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Global Cryptosporidium Loads from Livestock Manure

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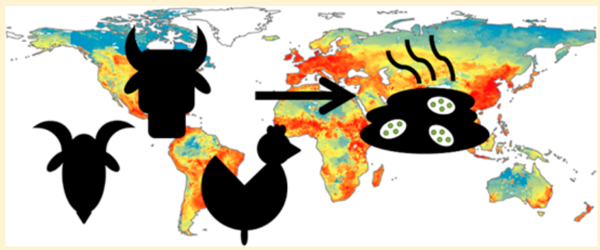
Supporting Information

ABSTRACT: Understanding the environmental pathways of *Cryptosporidium* is essential for effective management of human and animal cryptosporidiosis. In this paper we aim to quantify livestock *Cryptosporidium* spp. loads to land on a global scale using spatially explicit process-based modeling, and to explore the effect of manure storage and treatment on oocyst loads using scenario analysis. Our model GloWPa-Crypto L1 calculates a total global *Cryptosporidium* spp. load from livestock manure of 3.2×10^{23} oocysts per year. Cattle, especially calves, are the largest contributors, followed by chickens and pigs. Spatial differences are linked to animal spatial distributions. North America, Europe, and Oceania together account for nearly a quarter of the total oocyst load, meaning that the developing world accounts for the largest share. GloWPa-Crypto L1 is most sensitive to oocyst excretion rates, due to large variation reported in literature. We compared the current situation to four alternative management scenarios. We find that although manure storage halves oocyst loads, manure treatment, especially of cattle manure and particularly at elevated temperatures, has a larger load reduction potential than manure storage (up to 4.6 log units). Regions with high reduction potential include India, Bangladesh, western Europe, China, several countries in Africa, and New Zealand.



1. INTRODUCTION

Cryptosporidium is a protozoan parasite that is found all over the world and can cause diarrhea in humans and animals.[1−3] Every year around 1.3 million people die of the consequences of diarrhea.[4] *Cryptosporidium* has been identified as one of the six major pathogens responsible for diarrhea in children younger than 5 years in Africa and Asia.[5] *Cryptosporidium* is transmitted via the fecal-oral route. Direct contact with feces of humans or animals is a possible transmission route,[6] but often transmission occurs via an environmental route, such as drinking of or recreation in contaminated water[7] and eating of fresh produce that has been fertilized with manure or irrigated with contaminated water.[8] Livestock, particularly cattle, is considered to be an important reservoir of zoonotic *Cryptosporidium*.[1,2] Cryptosporidiosis has been reported in many important livestock species, including cattle, buffaloes, pigs, goats, sheep, horses, camels, donkeys, chickens, and ducks, and it has been reported on all continents except Antarctica.[1,2] Infection of livestock with *Cryptosporidium* can result in decreased production and loss of income for the livestock sector.[9,10] Not all *Cryptosporidium* species in livestock are of public health significance; the majority of human infections are caused by *C. parvum* and *C. hominis.*

Studying the environmental transmission routes of *Cryptosporidium* is important for assessing and mitigating disease risk, yet observational data of *Cryptosporidium* in the environment are very scarce, as sampling is costly and time-consuming. Especially quantitative information about diffuse sources, such as loads from livestock, is rare. This is where process-based modeling can help; process knowledge can provide insights relevant for managing human and animal cryptosporidiosis when observational data are scarce. Process knowledge on *Cryptosporidium* from livestock manure includes the following. Oocysts, the robust survival stage of the pathogen, are excreted in manure of infected animals, depending on cryptosporidiosis prevalence and the excretion rate (concentration) of oocysts in manure that can vary between livestock species and age groups.[11−13] Manure is either deposited directly on fields during grazing, stored or treated before it is applied to land, or it is used for other purposes such as burning for fuel. Oocysts can decay during storage and treatment (such as anaerobic digestion) of manure.[14−16] These are all factors that can be incorporated in a process-based model of *Cryptosporidium* loads from livestock manure. From the land, oocysts can spread further through the environment, they can, for example, be transported with runoff to surface waters.

In this paper we aim to quantify livestock *Cryptosporidium* spp. loads to land on a global scale using spatially explicit process-based modeling, and to explore the effect of manure storage and treatment on oocyst loads using scenario analysis. We present the model GloWPa-Crypto L1, a global spatially

