Apicomplexans in Goat: Prevalence of *Neospora caninum*, *Toxoplasma gondii*, *Cryptosporidium* spp., *Eimeria* spp. and Risk Factors in Farms from Ecuador

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Abstract: *Neospora caninum*, *Toxoplasma gondii*, *Cryptosporidium* and *Eimeria* species are parasites of phylum Apicomplexa, which includes several protozoa affecting animals and humans. In Ecuador, the maintenance of goat health is a matter of utmost importance because it affects the economic welfare of the breeders. *N. caninum* and *T. gondii* cause reproductive problems in goats, leading to abortions or weak offspring. Severe diarrhea in kids and delays in growth are due to the *Cryptosporidium* and *Eimeria* species. Moreover, *T. gondii* and *Cryptosporidium* are zoonotic parasites with serious consequences for human health. The aim of this work was to determine, by serological and parasitological tests, the prevalence of these parasites and the risk factors for goat populations in Southern Ecuador. On some farms, the prevalence of *N. caninum* and *T. gondii* reached more than 50%; up to 17% of the kids were positive for *Cryptosporidium* and 90% of the goats were positive for the *Eimeria* species. The analysis of risk factors revealed differences according to the parasite species. Considering the zoonotic significance of these results, control and prevention measures are essential and constitute a warning to veterinarians and governmental institutions.

Keywords: goat; *Neospora caninum*; *Toxoplasma gondii*; *Cryptosporidium* spp.; *Eimeria* spp.; prevalence; risk factors; apicomplexans; Ecuador
1. Introduction

The phylum Apicomplexa constitutes a broad group of microorganisms that includes more than 6000 named species of single-celled, obligate intracellular protozoan organisms that all have a parasitic life cycle [1], distributed among a wide diversity of animals. Many of these parasites have significant clinical and economic relevance since they cause important human and veterinary diseases worldwide [2]. There are apicomplexans that originally developed through an oral-intestinal cycle and are commonly referred to as coccidia, one of the most important groups of animal parasites. Coccidians in sensu stricto (e.g., *Eimeria*, *Cryptosporidium*, *Cystoisospora*) are considered host-specific with a simple one-host life cycle; infection is limited to the intestines and usually to the enterocytes. The life cycle of a coccidian parasite of cats, *Toxoplasma gondii*, probably evolved from a fecal-oral cycle. Some apicomplexans also acquired other forms of transmission, e.g., fecal-oral cycle, carnivorism and transplacentally, adapting to several hosts. The discovery of oocysts in cat feces led to the recognition of several new taxa of economically important *Toxoplasma*-like parasites (e.g., *Hammondia*, *Neospora*, *Sarcocystis*) [3]. Unlike *Eimeria* and *Cryptosporidium*, the life cycles of *Neospora caninum* and *Toxoplasma gondii* involve, in addition to the sexual stage in canids or felids as definitive hosts, tissue stages with multiple intermediate hosts.

Domestic dogs and wild canids are the definitive hosts of *Neospora caninum*, and several species participate in the life cycle as intermediate hosts, including goats [4]. *Neospora caninum* is responsible for reproductive problems in ruminants; the disease is considered a major cause of abortion in cattle worldwide, but embryonic resorption, fetal mummification, fetal maceration, stillbirth and clinically healthy (but infected) kids may also occur [5]. The results of a systematic review of 22,234 goats from 18 countries showed a higher proportion of seropositive animals in the Americas compared to other regions [6]. Ecuador was not included in that review and, until the present study, there was no published data about goat neosporosis.

*Toxoplasma gondii* is a zoonotic parasite that is able to infect probably all warm-blooded animals and humans; one-third of the human population is chronically infected with the parasite [3]. Oocysts of *T. gondii* are formed only in cats, including both domestic and wild felids, but less than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all shed oocysts after ingesting cysts present in tissues (e.g., meat, milk) of the intermediate hosts [7]. Infection with the parasite is an important cause of neonatal mortality in small ruminants, resulting in reproductive and economic losses worldwide. Among small ruminants, goats appear to be more susceptible to clinical toxoplasmosis, and even adult goats have died from acute infection. Congenital toxoplasmosis in small ruminants can kill the fetus [7]. Reproductive failure is one of the important clinical consequences of *T. gondii* infection acquired during primary infection. Kids may be mummified, macerated, aborted, stillborn, or may be born weak or die soon after birth [3]. Sheep and goats are important sources of infection for humans due to their role as intermediate hosts; the consumption of infected milk, raw fresh cheese or meat can facilitate the zoonotic transmission; ingestion of undercooked infected lamb is recognized as risk factor for *T. gondii* infection in Europe and particularly in pregnant women [3]. In Guayaquil, Ecuador, a study of pregnant women receiving prenatal care revealed a prevalence of toxoplasmosis of up to 73%; consumption of undercooked meat was the main risk factor for acquiring the infection [8].

*Cryptosporidium* and *Eimeria* species are transmitted by accidental ingestion of highly resistant and environmentally stable oocysts present in food and water [9,10]. *Cryptosporidium parvum*, *C. hominis*, *C. ubiquitum* and *C. xiaoii* have been identified in goats; the common occurrence of zoonotic *C. parvum* and *C. ubiquitum* in small ruminants has raised public health concerns over cryptosporidiosis [11]. The impact of the disease depends on several factors, e.g., the susceptibility of the animals, the presence of carrier status and the stability of infection. The disease is difficult to control due to the reproductive ability of the parasite, the lack of vaccines, effective drugs and cumbersome diagnostic procedures [12]. In ruminant livestock, including goats, parasitic infection affects growth and production. The disease also exerts a substantial burden on the health and growth of children in develop-
Cryptosporidiosis is characterized by self-limiting diarrhea in immune competent individuals, but in immune compromised patients, the disease could be fatal. The disease caused by the genus *Eimeria* is commonly known as coccidiosis. *Eimeria* species are found worldwide under different habitats and husbandry conditions and may be considered a highly relevant factor in intestinal disease of young animals [10]. Several species of *Eimeria* parasite cattle, small and wild ruminants, but there is no cross infection between species due to the strict host specificity [13]. Many factors influence the pathologic and clinical outcome of coccidiosis: mixed infections with several species, a short life cycle associated with an extraordinary reproductive capacity of the parasite, inflammatory immune responses, concomitant infections with other pathogens, management practices and stress [10]. Depending on the type of management, coccidiosis might affect 100% of goat kids of 4–10 weeks, disturbing animal health and the productivity of farms. *Eimeria*-infected goat kids show intestinal symptoms, from non-hemorrhagic to severe hemorrhagic diarrhea, dehydration, weight loss and growth delay, particularly during the weaning period [14].

In the countries where the majority of goats are found, most farmers are of lower socioeconomic status; locally adapted goat breeds are raised for milk and meat, and in dry and drought-prone areas, goat milk is often the only protein source in children’s diets [15]. In Southern Ecuador, the province of Loja is characterized by a pronounced dry season and limited natural resources [16]. In Zapotillo Canton, most of the livestock activity corresponds to goat breeding; 28,000 goats are distributed in parishes named Limones (50.22%), Cazaderos (15.94%), Zapotillo (14.21%), Garza Real (10.97%), Paletillas (4.47%) and Bolaspamba (4.17%) [17]. Farms are characterized by extensive management with animals of low genetic quality; the main activity is the production and commercialization of milk. Goat breeding is managed under the traditional extensive system; occasionally, animals receive corn husks, corn cobs, carob beans and crop residues as a supplementary diet [18].

Due to the impact of these parasitic diseases on goat health, the economic performance of farms and the human population that depends on goat breeding, the present work aimed to estimate the seroprevalence of *Neospora caninum* and *Toxoplasma gondii* by ELISA tests and the prevalence of *Cryptosporidium* spp. and *Eimeria* spp. by parasitological techniques; to evaluate a rapid immunochromatographic test for the diagnosis of *Cryptosporidium* and to identify the risk factors associated with infections in goat herds from Ecuador.

