Age of calf at weaning of spring-calving beef cows and the effect on cow and calf performance and production economics,” Journal of Animal Science, vol. 78, no. 6, pp. 1403–1413, 2000.

[41] S. H. Kilborn, G. Trudel, and H. Uhthoff, “Review of growth plate closure compared with age at sexual maturity and lifespan in laboratory animals,” Contemporary Topics in Laboratory Animal Science, vol. 41, no. 5, pp. 21–26, 2002.

[42] P. R. Donahue, E. A. Hoover, G. A. Beltz et al., “Strong sequence conservation among horizontally transmissible, minimally pathogenic feline leukemia viruses,” Journal of Virology, vol. 62, no. 3, pp. 722–731, 1988.

[43] K. S. Swanson, K. N. Kuzmuk, L. B. Schook, and G. C. Fahey Jr., “Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs,” Journal of Animal Science, vol. 82, no. 6, pp. 1713–1724, 2004.

[44] M. M. Clark, C. A. Spencer, and B. G. Galef Jr., “Reproductive life history correlates of early and late sexual maturation in female Mongolian gerbils (Meriones unguiculatus),” Animal Behaviour, vol. 34, no. 2, pp. 551–560, 1986.

[45] A. F. Ryan, N. K. Woolf, and F. R. Sharp, “Functional ontogeny in the central auditory pathway of the mongolian gerbil,” Experimental Brain Research, vol. 47, no. 3, pp. 428–436, 1982.

[46] M. Müller, “The cochlear place-frequency map of the adult and developing mongolian gerbil,” Hearing Research, vol. 94, no. 1-2, pp. 148–156, 1996.

[47] R. A. Vilchez, C. F. Brayton, C. Wong et al., “Differential ability of two simian virus 40 strains to induce malignancies in weanling hamsters,” Virology, vol. 330, no. 1, pp. 168–177, 2004.

[48] H. Würbel and M. Stauffacher, “Physical condition at weaning affects exploratory behaviour and stereotypy development in laboratory mice,” Behavioural Processes, vol. 43, no. 1, pp. 61–69, 1998.

[49] G. Xiccato, A. Trocino, A. Sartori, and P. I. Queaque, “Effect of weaning diet and weaning age on growth, body composition and caecal fermentation of young rabbits,” Animal Science, vol. 77, no. 1, pp. 101–111, 2003.

[50] C. de Blas and J. Wiseman, Nutrition of the Rabbit, 2nd edition, Cambridge, Mass, USA, 2010.

[51] N. W. Bond and E. L. di Giusto, “Prenatal alcohol consumption and open field behaviour in rats: effects of age at time of testing,” Psychopharmacology, vol. 52, no. 3, pp. 311–312, 1977.