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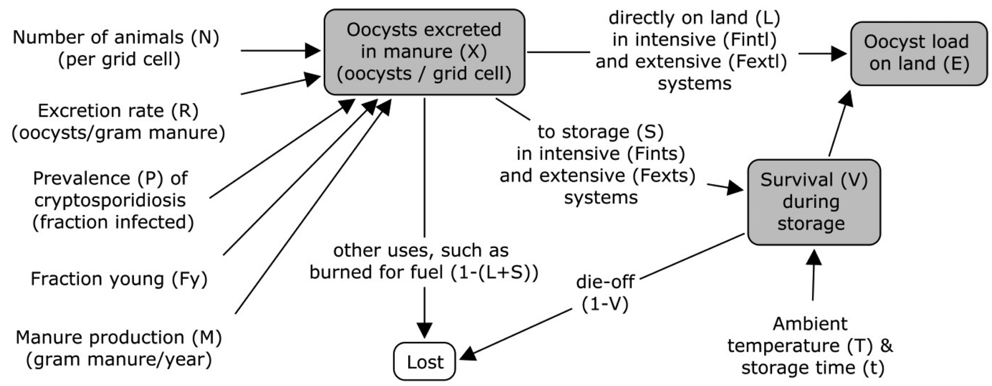

Figure 1. Schematic overview of major model components of GloWPa-Crypto L1. Gray boxes represent the major subcomponents that are calculated, the white box represents the oocysts that are lost, and the text without boxes are model inputs. The oocyst load to land (E) is the main model output.

explicit model at a 0.5° × 0.5° grid that calculates total annual oocyst loads to land from manure of 11 livestock species.

2. MATERIALS AND METHODS

GloWPa-Crypto L1 calculates livestock oocyst loads to land worldwide. We define "load" as the annual total number of oocysts from livestock manure that end up on land. This accounts for all oocysts in manure that is dropped directly on land, and the proportion of oocysts that survive in manure that is stored before it is applied to land. GloWPa-Crypto L1 is programmed in R,[17] it operates on a 0.5° × 0.5° grid and on an annual time step. The model is considered representative for approximately the year 2005. Figure 1 shows a schematic overview of major model components. The main input data for the model include: number of animals (from the Gridded Livestock of the World v2.0[18]), cryptosporidiosis prevalence and oocyst excretion rates (from an extensive literature review[19]), manure production and storage estimates (from IPCC and USEPA[20,21]), intensive and extensive farming systems (from the IMAGE model[22]) and ambient temperature (from the WATCH forcing data[23]). GloWPa-Crypto L1 is partly based on an exploratory global model of *Cryptosporidium* loads to surface water.[24] In addition to the earlier work, GloWPa-Crypto L1 includes manure storage and oocyst decay, and prevalence and oocyst excretion are now based on an extensive literature review. GloWPa-Crypto L1 does not differentiate between different species of *Cryptosporidium*, as there is insufficient data available on prevalence and excretion rates of the different species in different livestock animals. Moreover, observational studies on oocysts in the environment usually do not distinguish between different species either, as the antibodies used for environmental surveillance are not specific to human or zoonotic *Cryptosporidium* species only.

2.1. Calculating Oocyst Excretion in Livestock Manure (*X*).

GloWPa-Crypto L1 includes 11 livestock species: cattle, buffaloes, pigs, sheep, goats, horses, camels, donkeys, mules, chickens, and ducks. Oocyst excretion (X) per grid cell for each animal species (i) is calculated as follows:

$$X_i = N_i \times \mathrm{Fy}_i \times \mathrm{My}_i \times \mathrm{Py}_i \times \mathrm{Ry}_i + N_i \times (1 - \mathrm{Fy}_i) \times \mathrm{Ma}_i \times \mathrm{Pa}_i \times \mathrm{Ra}_i \quad (1)$$

where X_i is the oocyst excretion in a grid cell (oocysts/year) for animal species i; N_i is the number of animals of species i in the grid cell; Fy_i is the fraction of animals of species i that is young (defined as under three months old); My_i and Ma_i are the manure production (M) per head for young (y) and adults (a) of species i (gram manure/year), respectively; Py_i and Pa_i are the prevalence (P) of cryptosporidiosis for young (y) and adults (a) of species i (fraction infected); and Ry_i and Ra_i are the excretion rates (R) of oocysts for an infected young (y) or adult (a) animal of species i (oocysts/gram manure)

The numbers of animals on a grid cell (N_i) are taken from the Gridded Livestock of the World v2.0[18] for six animal species: cattle, sheep, goats, pigs, chickens, and ducks. These data were aggregated to a 0.5° × 0.5° grid. For the five other livestock species only country totals were available.[25] We assumed that buffaloes are distributed over countries similar to cattle, and that horses, camels, mules, and donkeys are distributed similar to sheep and goats, see the Supporting Information (SI). Figures S1 and S2 visualize the distribution of livestock over the world. We define young animals as those under three months old, as prevalence and excretion rates for this group were found to differ from those of adult animals (Table S4). The young fraction of animals is estimated based on the fertility rates[26] and average number of offspring per parturition of the different animal species (Table S3). Manure production (Ma_i and My_i) was calculated from average body mass of adult livestock, birth weight of young livestock, and manure production per 1000 kg mass (Tables S1 and S2).[20,21] Average prevalence of cryptosporidiosis (Py_i and Pa_i) and average oocyst excretion rate of infected animals (Ry_i and Ra_i) are based on an extensive systematic literature review[19] (see Table S4).