### 2. Materials and Methods

#### 2.1. Study Area

The research was carried out on 24 goat farms from Zapotillo, Garza Real, Cazaderos, Limones and Paletillas, parishes of Zapotillo Canton, Loja Province, Southern Ecuador (Figure 1). The farms were selected by the RAND function in Excel® (v. 17.0, Microsoft, Redmond, WA, USA) using the database provided by the Agricultural Public Information System (SIPA, by the acronym in Spanish) of the Ministry of Agriculture and Livestock of Ecuador [19]. Local breeders used to release the goats every day to forage freely in the forest, and at sunset, the animals returned to the dirt-floored stables located near the owner’s houses [20].

#### 2.2. Sampling

A total of 388 blood samples for serological diagnosis of *N. caninum* and *T. gondii* were collected from the jugular vein using the BD-Vacutainer® (Becton Dickinson, Mississauga, ON, Canada) system in tubes without anticoagulant. Sixteen goats aged over six months were sampled on each farm. After centrifugation of the blood samples, sera were collected and stored in aliquots at −20 °C until use.
A total of 391 fecal samples were collected directly from the rectum using clean plastic bags. A minimum of sixteen samples were taken per farm: eight samples from kids under six months old and eight samples from adults over six months old [21]. Some farmers accepted the sampling of blood but did not accept the sampling of feces from their animals, so the minimum sample number was completed on other farms. The fecal samples to be used in the detection of *Eimeria* spp. and *Cryptosporidium* spp. by flotation and McMaster techniques were preserved in 10% formalin. Fecal samples to be used in the *Cryptosporidium* rapid test were stored without any additives at $-20 \, ^\circ\mathrm{C}$ until use, according to the manufacturer’s instructions. A FAMACHA card was used to assess the level of anemia in goats by measuring the color of the ocular mucosa [22].

Data concerning each animal were registered on a separate sheet; each of the blood and fecal samples were numbered and registered along with the animal’s age (verified by dentition), sex, FAMACHA score and any comments about the animal.

2.3. Questionnaire

An organized questionnaire was designed to collect general information about the farms (area, pastures, soil irrigation, number, sex and age of goats), management procedures (presence of other species, drinking and feeding troughs, cleaning of pens, mineral/vitamins or food supplementation, goat milk and meat consumption), disposal of abortion products) and animals (sanitary management, abortions, symptoms such as diarrhea, emaciation or anorexia). All of the questionnaires were completed face-to-face by the same interviewer. Participation was voluntary.

2.4. Serological Assays

Detection of Antibodies Anti-*Neospora caninum* and *Toxoplasma gondii* by ELISA

Serological analysis of goat sera was performed using indirect ELISA kits for the detection of goat IgG antibodies against *N. caninum* and *T. gondii* (IDEXX® NeosporaAb and IDEXX® ToxotestAb, Westbrook, ME, USA, respectively). A total of 388 goat blood sera were tested in *N. caninum* ELISA and 368 in *T. gondii* ELISA, excluding females which had not reached the minimum reproductive age of six months and males.
To detect antibodies against *N. caninum*, 90 µL of sample diluent and 10 µL of undiluted sample sera, positive and negative control sera were dispensed into appropriate wells of the plates precoated with antigen. Plates were incubated at 37 °C for 60 min. Following incubation, three washes with 300 µL per well were carried out using an ELISA plate washer (BioTek® 50 TS8, Friedrichshall, Germany). After washing, 100 µL of conjugate was dispensed in each well and incubated at 37 °C for 60 min. The wells were washed again as described, and 100 µL of TMB-substrate was added; plates were incubated at 18–26 °C for 15 min. The reaction was stopped with 100 µL per well of stopping solution. Plates were read at 450 nm in an ELISA reader (BioTEk® ELx800, Friedrichshall, Germany) using Gen5® software (BioTek®, Friedrichshall, Germany). Positive and negative control sera were tested in duplicate according to the commercial test.

For detection of antibodies against *T. gondii*, 100 µL of pre-diluted (1:400) sera samples and controls were dispensed into wells of the plate precoated with antigen. The rest of the procedure was conducted as described above for *N. caninum*.

**Interpretation of results was made using the formula:**

\[
\text{Sample/Positive (S/P) %} = 100 \times \left( \frac{\text{(sample OD value at 450 nm} - \text{X OD negative control)}}{\text{(X OD positive control} - \text{X OD negative control})} \right)
\]

A sample was considered negative to *N. caninum* if S/P % < 30; suspect, 30 ≤ S/P % < 40 or positive, if S/P % ≥ 40.

A sample was considered negative to *T. gondii* if S/P % < 20; suspect 20 ≤ S/P % < 30; weak positive 30 ≤ S/P % < 100 or positive if S/P % ≥ 100.

**2.5. Parasitological Tests**

**2.5.1. Flotation Technique**

The sucrose flotation method [23] was used to detect oocysts of the genus *Eimeria*. Briefly, two grams of feces were mixed thoroughly with 30 mL of Sheater’s flotation solution (density 1.27) in a cup and strained through two layers of gauze; the filtered solution was placed into a test tube to completely fill it, ensuring that a slightly convex meniscus was formed. A 22 × 22 mm coverslip was placed on the tube, left for 10 min, removed and placed on a glass slide. The entire coverslip was examined under a light microscope at 400x magnification.

**2.5.2. McMaster Technique for *Eimeria* Oocyst Counting**

The filtered solution of feces obtained in the flotation technique was also used to be transferred into the McMaster chamber for oocyst counting [23]. The quantitative results were recorded as oocysts per gram of fecal (opg) value. The level of infection was estimated as a Bangoura and Daugschies score [24]: 0, no oocysts detectable; 1, ≤100 opg; 2, ≤1000 opg; 3, ≤10,000 opg; 4, ≥10,000 opg.

**2.5.3. Identification of *Cryptosporidium* spp. in Fecal Samples**

The formalin-ethyl acetate concentration method (FEA) modified by Weber et al. [25] was used to confirm the presence of *Cryptosporidium* in fecal samples. Briefly, four milliliters of the formalin-fixed feces suspension, 6 mL of 10% formalin and 3 mL of ethyl acetate were placed into a 15-mL centrifuge tube, shook thoroughly and centrifuged at 500× g for 5 min. The supernatant was decanted and sediment was suspended in 5 mL of deionized water, layered carefully over 5 mL of saturated sodium chloride and centrifuged at 500× g for 10 min. Approximately 3 mL of the top layer was removed and discarded. The remainder of the top layer and approximately 0.5 mL of the medium layer containing saturated sodium chloride were removed and washed in 13 mL of deionized water by centrifugation at 500× g for 10 min. The smears were made from the sediment and stained by the modified Ziehl-Neelsen (ZN) procedure for *Cryptosporidium* [26]. Slides were dried out at room temperature and fixed with methanol for 3 min, washed with distilled water and covered with carbofuchsin for 10 min; after washing with tap water, acid alcohol was
placed on the slides for 30 s, washed again and counterstained with 1% methylene blue for 1 min. After washing, the slides were allowed to dry at room temperature and checked under the microscope at 1000× magnification. A fecal sample of a previously tested-positive animal was used as a control.

2.6. Detection of Cryptosporidium spp. in Fecal Samples by a Rapid Test

The RIDA® QUICK Cryptosporidium test (R-Biopharm AG, Darmstadt, Germany) was used to evaluate the feasibility of Cryptosporidium diagnosis in the field. Fecal pools from each farm from two groups of age (animals under six months and over six months old) were used. Fifty milligrams of pooled, thawed out feces, manually homogenized, were suspended in buffer (included in the kit) and allowed to settle down for three minutes until a clear supernatant was formed; 200 µL of supernatant was placed in the inlet hole of the cassette. Reading was carried out five minutes later; the control line and the line corresponding to a positive result were verified and recorded. The results were compared with those obtained by ZN.