2.2. Calculating Oocyst Survival during Manure Storage (*V*).

For oocyst in manure that is dropped during grazing (L), we assume that everything ends up directly on land, according to the following equation:

$$L_i = X_i \times (F_{\mathrm{intl}_i} + F_{\mathrm{extl}_i}) \quad (2)$$

where X_i is the oocyst excretion (oocysts/grid/year) for animal species i (see eq 1); and F_{intli} and F_{extli} are the fraction of animals of species i that are kept in respectively intensive and extensive systems and drop manure on land during grazing.

However, if manure is stored before it is applied to land, then oocysts will die off during the storage period. The following equation calculates the number of oocysts in manure that is stored and then spread on land (S):

$$S_{ij} = X_i \times (F_{\mathrm{ints}_i} + F_{\mathrm{exts}_i}) \times \mathrm{FS}_j \times \overline{V}_j \quad (3)$$

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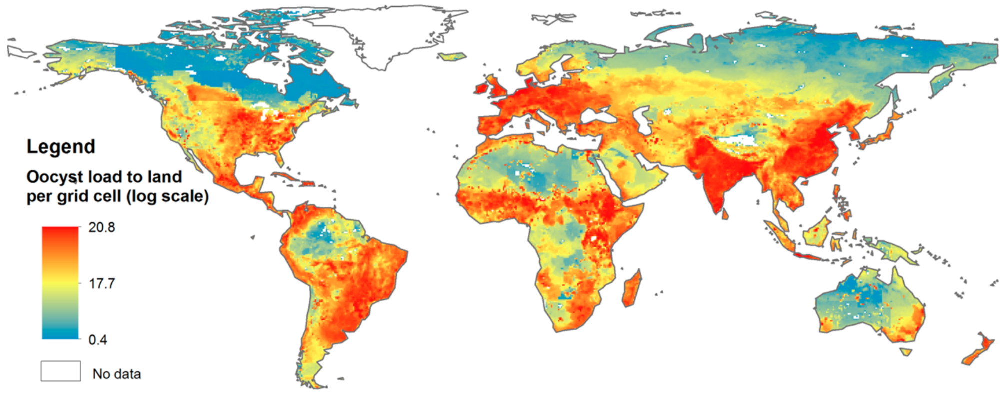

Figure 2. Oocyst loads to land (E) per grid cell per year. Grid cell size is 0.5° × 0.5°.

where X_i is the oocyst excretion (oocysts/grid/year) for animal species i (eq 1); F_{intsi} and F_{extsi} are the fractions of animal species i that are kept in respectively intensive and extensive systems of which the manure goes to storage; FS_j is the fraction of stored manure kept in storage system j; and $\overline{V}_J$ is the fraction of oocysts that survives during storage in system j, averaged over time (eqs 4 and 5)

Data on whether animals are kept in intensive or extensive farming systems (F_{intli}, F_{extli}, F_{intsi}, F_{extsi}) were taken from the Integrated Model to Assess the Global Environment (IMAGE) according to Bouwman et al.[22] Data on the use of different storage systems (FS_j) for the different animal species are from the 2006 IPCC Guidelines for National Greenhouse Gas Inventories[20] and underlying data from a USEPA report on Global methane emissions from livestock and poultry manure[21] (see SI S5). We assumed that all manure that is stored is applied to land in the same grid cell after storage. Manure trade was thus ignored.

Average oocyst survival ($\overline{V}_J$) in each storage system depends on storage time and temperature (eqs 4 and 5). We use an exponential decay function to calculate survival of oocysts over time (V):

$$V = e^{-K \times t} \tag{4}$$

Where:

t is the time (days)

K is a constant, that is dependent on temperature

We derived a value for K for each grid cell, based on a relation between temperature (°C) and oocyst survival in livestock manure (measured as viability or infectivity) using data from seven studies,[14−16,27−30] see the Supporting Information S6 for more detail.

Data on the duration of manure storage worldwide are unavailable to our knowledge. Unless a short storage time was explicitly indicated (e.g., systems "daily spread" and 'Pit storage shorter than one month', see Supporting Information S5) we assumed that manure is on average stored for 9 months (274 days), based on an assumed one or two harvests per year per location and spreading of manure at the start of the growing season. We integrated eq 4 over the estimated storage time to get the average survival rate ($\overline{\overline{V}}_J$) in every grid cell (eq 5). We do an integration, because we assume that a manure storage system continually receives manure, as livestock produce manure every day. To clarify, when manure is accumulated for 9 months and then spread on a field, this manure will be between 1 day and 9 months old.

$$\overline{V}_j = \frac{\int_0^{t_j} V \, dt}{t_j} \tag{5}$$

We do not differentiate for other characteristics of manure storage systems because of a lack of data (see Table S7), although conditions under which manure is stored may differ per system, and these conditions might influence *Cryptosporidium* survival. Only anaerobic digesters are considered a special case, as they are a type of manure treatment rather than mere storage. Anaerobic digesters can be operated at medium or high temperatures (mesophilic or thermophilic digestion). Garces et al.[16] found that oocyst infectivity was reduced by 2 log units after mesophilic digestion, and by over 5 log units after thermophilic digestion. Data on the relative use of mesophilic and thermophilic digestion worldwide are not available, but mesophilic systems are reported to be more stable and most commercial-scale anaerobic digesters are operated at mesophilic temperatures.[31] Therefore, we took the conservative estimate that all digestion is mesophilic and assume a 2 log reduction ($\overline{\overline{V}}_J$ = 0.01).

2.3. Calculating the Total Oocyst Load (*E*). In GloWPa-Crypto L1, the total oocyst load (E) is defined as the number of *Cryptosporidium* oocysts in a grid cell ending up on land annually (Figure 1). The oocyst load in a grid cell is calculated as follows:

$$E = \sum_{i} L_i + \sum_{ij} S_{ij} \tag{6}$$

where E is the total oocyst load in a grid cell (oocysts/year); L_i are the oocyst loads (oocysts/year) in a grid cell from manure that is dropped directly on land by animal species i (eq 2) (these are summed for all animal species); and S_{ij} are the oocyst loads (oocysts/year) in a grid cell from manure of animal species i that has been stored in storage system j (eq 3) (these are summed for all animal species and storage systems).

2.4. Sensitivity Analysis and Scenario Analysis. We test the sensitivity of our model to variation in the input variables in a nominal range sensitivity analysis. We change our input variables one at a time, based on the lower and upper end of a reasonable range the value can take. Tables S8−S10 show the input data of the sensitivity analysis.

We compare our baseline model to four alternative management scenarios, assuming that all manure goes directly to land (Scenario 1), all manure goes to storage (Scenario 2), all manure is treated by mesophilic anaerobic digestion (Scenario 3), and all manure is treated by thermophilic

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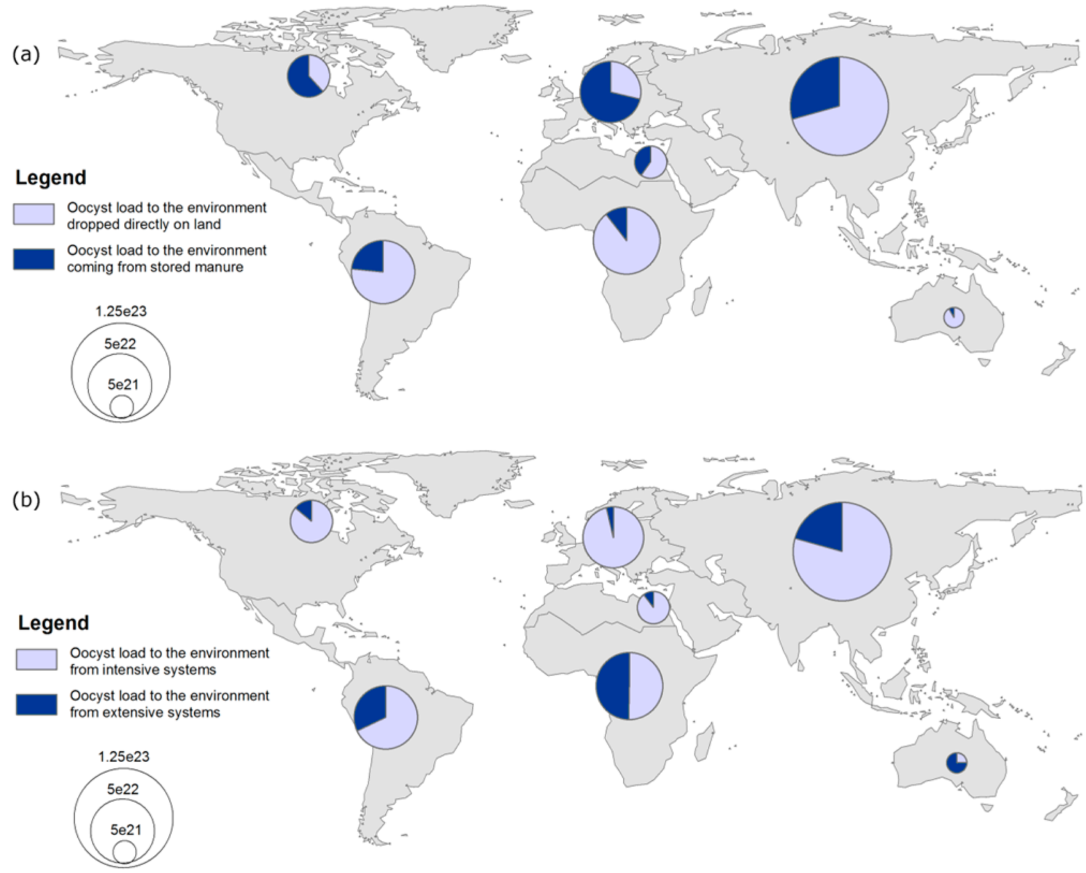