2.7. Data Analyses

The SPSS® IBM® package for Windows (version 24; SPSS, Chicago, IL, USA) was used for statistical analyses. The results of serological and parasitological tests were included in a data sheet (Excel® v. 17.0, Microsoft, Redmond, WA, USA) along with the variables obtained through the questionnaire (Table A1). The SI function was applied to assign a value of one if a positive number different from zero was found in the cell of the previous column. For descriptive analyses, parish and farm level prevalence were calculated. In addition, non-parametric tests were carried out because seroprevalence of N. caninum and T. gondii and prevalence of Cryptosporidium spp. and Eimeria spp. did not follow a normal distribution (Kolmogorov–Smirnov Z = 0.205, p < 0.05; 0.529, p < 0.05; 0.523, p < 0.05, respectively).

To establish differences between variables of two groups, e.g., animals <6 months and >6 months, a non-parametric Mann–Whitney U test was used. A non-parametric ANOVA (Kruskal–Wallis) was used to establish differences between variables with more than two groups, e.g., parishes. To assess the significance of each variable related to positivity, a Kruskal–Wallis test was used [21] with a 95% confidence interval and a p-value < 0.05 [27].

Nominal variables were coded numerically, assigning a number to each category with the mean as the cut-off point. A binary value was assigned to the dichotomous variables (1 if the answer was yes, and 0 otherwise). Regarding numerical variables, central tendencies and dispersion statistics were calculated. Variables with more than two categories were transformed into dichotomous variables using a reference category. In the cases of N. caninum and T. gondii, the serum which resulted as “suspect” or “weak positive” by ELISA was considered as positive due to the impossibility of obtaining duplicated samples. Consequently, the dichotomy of variables was maintained in the statistical analysis.

To determine risk factors, 49 variables (Table S1) related to management and characteristics of the farm and animals were paired to the prevalence of each of the parasites. Some variables were excluded at the time of analysis (those related to the farm or dichotomous variables with a frequency value higher than 95%).

A selection of variables was carried out to accomplish the multivariable model. For categorical variables, a Chi-square test was used, selecting variables with p < 0.2. For numerical variables, a Kolmogorov–Smirnov test was used, selecting variables with a p-value < 0.05. The variables selected by univariable analysis were subjected to a multinomial logistic regression model [28]. The logistic regression analysis was carried out using non-automatic variable selection and stepwise entry to determine the significance (p < 0.05) of each variable. Results were presented as odds ratios (OR) adjusted to a 95% confidence interval (CI).
3. Results

3.1. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii*

The overall seroprevalence of *N. caninum* was 12.11% (47/388) in 10 of the 24 farms. Seroprevalence values per farm ranged from no seropositive animals in 14/24 of the farms to 81.25% of seropositive animals in a single farm from Cazaderos parish (Table A1).

The seroprevalence value of *T. gondii* was 18.20%; values ranged between 11.11 (Cazaderos parish) and 23.03% (Zapotillo parish) (Table 1) ($p > 0.05$) in 20/24 farms. The maximum value of *T. gondii* seroprevalence per farm was 61.11% (Table A1). No significant differences were found between sex of the animals or breed phenotype ($p > 0.05$).

**Table 1. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* and prevalence of *Cryptosporidium* spp. and *Eimeria* spp. in goats from parishes of Zapotillo Canton, Ecuador.**

| Parish       | Seroprevalence | Prevalence          |
|--------------|----------------|---------------------|
|              | *N. caninum*¹ ² | *T. gondii*¹ ²      |
|              |                 | *Cryptosporidium*² | *Eimeria*² |
| Garza Real   | 17.78 (16/90)   | 16.67 (15/90)        |
| Zapotillo    | 4.79 (8/167)    | 23.03 (38/165)       |
| Limones      | 2.78 (1/36)     | 14.81 (4/27)         |
| Pailetillas  | 0 (0/14)        | 14.29 (2/14)         |
| Cazaderos    | 27.16 (22/81)   | 11.11 (8/72)         |
| Total        | 12.11 (47/388)  | 18.20 (67/368)       |

¹ Values presented as percentages. ² Inside the brackets, number of positive goats/total of goats sampled in the parish.

3.2. Prevalence of *Cryptosporidium* spp. and *Eimeria* spp.

*Cryptosporidium* spp. oocysts were detected in 10.49% (41/391) of the samples and in 91% (20/22) of the farms. The highest prevalences of *Cryptosporidium* spp. were found in Garza Real (12.96%) and Zapotillo (10.70%); prevalence values per farm ranged from 3.85% to 35.29% (Table 1). Oocysts were observed in 16.88% (27/160) of kids <6 months and 6.06% (14/231) of goats >6 months.

*Eimeria* oocysts were observed in 89.51% (350/391) of the samples; 85.71% (198/231) in adult animals and 95% (152/160) in kids; within the farms, the prevalence ranged from 58.82% to 100%. The highest prevalence was observed in Pailetillas (100%, 17/17) and Garza Real (94.43%, 102/108) and the lowest in Limones (80%; 12/15) (Table 1). Significant differences were found between parishes, farms ($p < 0.05$), adults (>6 months) and kids (<6 months) (Z = −2.943, $p < 0.05$), with a higher frequency of positivity in kids ($p < 0.01$). There were no significant differences between males and females or animal phenotype ($p > 0.05$).

The average oocyst count was 33.54 ± 100.9. Of the 391 feces samples, 41 showed no oocysts and were classified as score 0; 325 samples were classified as score 1 ($\leq 100$ opg), 24 samples as score 2 ($\leq 1000$ opg) and 1 sample with 1012 opg as score 3 ($\leq 10,000$ opg).

3.3. Prevalence of *Cryptosporidium* spp. Using the Rapid Test

The rapid tests were performed on pools of feces from two age groups on each farm. Of the 22 farms tested, seven were positive to the rapid test (3 < 6 months; 4 > 6 months). No correlation was found between the results obtained by Ziehl-Nielsen and those obtained by the rapid test in samples from kids ($r = −0.043$, $p > 0.05$). There was a weak but non-significant correlation between the prevalence of *Cryptosporidium* spp. in adults and the rapid test ($r = 0.327$, $p > 0.05$). Significant differences were found between results obtained by both Ziehl-Nielsen and rapid tests on the same farms ($p < 0.05$).

3.4. Mixed Infections

Two hundred and forty-four animals were infected with two of the four apicomplexans; 30 animals were infected with three parasites and six with all four species (Table 2).
Regarding infections with two species, the highest prevalence value was 38.62% (151 animals from 22 farms with both Cryptosporidium spp. and Eimeria spp.); the lowest was 2.72% with T. gondii and N. caninum (10 animals from 6 farms). T. gondii, Cryptosporidium spp. and Eimeria spp. were observed in 4.62% of the animals from 10 farms; lower prevalences were observed with N. caninum, T. gondii and Eimeria spp. (1.90%) or N. caninum, T. gondii and Cryptosporidium spp. (1.63%) (Table 2).

Table 2. Prevalence of mixed infections with Neospora caninum, Toxoplasma gondii, Cryptosporidium spp. and Eimeria spp. in sampled goats from canton Zapotillo, Ecuador.

| Mixed Infections | Prevalence (%) | Animals/Farms |
|------------------|----------------|---------------|
| Cryptosporidium spp. and Eimeria spp. | 38.62 | 151/22 |
| T. gondii and Eimeria spp. | 7.88 | 29/13 |
| N. caninum and Eimeria spp. | 5.93 | 23/8 |
| T. gondii and Cryptosporidium spp. | 4.35 | 16/9 |
| N. caninum and Cryptosporidium spp. | 3.87 | 15/8 |
| T. gondii and N. caninum | 2.72 | 10/6 |
| T. gondii, Cryptosporidium spp. and Eimeria spp. | 4.62 | 17/10 |
| N. caninum, T. gondii and Eimeria spp. | 1.90 | 7/5 |
| N. caninum, T. gondii and Cryptosporidium spp. | 1.63 | 6/5 |
| N. caninum, T. gondii, Cryptosporidium spp. and Eimeria spp. | 1.63 | 6/5 |

4. Analysis of Risk Factors

4.1. Risk Factors for Neospora caninum and Toxoplasma gondii Infections

The frequencies of 49 categorical variables are presented in Table S1. Univariable analysis revealed 26 variables associated with farm management and six related to animals (e.g., body condition, ectoparasites, abortions, milk production) as risk factors for N. caninum seropositivity (Table A1 by a Chi-square test). Ten numerical variables (\(p < 0.05\)) were selected by a Kolmogorov–Smirnov analysis: animal age, farm area, pasture area, number of animals (males, females and kids), mortality/\(\text{year}\) (adults, kids) and milk production.