Figure 3. Oocyst load per world region, (a) from manure dropped directly on land and from stored manure, and (b) from intensive and extensive systems. Pie chart sizes are proportional to the size of the oocyst load. We distinguish seven world regions: Europe, Asia, Africa, North America, Latin America, and the Middle East—North Africa (MENA), see SI S1.

anaerobic digestion (Scenario 4). The difference between Scenario 1 and the baseline model represents the reduction in oocyst loads that is currently achieved by manure storage. The difference between the baseline model and Scenarios 2−4 represent the reduction potential. In all scenarios, the fraction of manure that is used for other purposes (e.g., burned for fuel) and leaves the system is unchanged. The assumptions on storage time and temperature in Scenario 2 are the same as in the baseline model. The assumption on the effect of oocyst survival of mesophilic anaerobic digestion (2 log reduction) and thermophilic anaerobic digestion (5 log reduction) is the same as that in the baseline model.

3. RESULTS AND DISCUSSION

3.1. Oocyst Loads to Land. Our model calculates a total global oocyst load from animal manure of 3.2×10^{23} oocysts per year. Figure 2 shows how this is distributed over the world. The patterns are mostly determined by the distribution of cattle, chickens, and pigs in the Gridded Livestock of the World V2.0 data. North America, Europe, and Oceania together only account for nearly a quarter of the total oocyst load, meaning that the developing world accounts for the largest share. This is likely mostly due to high animal numbers (Figure S1) and limited manure storage in developing countries, as cryptosporidiosis prevalence does not differ greatly between different world regions[19] (Table S4).

Compared to other studies calculating livestock oocyst loads to land, GloWPa-Crypto L1 model outcomes are in the same range. Atwill et al.[32] estimate a load of about 2.8×10^4 to 1.4×10^5 oocysts/animal/day for beef cattle from 22 feedlots in 7 states in the U.S.A. With GloWPa-Crypto L1, we estimate for North American adult beef cattle a daily load of 2.9×10^5 oocysts/animal/day. Starkey et al.[33] estimate the daily *C. parvum*-like oocyst load from dairy cattle across all ages in the New York City Catskill/Delaware watershed to be 4.15×10^{10}. They assume 258 herds within this watershed with an average of 125.3 animals per herd, hence, the oocyst load/animal/day is 1.28×10^6. They estimate that preweaned calves (<2 months) produce 99.5% of this load. With GloWPa-Crypto L1, we estimate for North American cattle an average of 5.89×10^6 oocysts/animal/day, of which 93% comes from young cattle (<3 months). In both cases, our load estimate is somewhat higher, and our proportion attributed to calves is somewhat lower, but still in the same order of magnitude. All of these estimates are excluding die-off during storage.

3.2. Sources of Oocyst Loads. In Europe and North America most oocysts come from stored manure, in the other regions oocyst are predominantly excreted directly on land. Asia has the highest total oocyst load, followed by Africa, Latin America, and Europe (Figure 3a) In Oceania, the majority of oocysts come from extensive systems, in Africa it is approximately equal, and in the other regions intensive systems dominate (Figure 3b). We did not assume different cryptosporidiosis prevalence for animals kept in different systems, although there is some evidence that in systems where large numbers of animals live closely together, disease prevalence is higher, but the evidence was too weak and the input data too variable to provide a quantitative estimate.[34−39]

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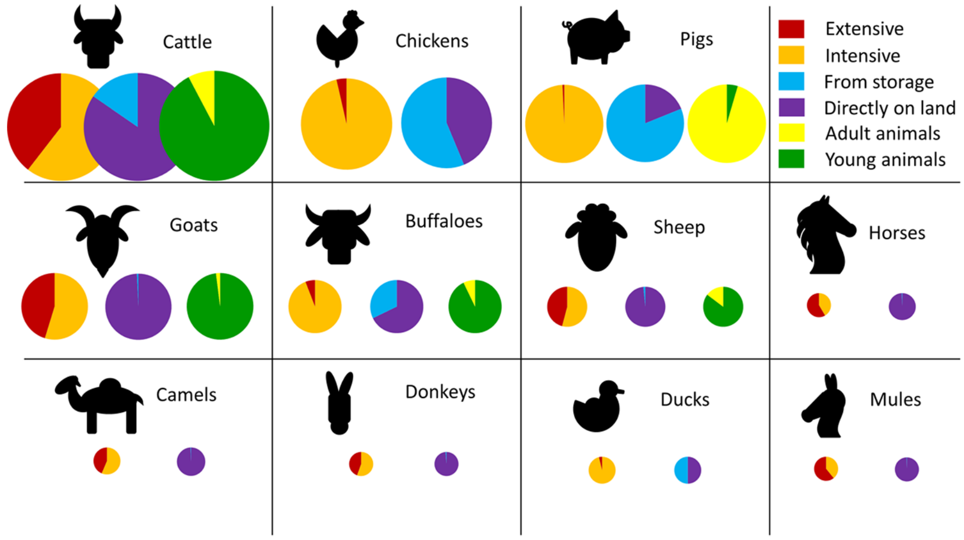

Figure 4. Oocyst load (E) per animal species attributed to intensive (F_{int}) or extensive (F_{ext}) systems, coming from storage (S) or excreted directly on land (L), and coming from adult or young animals. Pie chart sizes are an indication of the total annual oocyst load per animal species.

On a global scale, cattle are the dominant source of oocysts, followed by chickens and pigs (Figure 4). Intensive systems are the largest source of oocysts for most animal species, especially for pigs and chickens. Manure dropped directly on land is the largest source of oocysts for most animal species, except for chickens, pigs, and ducks. For cattle, buffaloes, goats, and sheep, young animals are the largest source of oocysts. For pigs, adults are the largest source of oocysts, although prevalence and oocyst excretion rates are higher for young pigs than for adults. The reason for this is that adult pigs produce much more manure than young pigs. It should be noted that the literature indicates that cryptosporidiosis is more prevalent among dairy calves than among beef calves,[40−42,34,43] although the literature is not fully consistent on this.[36,44−46] We did not make a distinction for dairy and beef cattle because the Gridded Livestock of the World v2.0 does not distinguish between these.

3.3. Sensitivity Analysis. Due to the lack of observational data of *Cryptosporidium* in the environment, a full model validation (in the meaning of comparing model outcomes to an independent set of observational data) is not possible for GloWPa-Crypto L1, as is the case for many large scale (ecological) models.[47] Yet there are other ways to build trust in a model, that can be summarized in the process "evaludation",[47] several of which we have incorporated in this study. These include transparency about model input data and assumptions (Section 2), comparing model outcomes to other studies calculating loads to land (Section 3.1), and doing a sensitivity analysis to study model performance.

The sensitivity analysis (Tables S8−10) shows that the model is most sensitive to changes in the excretion rates, especially the excretion rates of young cattle (factor 27.9 or ^{10}log 1.4), young goats (factor 4.9), and young buffaloes (factor 3.8). This means that the absolute size of oocyst loads to land, the relative importance of the different animal species and the patterns on the maps should be interpreted with this in mind. Regarding prevalence, the model is most sensitive to changes in the prevalence among young cattle, adult pigs, and chickens (factor 2.17, 1.45, and 1.41, respectively). For variables other than excretion rates and prevalence, the model is most sensitive to changes in the fraction of manure going to storage and to land (factor 1.96). The model is not very sensitive to changes in storage time and temperatures in different seasons, and to the excretion rates and prevalence among animal species that do not contribute much to the global total oocyst loads (e.g., mules, donkeys, and ducks).

It is not surprising that the sensitivity analysis shows that the model is most sensitive to changes in the oocyst excretion rates, because the range over which they were varied in the sensitivity analysis is large, as excretion rates exhibit strong variation (over several orders of magnitude).[19] Starkey et al.[33] also report that their model to calculate oocyst loads is most sensitive to oocyst excretion. A source of uncertainty for excretion rates for all animal species is that recovery efficiencies of oocysts are often not determined, and this can affect fecal concentration estimates strongly.[48] The literature review[19] did not identify any studies for the excretion rates for mules, donkeys, camels, buffaloes, chickens, and ducks. For mules, donkeys, and camels, the value for horses was taken as model input, and for buffaloes the value for cattle. For chickens and ducks, we determined model input values based on additional literature, all inoculation studies (i.e., not natural infection) with very young animals (see Table S5). This may lead to an overestimation of excretion rates, as inoculation with higher oocyst numbers can lead to higher shedding,[49,50] although not all studies observe this,[51] and one study indicates that younger chickens shed more oocysts and for a longer period than older chickens.[52] Furthermore, nearly all studies in chickens were done with *C. baileyi*, and excretion rates can also differ for different *Cryptosporidium* species; one study found that *C. meleagridis* was shed by chickens in 2−3 times lower numbers than *C. baileyi*.[53]