Twenty-two variables were considered as risk factors for T. gondii seropositivity by univariable analysis: 19 related to farm characteristics and management and three to animals (age of animals with diarrhea and duration of diarrhea, infection with louses) (Table A1); from these, 16 variables are common for both N. caninum/T. gondii, three of them related to goats (age of animals with diarrhea and duration, infection with louses). By a Kolmogorov–Smirnov analysis, nine variables (\(p < 0.05\)) were selected: animal age (months), farm area, pasture area, number of animals (males, females), abortions, total area of the farm, partial area, mortality/\(\text{year}\) (adults, kids) and mortality of adult goats.

The multivariable logistic regression analysis suggested four variables as risk factors for N. caninum infection (vitamin supplementation, age of diarrhea, frequency of deworming and known pasture area) (Table 3); for T. gondii infections: presence of artiodactils, presence of domestic fowl in the farms and administration of sulfas (Table 3).

Table 3. Risk factors associated with Neospora caninum and Toxoplasma gondii in goats from Ecuador obtained by multivariable logistic regression analysis.

| Variable | Category | N. caninum | | T. gondii | |
|----------|----------|------------|-----------------|-----------|------------|-----------------|-----------|
| Supplementation with vitamins | Yes | | | | 0.001 | 40.96 | 2.4–700.6 | | | | |
| Supplementation with vitamins | No | | | | | | | | | | | | |
| Age of diarrhoea | >30 days | | | | | | | | | | | | |
| Frequency of deworming | Regular | | | | | | | | | | | | |
| Frequency of deworming | Irregular | | | | | | | | | | | | |
Table 3. Cont.

| Variable Category | N. caninum |   | T. gondii |   |
|-------------------|-----------|---|-----------|---|
| Known pasture area | Yes | 0.021 | 25.16 | 1.6–390.4 | * | * | * | * |
| Presence of artiodactyls | Yes | – | – | 0.001 | 2.943 | 1.5–5.7 | * | * |
| Domestic fowl | Yes | – | – | 0.009 | 2.428 | 1.3–4.7 | * | * |

a p value-Wald test; b Odds ratio; c Confidence interval; * Reference category.

4.2. Risk Factors for Cryptosporidium spp. and Eimeria spp.

Ten variables were revealed as risk factors for Cryptosporidium by univariable analysis: age group, body condition, dairy farm, irrigation, presence of cats, facilities, ventilation, infection with louses, abortion products in pastures and technical visits; animal age (p < 0.05) was selected by Kolmogorov–Smirnov analysis. Nineteen categorical variables were selected for Eimeria infections: age group, FAMACHA results, presence of cattle, dairy farms, irrigation, presence of cats, frequency of cleaning, water troughs, plastic troughs, infection with louses, food supplementation, tire troughs, administration of sulfas, abortion products in pastures, milk production values, abortions (%), type of pasture, fertilization and dogs’ consumption of abortion products (Table A3); animal age and pasture area (p < 0.05) were selected by Kolmogorov–Smirnov analysis to be further considered as risk factors.

The multivariable logistic regression analysis suggested two variables as risk factors for Cryptosporidium spp. infection (age group and body condition) (Table 4); for infections with Eimeria spp., percentage of abortions/total females, type of pastures and presence of cattle (Table 4).

Table 4. Risk factors associated with Cryptosporidium spp. and Eimeria spp. in goats from Ecuador, obtained by multivariable logistic regression analysis.

| Variable Category | Cryptosporidium spp. | Eimeria spp. |
|-------------------|----------------------|--------------|
|                   | p a | OR b | CI c 95% | p a | OR b | CI c 95% |
| Age group <6 m    | 0.001 | 3.06 | 1.5–6.1 | 0.013 | 2.87 | 1.3–6.6 |
| Age group >6 m    | * | * | * | * | * | * |
| Body condition Regular | 0.016 | 2.48 | 1.2–5.2 | – | – | – |
| Body condition Good | * | * | * | – | – | – |
| % of abortions/total females <10% | – | – | – | <0.0001 | 6.98 | 2.9–16.6 |
| % of abortions/total females >10% | – | – | – | * | * | * |
| Type of pasture Cultivated | – | – | – | 0.039 | 8.76 | 1.1–68.4 |
| Type of pasture Natural | – | – | – | * | * | * |
| Presence of cattle No | – | – | – | 0.002 | 3.98 | 1.7–9.4 |
| Presence of cattle Yes | – | – | – | * | * | * |

a p value-Wald test; b Odds ratio; c Confidence interval; * Reference category.

4.3. Risk Factors for Mixed Infection

Three variables related to general and sanitary management of the farms were found as risk factors for mixed infections: intensive management, frequency of cleaning >3 months and administration of sulfas (Table 5). No statistical differences were found between the other variables and infections with three or four parasites.
Table 5. Risk factors of mixed infections with *Neospora caninum*, *Toxoplasma gondii*, *Cryptosporidium* spp. and *Eimeria* spp. in sampled goats from Zapotillo Canton, Ecuador.

| Mixed Infections                        | Variable               | Category     | p  a | OR  b | CI  c 95% |
|-----------------------------------------|------------------------|--------------|------|-------|-----------|
| *N. caninum* and *Eimeria* spp.         | Management             | Intensive    | 0.005| 3.546 | 0.12–0.68 |
|                                         |                        | Extensive    | *    | *     | *         |
| *N. caninum* and *Cryptosporidium* spp.| Management             | Intensive    | 0.013| 3.891 | 0.09–0.75 |
|                                         |                        | Extensive    | *    | *     | *         |
| *T. gondii* and *Cryptosporidium* spp.  | Administration of sulfas | Yes          | 0.003| 4.713 | 1.69–13.13 |
|                                         |                        | No           | *    | *     | *         |
| *T. gondii* and *Eimeria* spp.          | Frequency of cleaning  | >3 months    | 0.015| 5.912 | 1.20–5.54 |
|                                         |                        | <3 months    | *    | *     | *         |
| *Cryptosporidium* spp. and *Eimeria* spp.| Management             | Intensive    | 0.042| 1.845 | 0.30–0.98 |
|                                         |                        | Extensive    | *    | *     | *         |
|                                         | Frequency of cleaning  | <3 months    | 0.037| 1.901 | 0.29–0.96 |
|                                         |                        | >3 months    | *    | *     | *         |
| *T. gondii*. *Cryptosporidium* spp. and *Eimeria* spp. | Administration of sulfas | Yes          | 0.010| 4.375 | 1.42–13.51 |
|                                         |                        | No           | *    | *     | *         |

a *p* value-Wald test; b Odds ratio; c Confidence interval; * Reference category.

5. Discussion

This work reported the prevalence of four apicomplexan parasites that affect goats in farms in Ecuador; the risk factors associated with the infections are also presented. They all have negative consequences on the farms’ economy, either due to the effect on weight gain, secondary infections and mortality because of the intestinal damage by *Cryptosporidium* and *Eimeria* species or to abortions and weak offspring by *Neospora caninum* and *Toxoplasma gondii* [5,7,10,12]. Furthermore, as zoonotic parasites (some species of *Cryptosporidium*, *T. gondii* and probably *N. caninum* [29]), they pose a serious concern to human populations.

It is well-known that bovine neosporosis is an abortigenic parasitic disease that causes severe economic losses on cattle farms. However, the economic and epizootiologic importance of *Neospora caninum* infection in goats remains in doubt as the pathogenesis of caprine neosporosis is mostly unknown [30]. Until the present work, there have been no reports of neosporosis in goats from Ecuador; 21–23% of seroprevalence values were shown in cattle herds from Santo Domingo, Cañar and Azuay provinces [31,32]. The seroprevalence value of *N. caninum* found in this work (12.11%) was higher than those reported in Italy (5.7%), China (8.55%) or in Romania (2.3%) [33–35]. In Brazil, 26.11% of seroprevalence was described [36]. It is remarkable that two farms in the present study showed more than 50% seropositivity to *N. caninum*.