Observed prevalence is also uncertain, as different measurement methods can give different outcomes, in part due to the detection limit of the methods used that may cause "low level shedders" to be missed.[54,55] Detection limits are often not discussed in studies measuring *Cryptosporidium* in animal feces. Furthermore, we assumed that observed prevalence, usually measured at a point in time or over a short period, can be generalized to reflect the average prevalence throughout the year. If observed prevalences are biased toward seasons, regions, or herds that experience higher than average levels of

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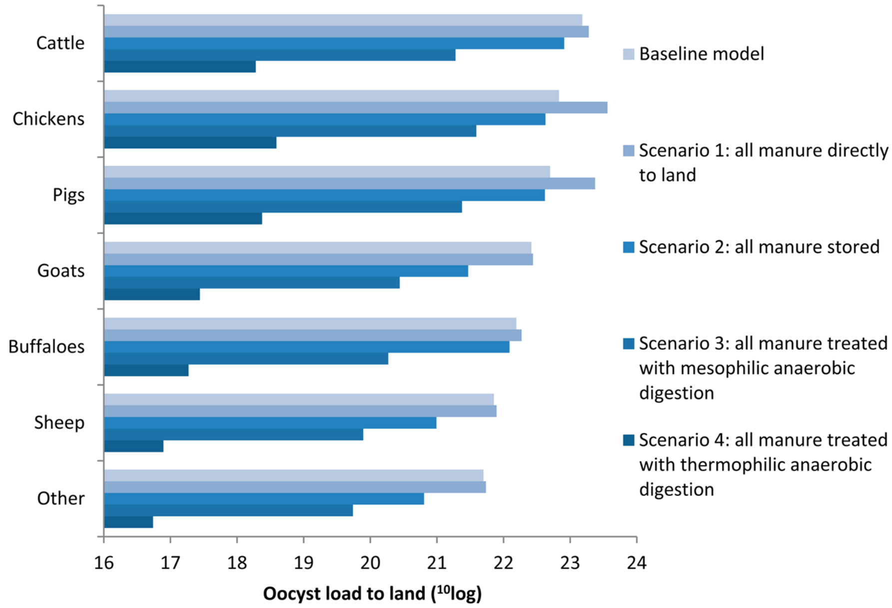

Figure 5. Oocyst load per animal species for the baseline model and four alternative management scenarios. The scenarios are assuming that all manure goes directly to land (Scenario 1), all manure goes to storage (Scenario 2), all manure is treated by mesophilic anaerobic digestion (Scenario 3), and all manure is treated by thermophilic anaerobic digestion (Scenario 4). The category "Other" combines the results from horses, camels, donkeys, mules, and ducks.