In the present study, vitamin supplementation and regular deworming were determined as risk factors. A common management practice is to place supplements (dried feeds, vitamins or minerals) in a container and replace it when depleted; deworming products or dried supplements are also kept in a warehouse where dogs and domestic fowl used to sleep. In most goat farms in Ecuador, no aseptic measures are taken in drug administration by farmers; bottles containing medicines are found in pens or bins; thus, oocysts present in dried feces can be carried by the wind, insects or birds and can be attached to them [37,38]. Diarrhea episodes 30 days after birth and a known grazing area were also found as risk factors. In cattle, co-infection of *N. caninum* with other pathologies, such as herpesvirus BHV-1 and stress-causing immunosuppression, has been reported [33,39]. The animals that are not allowed to graze freely or with a grazing area delimited by physical barriers are more exposed to oocyst ingestion [40].

Due to the high frequency of domestic dogs (95.8%), the odds ratio could not be determined. Canids are the final hosts of *N. caninum*, so their presence would be expected to be a risk factor for goats [30,41]. Thus, this variable should not be ruled out as a risk factor for *N. caninum*.
There are a large number of papers regarding *Toxoplasma gondii* seroprevalence in goats using techniques such as ELISA, immunofluorescence or microagglutination tests; despite several common antigens, there are no cross-reactions between *N. caninum* and *T. gondii* [40]. The findings of *T. gondii* seroprevalence around the world in the period 2009–2020 have been excellently summarized by Dubey [3]. Seroprevalence values ranged between 1.5 and 73.8% worldwide (including those obtained in the present work), but it is difficult to compare the findings due to differences, e.g., in diagnostic methods, farm and animal management and the presence of domestic and wild felids. A real zoonotic risk was found in two goat farms sampled in this investigation, with seroprevalence values of 61.11% and 50%. Recently, a systematic review of the literature and a meta-analysis regarding the seroprevalence of *T. gondii* in 55,317 goats from 75 reports did not include data from Ecuador, which emphasizes the importance of the present study [42].

The only known definitive hosts for *Toxoplasma gondii* are members of the family Felidae. Domestic cats are the major source of environmental contamination with oocysts; however, it is a difficult matter to prevent cats from living or loitering on farms that have grazing stock [7]; farmers also have cats at farms to avoid rats. In our study, the presence of domestic fowl and artiodactyls and administration of sulfa drugs were found to be risk factors. The presence of domestic fowl is a risk factor due to their consumption habits and the possibility of being vectors of oocysts from cat feces, similar to what occurs with the dispersion of *Neospora* oocysts from dog feces. It has been shown that domestic chickens can host *T. gondii* [43] and so contribute to the cycle in a predator–prey relationship or by consumption of raw chicken meat provided to the cat by the farmer. A similar reason for poor sanitary management through treatment administration as a risk factor in *N. caninum* applies to the administration of sulfa drugs for *T. gondii* [37]; Sporulated oocysts survive for long periods under moderate environmental conditions and can be spread by the erosion of topsoil and mechanically by flies, cockroaches, dung beetles and earthworms [7]. Otherwise, sulfonamides, such as sulfadiazine, were found to have important inhibitory effects on *T. gondii* [44]; however, it should not be discarded that an induced resistance to sulfas was previously described [45]. In Ecuador, the resistance to drugs is a concern for veterinarians; veterinary drugs are sold over-the-counter without supervision.

The presence of artiodactyls as a risk factor for *Toxoplasma gondii* is an interesting topic of discussion, and no information is available from Ecuador. Aston et al. [46] evaluated *T. gondii* seropositivity in artiodactyls hunted in Peru and found antibodies in peccaries *Pecari tajacu*, *Taijazu pecari*, brocket deer species *Mazama americana*, *Mazama gouazoubira* and *Tapirus terrestris* (lowland tapir), which probably served as reservoirs in their natural habits. Farmers used to consume some wild species as bush meat and eventually feed the domestic cats with the remains, thus maintaining the cycle on the farms. The main wild ruminant species, hunted and destined for human consumption in Spain (red deer and roe deer), showed high antibody titers against *T. gondii*, suggesting that undercooked game meat should not be consumed by humans or be used to feed cats [47]. Further studies are necessary to elucidate the *T. gondii* infection levels in meat and derived products from wild artiodactyl species in Ecuador.

Cryptosporidiosis is one of the major health problems in neonatal goats kids because it causes retarded growth, decreased feed efficiency, delayed maturity, loss of fertility and mortality levels could reach up to 40% [48]. The presence of *Cryptosporidium* species is also a risk for the human population because there are species with zoonotic potential [15]. Several works reported higher prevalence values in kids compared to adult goats [49–52]. The prevalence of *Cryptosporidium* spp. found in this work ranged between 5.26 to 20% (there were no positive samples in 2/22 farms), indicating that there is a widespread dissemination of *Cryptosporidium* in goat farms from Zapotillo Canton.

In the present study, a rapid commercial assay was used to detect *Cryptosporidium* without requiring analysis in a laboratory. A low coincidence was found between the ZN technique and rapid tests in both kids’ and adults’ samples. The RIDA®QUICK Cryptosporidium test identifies an antigen of *Cryptosporidium parvum*; however, there may be
other species of Cryptosporidium parasitizing goats. In Spain, a report showed that 71.4% of the samples were positive for species other than C. parvum: C. xiaoi and C. ubiquitum [47]. In the United Kingdom, C. hominis has been reported in goats with a potential risk for people in contact with infected animals [53]. Other reasons may justify the low concordance between both assays. According to the manufacturer, a negative result of the immunochromatographic test does not exclude a possible infection with C. parvum, which may be due to a low amount of antigen in the sample. In Spain, two commercial immunochromatographic methods were reported to have significant differences in sensitivity and specificity [54]. Other studies suggested that immunochromatographic assays used for detection of Cryptosporidium in feces were specific to a certain antigen and did not provide information on other species or genotypes involved [55,56], for which molecular techniques should be performed [15].

Two variables were found as risk factors for Cryptosporidium spp.: age group (kids under 6 months old) and a “regular” body condition of the animals. The main source of infection for newborn kids is contact with feces from adult animals with subclinical infections [57], especially in females during the peripartum period, when oocysts are most frequently shed. A “fair” body condition (<3) was determined as a risk factor for Cryptosporidium: in one month, the difference in weight gain of an infected animal compared to a healthy one can be up to 1.48 kg [11].

In this study, each farm in each parish was positive for Eimeria spp. as expected and reported worldwide. The genus Eimeria is distributed globally, and the infection rates can reach more than 90% in some areas. The prevalence is largely dependent on farm management because oocysts require moist and dark environments to sporulate, which is a typical situation in goat pens in Ecuador. Under conditions that promote Eimeria development, the accompanying clinical symptoms include low feed conversion rate, weight loss and lethargy [58].

In this research, most of the samples were ranked at score 1 (less than 100 opg), meaning that goats were in subclinical infection. The economic impact of coccidiosis in small ruminants is not well documented; in tropical regions with extensive breeding zones and animals of local origin, subclinical coccidiosis with poor weight gain as a symptom is probably not of major importance compared to other infections [14]. The coprological examinations should be quantitative and allow the diagnosis of the most pathogenic species of Eimeria found in feces. Such identification is done on the basis of the morphology of the sporulated oocysts and their structures and estimation of sporulation periods [10]. However, despite the general relationship between clinical coccidiosis and high excretion of oocysts, a cut off for oocysts values is difficult to calculate because of the differences in pathogenicity of each of the nine Eimeria species identified in goats [14]. Certainly, the identification of the Eimeria species would have offered invaluable information about Eimeria goat species from Ecuador, but this goal could not be achieved. It is important to highlight that coccidial infection causes an imbalance in the intestinal microbiota, which not only leads to a decrease in food intake and reduced absorption but also increases the susceptibility of the organism to secondary infections and impairs the intestinal mucosal barrier function [59]. Control measures for coccidiosis should be taken at farm level no matter which species are involved.

Four variables were identified as risk factors for Eimeria in parishes from canton Zapotillo: kids younger than 6 months, farms with abortion problems, the presence of cultivated pastures and the absence of cattle. Coccidiosis is a disease that primarily affects young animals in all species, and goats are not an exception [10,60,61]. Abortions were identified as a risk factor because the concomitant immunity developed by ruminants against Eimeria spp. is impaired and the peak of excretion occurs in the peripartum period [62]. A large number of Eimeria oocysts released in feces by immune compromised animals act as a source of contamination for other members of the herd. The use of cultivated grassland is related to a medium-high level of breeding “intensification” which is considered a risk factor for the presence of Eimeria spp. [14]. In Ecuadorian goat farms,
corn husks and cobs from domestic crops are also placed on the ground or in the feeders as a supplement, increasing the risk of infection.

The sampling was carried out during the transition from the dry to the rainy season and some tree and shrub species were releasing their seeds to the ground. Goats living in arid or semi-arid areas with seasonal rainfall encounter abundant leaf supplies in the rainy season, but they are scarce in the dry season, being forced to graze at ground level in search of small plants and tree seeds [63]. If cattle are not present, there is no competition for food, and goats are more prone to ingesting oocysts that are at ground level [40].

In this study, mixed infections were also considered. Few studies show co-infections by protozoan in goats, despite the consequences they may have in the productivity of farms. For example, co-infections with *Neospora caninum* and *Toxoplasma gondii* have been scarcely reported. Hassig et al. [64] detected reproductive problems in a flock of sheep that had been evaluated independently for *T. gondii* or *N. caninum* and subsequently turned out to be mixed infections. In buffaloes, infection with *N. caninum* is associated with abortion and the presence of retained fetal membranes. Infection with *T. gondii* has been associated with an increase in days open; in the case of co-infections with both pathogens, the effects on the animals could be related to both abortion and embryonic death [65]. In this study, mixed infections for both species were found in 2.72% of the goats, but when assessing infections with three or four of the parasites, it was found that both *T. gondii* and *N. caninum* co-infected a greater number of animals (7.88%). In the state of Paraná, Brazil, the frequency of antibodies to both species was 3.0% (19/629) from 32 goat farms [66].

A small number of reports included estimations of co-infections by *Cryptosporidium* spp. and *Eimeria* spp. in goats. The prevalence of both parasites is age-related, but simultaneous infection seems not to be a very common scenario [67]. However, in this study, 150 samples/391 had mixed infection with *Eimeria* and *Cryptosporidium*, which represents an important number to consider in control measures.

To the best of the authors’ knowledge, there are no reports of co-infections with the four apicomplexans in goats as carried out in this study. All the risk factors are related to sanitation and farm management. The four apicomplexans have an oral route of infection: restricted grazing/bedding areas, poor cleaning of pens and keeping treatment bottles in inadequate places increase the exposure of animals to infective oocysts.

Finally, it would be interesting to assess whether host competition (host resources, host immune responses or interference between species) exists in these mixed infections. This is a topic that remains open for further research.

6. Conclusions

In this work, the prevalence of four parasites of phylum Apicomplexa, *Neospora caninum*, *Toxoplasma gondii*, *Cryptosporidium* spp. and *Eimeria* spp., and the risk factors associated with infection in goat farms in Ecuador are reported. The results showed high prevalence values in some farms, and in the case of zoonotic *T. gondii*, the results revealed a severe risk for human consumers of milk and undercooked meat from goats. Low association values were found between the Ziehl Neelsen assay and the rapid test used to detect *Cryptosporidium* in both kids’ and adults’ samples. Risk factors for these parasitic diseases include several variables related to farm management and individual characteristics such as age of animals, presence of diarrhea or abortions. These results suggest the importance of control measures, epidemiological surveillance and education of farmers with the participation of governmental entities and private veterinarians to reduce the economic losses in goat farms and to maintain public health.

Based on the authors’ knowledge, this is the first report of *Neospora caninum*, *Toxoplasma gondii* and *Cryptosporidium* spp. and mixed infections in goat farms from Ecuador.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani12172224/s1. Table S1. Frequency of categorical variables recorded in coprological and serological databases.
Author Contributions: Conceptualization, L.G. and C.R.-V.; methodology, L.G. and C.R.-V.; validation, L.G. and C.R.-V.; formal analysis, K.C., L.G. and C.R.-V.; investigation, K.C., L.G. and C.R.-V.; resources, K.C. and L.G.; data curation, K.C., L.G. and C.R.-V.; funding acquisition, L.G.; writing—original draft preparation, C.R.-V.; writing—review and editing, C.R.-V.; supervision, L.G. and C.R.-V. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This article does not contain any studies with human subjects. All the animal experimental procedures (sampling of blood and feces from goats) were conducted in accordance to the precepts established by the Code of Bioethics and Biosafety of the Ministry of Science and Technology of the Republic of Venezuela, since the Republic of Ecuador and the institutions that participated in this work (Universidad Nacional Experimental Francisco de Miranda, Venezuela and Universidad Técnica Particular de Loja, Ecuador) have no established ethics committees and/or regulations in this regard. The samples were taken from the goats by experienced veterinarians under the rules established in Guide for the Care and Use of Agricultural Animals in Research and Teaching (https://www.aaalac.org/pub/?id=E900BD6-CCCF-AB13-89B6-DA98A4B52218, accessed on 14 June 2022). The study was performed among domestic animals from rural areas only and no damage to endangered species of wild flora and fauna occurred during the sampling, according to the Convention on Biological Diversity.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data is available upon request to corresponding authors.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Prevalence per farm of Apicomplexans parasites in Zapotillo Canton, Ecuador.

| Farm (n) | Feces (n) | Sera (n) | N. caninum | T. gondii | Cryptosporidium spp. | Eimeria spp. |
|----------|-----------|----------|------------|-----------|---------------------|--------------|
| 1        | 25        | 11       | 9.09       | 9.09      | 16.00               | 96.00        |
| 2        | 17        | 19       | 10.53      | 10.52     | 5.88                | 58.82        |
| 3        | 26        | 18       | 22.22      | 61.11     | 3.85                | 100.00       |
| 4        | 25        | 12       | 0.00       | 0.00      | 8.00                | 72.00        |
| 5        | 17        | 16       | 81.25      | 0.00      | 5.88                | 100.00       |
| 6        | 15        | 15       | 64.29      | 13.33     | 20.00               | 100.00       |
| 7        | 15        | 18       | 33.33      | 27.77     | 12.33               | 86.67        |
| 8        | 18        | 16       | 43.75      | 18.75     | 16.67               | 94.44        |
| 9        | 19        | 16       | 12.50      | 0.00      | 5.26                | 89.47        |
| 10       | 15        | 16       | 0.00       | 12.50     | 0.00                | 80.00        |
Table A1. Cont.

| Farm (n) | Feces (n) | Sera (n) | N. caninum | T. gondii | Cryptosporidium spp. | Eimeria spp. |
|----------|-----------|----------|------------|-----------|----------------------|--------------|
| 11       | 16        | 15       | 0.00       | 26.66     | 12.50                | 100.00       |
| 12       | 15        | 14       | 0.00       | 14.29     | 13.33                | 100.00       |
| 13       | 17        | 14       | 0.00       | 14.29     | 5.88                 | 100.00       |
| 14       | 14        | 13       | 0.00       | 0.00      | 14.29                | 92.86        |
| 15       | 15        | 15       | 0.00       | 12.50     | 6.67                 | 93.33        |
| 16       | 15        | 15       | 0.00       | 33.33     | 6.67                 | 100.00       |
| 17       | 18        | 17       | 0.00       | 50.00     | 16.67                | 88.89        |
| 18       | 17        | 17       | 0.00       | 29.41     | 35.29                | 94.12        |
| 19       | 18        | 18       | 0.00       | 6.25      | 5.56                 | 83.33        |
| 20       | 18        | 19       | 10.53      | 10.53     | 16.67                | 66.67        |
| 21       | 18        | 19       | 0.00       | 10.53     | 5.56                 | 94.44        |
| 22       | 18        | 19       | 0.00       | 16.66     | 0.00                 | 83.33        |
| 23       | N/A       | 20       | 5.00       | 18.18     | N/A                  | N/A          |
| 24       | N/A       | 18       | 0.00       | 25.00     | N/A                  | N/A          |

Table A2. Univariable analysis of variables associated with Neospora caninum and Toxoplasma gondii infections in goats from Zapotillo Canton, Ecuador.

| Variable                  | Category | Neospora caninum | Toxoplasma gondii |
|---------------------------|----------|------------------|-------------------|
|                           |          | Pos (n)          | Neg (n)           | Chi² | p-Value | Pos (n) | Neg (n) | Chi² | p-Value |
| Main activity             | Agriculture | 1                | 74               | 10.15 | 0.001   | –       | –       | –    | –       |
|                           | Cattle raising | 30               | 140              | Ref   | –       | –       | –       | –    | –       |
| Total area                | <15 ha | 17                | 201              | 8.70  | 0.003   | 19      | 133     | 4.75 | 0.029   |
|                           | >15 ha | 32                | 157              | Ref   | 46      | 170     | Ref     | –    | –       |
| Grazing area              | <15 ha | 15                | 184              | 8.03  | 0.005   | 21      | 150     | 6.36 | 0.012   |
|                           | >15 ha | 43                | 225              | Ref   | 44      | 153     | Ref     | –    | –       |
| Grazing area              | Known | 4                 | 116              | 12.58 | <0.001  | –       | –       | –    | –       |
|                           | Unknown | 20                | 97               | Ref   | –       | –       | –       | –    | –       |
| Management                | Intensive | 27                | 244              | 3.9   | 0.048   | –       | –       | –    | –       |
|                           | Extensive | 1                 | 74               | Ref   | –       | –       | –       | –    | –       |
| Body condition            | Good     | 45                | 278              | 5.99  | 0.014   | –       | –       | –    | –       |
|                           | Regular  | 2                 | 63               | Ref   | –       | –       | –       | –    | –       |
| Dairy farm                | Yes      | –                 | –                | –     | –       | –       | –       | –    | –       |
|                           | No       | –                 | –                | –     | 12      | 25      | 6.17    | 0.013  |
| Dual purpose              | Yes      | –                 | –                | –     | –       | –       | –       | –    | –       |
|                           | No       | –                 | –                | –     | 52      | 268     | 3.37    | 0.066  |
| % of kids/total           | <22%    | –                 | –                | –     | –       | –       | –       | –    | –       |
|                           | >22%    | –                 | –                | –     | 40      | 137     | 5.71    | 0.017  |
| Irrigation                | Yes      | –                 | –                | –     | –       | –       | –       | –    | –       |
|                           | No       | –                 | –                | –     | 25      | 166     | Ref     | –    | –       |
| Presence of artiodactyls  | Yes      | 3                 | 113              | 14.11 | <0.001  | 23      | 82      | 1.82 | 0.178   |
|                           | No       | 44                | 228              | Ref   | 45      | 240     | Ref     | –    | –       |
| Presence of cattle        | Yes      | 1                 | 89               | 13.32 | <0.001  | –       | –       | –    | –       |
|                           | No       | 46                | 252              | Ref   | –       | –       | –       | –    | –       |
| Presence of cats          | Yes      | 13                | 152              | 4.84  | 0.028   | –       | –       | –    | –       |
|                           | No       | 34                | 189              | Ref   | –       | –       | –       | –    | –       |
| Domestic fowl             | Yes      | 43                | 209              | 16.55 | <0.001  | 48      | 197     | 1.88 | 0.171   |
|                           | No       | 4                 | 132              | Ref   | 17      | 106     | Ref     | –    | –       |
| Facilities                | Yes      | 46                | 271              | 9.35  | 0.002   | –       | –       | –    | –       |
|                           | No       | 1                 | 70               | Ref   | –       | –       | –       | –    | –       |
| Ventilation               | Yes      | 47                | 308              | 4.97  | 0.026   | –       | –       | –    | –       |
|                           | No       | 0                 | 33               | Ref   | –       | –       | –       | –    | –       |
Table A2. Cont.

| Variable                        | Category            | Neospora caninum |  |  |  | Toxoplasma gondii |  |  |  |
|---------------------------------|---------------------|------------------|---|---|---|-------------------|---|---|---|
|                                 |                     | Pos (n) | Neg (n) | Chi² | p-Value | Pos (n) | Neg (n) | Chi² | p-Value |
| Frequency of cleaning           | <3 months           | 36       | 156     | 15.73 | <0.001 | –        | –       | –    | –       |
|                                 | >3 months           | 11       | 185     | Ref   | –       | –        | –       | –    | –       |
| Water troughs                   | Yes                 | 18       | 223     | 12.88 | <0.001 | 47       | 192     | 1.88 | 0.170   |
|                                 | No                  | 29       | 118     | Ref   | –       | 18       | 111     | Ref  | –       |
| Type of water                   | Puddled             | 14       | 111     | 25.10 | <0.001 | –        | –       | –    | –       |
|                                 | Running             | 33       | 230     | Ref   | –       | –        | –       | –    | –       |
| Origin of water                 | Natural             | 29       | 97      | 20.83 | <0.001 | 14       | 101     | 3.47 | 0.063   |
|                                 | Catchment           | 18       | 244     | Ref   | 51       | 202     | Ref     | –    | –       |
| Type of water troughs           | Plastic             | 4        | 88      | 6.83  | 0.009   | –        | –       | –    | –       |
|                                 | Cement              | 43       | 253     | Ref   | –       | –        | –       | –    | –       |
| Food supplementation            | Yes                 | 10       | 113     | 2.68  | 0.101   | 27       | 94      | 2.68 | 0.102   |
|                                 | No                  | 37       | 228     | Ref   | –       | 38       | 209     | Ref  | –       |
| Supplementation with minerals/vitamins | Yes | 44       | 165     | 34.00 | <0.001 | –        | –       | –    | –       |
|                                 | No                  | 3        | 176     | Ref   | –       | –        | –       | –    | –       |
| Troughs                         | Yes                 | –        | –       | –     | –       | 35       | 107     | 7.76 | 0.005   |
|                                 | No                  | –        | –       | –     | –       | 30       | 196     | Ref  | –       |
| Tire troughs                    | Yes                 | 0        | 30      | 4.48  | 0.034   | 9        | 21      | 3.42 | 0.064   |
|                                 | No                  | 47       | 311     | Ref   | –       | 56       | 282     | Ref  | –       |
| Cement troughs                  | Yes                 | 34       | 91      | 39.43 | <0.001 | –        | –       | –    | –       |
|                                 | No                  | 13       | 250     | Ref   | –       | –        | –       | –    | –       |
| Type of pasture                 | Natural             | –        | –       | –     | –       | 54       | 285     | 8.89 | 0.003   |
|                                 | Cultivated          | –        | –       | –     | –       | 11       | 18      | Ref  | –       |
| Pasture fertilization           | Yes                 | 11       | 36      | 6.40  | 0.011   | 16       | 31      | 9.94 | 0.002   |
|                                 | No                  | 36       | 305     | Ref   | –       | 49       | 272     | Ref  | –       |
| Ectoparasites                   | Yes                 | 11       | 170     | 11.61 | 0.001   | –        | –       | –    | –       |
|                                 | No                  | 36       | 171     | Ref   | –       | –        | –       | –    | –       |
| Infection with louses           | Yes                 | 0        | 82      | 14.33 | <0.001 | 24       | 58      | 9.77 | 0.002   |
|                                 | No                  | 47       | 259     | Ref   | 41      | 245     | Ref     | –    | –       |
| Application of sulfas           | Yes                 | –        | –       | –     | –       | 30       | 81      | 9.58 | 0.002   |
|                                 | No                  | –        | –       | –     | –       | 35       | 222     | Ref  | –       |
| Deworming                       | Yes                 | 23       | 118     | 3.67  | 0.055   | 32       | 109     | 3.98 | 0.046   |
|                                 | No                  | 24       | 223     | Ref   | –       | 33       | 194     | Ref  | –       |
| Frequency of deworming          | Regular             | 42       | 106     | 59.46 | <0.001 | –        | –       | –    | –       |
|                                 | Irregular           | 5        | 235     | Ref   | –       | –        | –       | –    | –       |
| Duration of diarrhoea           | <3 days             | 25       | 239     | 5.42  | 0.020   | 49       | 199     | 2.30 | 0.130   |
|                                 | >3 days             | 22       | 102     | Ref   | –       | 16       | 104     | Ref  | –       |
| Age of diarrhoea                | <30 days            | 24       | 294     | 34.52 | <0.001 | 60       | 254     | 3.073| 0.080   |
|                                 | >30 days            | 23       | 47      | Ref   | –       | 5        | 49      | Ref  | –       |
| Vaccination                     | Yes                 | –        | –       | –     | –       | 13       | 35      | 3.37 | 0.066   |
|                                 | No                  | –        | –       | –     | –       | 52       | 268     | Ref  | –       |
| Milk production                 | <770 mL             | 7        | 184     | 25.22 | <0.001 | –        | –       | –    | –       |
|                                 | >770 mL             | 40       | 157     | Ref   | –       | –        | –       | –    | –       |
| Abortions                       | Yes                 | 40       | 319     | 4.26  | 0.039   | –        | –       | –    | –       |
|                                 | No                  | 7        | 22      | Ref   | –       | –        | –       | –    | –       |
| Dogs' consumption of abortion products | Yes | 0        | 43      | 6.67  | 0.010   | –        | –       | –    | –       |
|                                 | No                  | 47       | 298     | Ref   | –       | –        | –       | –    | –       |
| Abortions kept in the pasture   | Yes                 | 9        | 214     | 32.14 | <0.001 | 42       | 161     | 2.85 | 0.091   |
|                                 | No                  | 98       | 127     | Ref   | 23       | 142     | Ref     | –    | –       |
| Eimeria spp. prevalence         | Yes                 | 26       | 181     | 2.47  | 0.116   | –        | –       | –    | –       |
|                                 | No                  | 38       | 187     | 11.47 | 0.001   | –        | –       | –    | –       |
| Technical Visits                | Yes                 | 9        | 154     | Ref   | –       | –        | –       | –    | –       |
|                                 | No                  | –        | –       | –     | –       | –        | –       | –    | –       |

Pos positive; Neg negative; Ref reference category.
Table A3. Univariable analysis of variables associated with Cryptosporidium spp./Eimeria spp. infections in goats from Zapotillo Canton, Ecuador.

| Variable                  | Category          | Cryptosporidium spp. | Eimeria spp. |
|---------------------------|-------------------|----------------------|--------------|
|                           | Pos (n) | Neg (n) | Chi² | p-value | Pos (n) | Neg (n) | Chi² | p-Value |
| Age group                 | <6 m     | 27      | 133  | 11.78   | 0.001   | 152    | 8    | 8.68    | 0.003   |
|                           | >6 m     | 14      | 217  | Ref     |          | 198    | 33   | Ref     |          |
| Body condition            | Good     | 28      | 297  | 7.18    | 0.007   | –      | –    | –       | –       |
|                           | Regular  | 13      | 53   | Ref     |          | –      | –    | –       | –       |
| Famacha                   | No dose  | –       | –    | –       | –       | 81     | 14   | 2.42    | 0.120   |
|                           | Dose     | –       | –    | Ref     |          | 269    | 27   | Ref     |          |
| Dairy farm                | Yes      | 2       | 41   | 1.75    | 0.186   | 36     | 7    | 1.73    | 0.189   |
|                           | No       | 39      | 309  | Ref     |          | 314    | 34   | Ref     |          |
| Irrigation                | Yes      | 7       | 93   | 1.75    | 0.187   | 93     | 7    | 1.74    | 0.187   |
|                           | No       | 34      | 257  | Ref     |          | 257    | 34   | Ref     |          |
| Presence of cattle        | Yes      | –       | –    | –       | –       | 87     | 14   | 1.65    | 0.199   |
|                           | No       | –       | –    | Ref     |          | 263    | 27   | Ref     |          |
| Presence of cats          | Yes      | 10      | 137  | 3.41    | 0.065   | 126    | 21   | 3.62    | 0.057   |
|                           | No       | 31      | 213  | Ref     |          | 224    | 20   | Ref     |          |
| Facilities                | Yes      | 40      | 318  | 2.13    | 0.144   | –      | –    | –       | –       |
|                           | No       | 1       | 32   | Ref     |          | –      | –    | –       | –       |
| Ventilation               | Yes      | 40      | 318  | 2.84    | 0.092   | –      | –    | –       | –       |
|                           | No       | 1       | 32   | Ref     |          | –      | –    | –       | –       |
| Frequency of cleaning     | <3 m     | –       | –    | –       | –       | 152    | 8    | 8.68    | 0.003   |
|                           | >3 m     | –       | –    | Ref     |          | 198    | 33   | Ref     |          |
| Water troughs             | Yes      | –       | –    | –       | –       | 246    | 34   | 2.89    | 0.089   |
|                           | No       | –       | –    | Ref     |          | 104    | 7    | Ref     |          |
| Type of water troughs     | Plastic  | –       | –    | –       | –       | 70     | 18   | 12.02   | 0.001   |
|                           | Cement   | –       | –    | –       | –       | 280    | 23   | Ref     |          |
| Food supplementation      | Yes      | –       | –    | –       | –       | 117    | 9    | 2.21    | 0.137   |
|                           | No       | –       | –    | –       | –       | 233    | 32   | Ref     |          |
| Tire troughs              | Yes      | –       | –    | –       | –       | 31     | 0    | 3.94    | 0.047   |
|                           | No       | –       | –    | –       | –       | 319    | 41   | Ref     |          |
| Type of pasture           | Natural  | –       | –    | –       | –       | 300    | 40   | 4.54    | 0.033   |
|                           | Cultivated| –       | –    | –       | –       | 50     | 1    | Ref     |          |
| Pasture fertilization     | Yes      | –       | –    | –       | –       | 63     | 3    | 2.96    | 0.084   |
|                           | No       | –       | –    | –       | –       | 287    | 38   | Ref     |          |
| Infection with louses     | Yes      | 13      | 71   | 2.84    | 0.092   | 80     | 4    | 3.73    | 0.053   |
|                           | No       | 28      | 279  | Ref     |          | 270    | 37   | Ref     |          |
| Application of sulfas     | Yes      | –       | –    | –       | –       | 128    | 8    | 4.71    | 0.030   |
|                           | No       | –       | –    | –       | –       | 222    | 33   | Ref     |          |
| Milk production           | <770 mL  | –       | –    | –       | –       | 191    | 30   | 5.17    | 0.023   |
|                           | >770 mL  | –       | –    | –       | –       | 159    | 11   | Ref     |          |
| % of abortions/total females | <10% | –       | –    | –       | –       | 316    | 25   | 28.27   | <0.001  |
|                           | >10%     | –       | –    | –       | –       | 34     | 16   | Ref     |          |
| Dogs consumption of abortion products | Yes | –       | –    | –       | –       | 45     | 1    | 3.84    | 0.050   |
|                           | No       | –       | –    | –       | –       | 305    | 40   | Ref     |          |
| Abortions kept in the pasture | Yes | 17      | 189  | 2.31    | 0.128   | 177    | 29   | 5.98    | 0.014   |
|                           | No       | 24      | 161  | Ref     |          | 173    | 12   | Ref     |          |
| Technical Visits          | Yes      | 18      | 206  | 3.35    | 0.067   | –      | –    | –       | –       |
|                           | No       | 23      | 144  | Ref     |          | –      | –    | –       | –       |

Pos positive; Neg negative; Ref reference category.
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