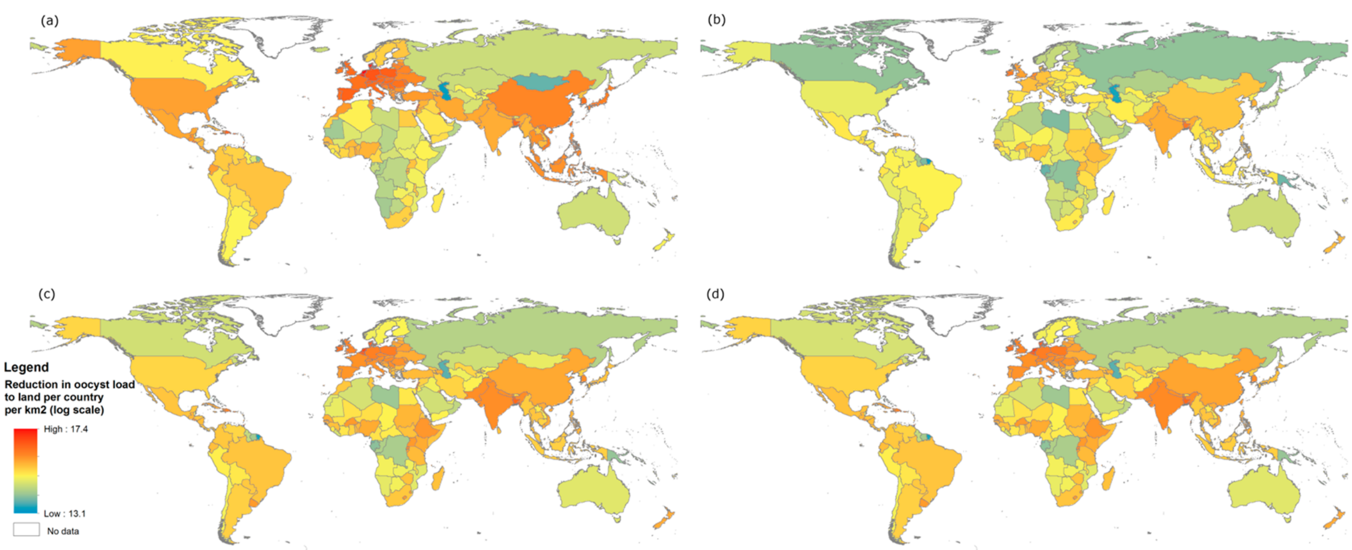

Figure 6. (a) Current reduction of oocyst load to land by storage (Scenario 1 minus baseline model run divided over country surface area in km^2). This map shows how much fewer oocysts end up on land because of current manure storage practices. High values indicate large reductions. (b–d) Reduction potential of oocyst load to land, showing respectively the baseline model run minus Scenario 2–4 divided over country surface area in km^2. These maps show how many fewer oocysts end up on land if all manure would go into storage (b), if all manure would be treated by mesophilic anaerobic digestion (c), and if all manure would be treated by thermophilic anaerobic digestion (d). High values indicate large reduction potential.

cryptosporidiosis infection, then we may overestimate prevalence in our model.

We want to stress the need for more observational data of *Cryptosporidium*, in fresh fecal material but also in manure in different types of storage facilities and on the field, from animals of different age groups, and from different countries. Recovery rates and detection limits should be assessed and published with these data.

3.4. Scenario Analysis: Effect of Manure Storage and Treatment. We compare the results for the four scenarios in Figures 5 and 6. The difference between the total oocyst load calculated in Scenario 1 and the baseline model was found to be a factor of 2.6. This represents the current reduction in oocyst loads due to manure storage. Around half of this current reduction is attributable to manure storage for chickens, around one-third to pigs, and approximately one tenth to cattle (Figure 5). Figure 6a is a map of the current reduction in oocyst load due to manure storage per country. High reduction is currently achieved in Europe, Bangladesh, China, countries in southeast Asia, and the U.S. Low reduction takes place in Mongolia, Russia, parts of Africa, and Australia.

The differences between Scenario 2–4 and the baseline model represent the oocyst load reduction potential of manure storage (2) and of manure treatment with mesophilic (3) and

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thermophilic (4) anaerobic digestion. If all manure would be stored under the conditions assumed in our model, then the environmental oocyst load would be a factor 2 lower than in our baseline run. If all manure would be treated with mesophilic anaerobic digestion, then the load would be reduced by a factor 37, and for thermophilic anaerobic digestion by a factor of nearly 37 000 (4.6 log units). Around half of the reduction potential in all three scenarios comes from cattle manure, after that chickens, pigs, goats, and buffaloes are most important. Figure 6b−d are maps of the oocyst load reduction potential per country, resulting from manure going to storage, treated with mesophilic anaerobic digestion, and thermophilic anaerobic digestion, respectively. The highest reduction potential can be found in India, Bangladesh, western Europe, China, several countries in Africa, and New Zealand. Low reduction potential is in Russia, Canada, and several countries in Africa. The spatial patterns in Figure 6b−d are similar, giving high values for countries with high livestock density and low current manure storage. The observation that especially western Europe, Bangladesh, and China have both a high current reduction (Figure 6a) and a high reduction potential (Figure 6b−d) follows from the high livestock density in these regions.

Manure storage alone is not a strategy by which oocyst loads to land are greatly reduced. In our model, we assumed continuous manure addition during the storage period, meaning that the manure going to land is ranging in age from fresh to old. Storing manure in batches, instead of with continuous addition of fresh manure, could improve oocyst load reduction.[14] Manure treatment with mesophilic or thermophilic anaerobic digestion can have a much larger impact as oocyst loads could be reduced by several log units. Our estimate of oocyst survival during anaerobic digestion is based solely on the findings of Garcés et al.,[16] but Kinyua et al.[29] confirm that treating manure by anaerobic digestion before it is applied to land can lower the risk of cryptosporidiosis from contaminated crops and soils significantly. It would be worthwhile to further investigate the effects of different manure treatments (such as anaerobic digestion, but also other possible treatments) on oocyst survival.

3.5. Importance of Livestock *Cryptosporidium* for Human Disease. Not all *Cryptosporidium* species are infectious for humans. Livestock harbor many *Cryptosporidium* spp. that have not been implicated in human infection.[1,2] The majority of human infections are caused by the species *C. hominis*, which predominantly infects humans, and *C. parvum*, which infects a variety of mammals. Ruminants are important reservoirs of *C. parvum*,[1,2] especially (preweaned) calves[6,17−20] and to a lesser extent adult cattle, lambs, and goat kids.[21−26] Livestock can also harbor other *Cryptosporidium* spp. that have occasionally been reported in humans, examples are *C. meleagridis* from chickens, *C. andersoni* and *C. bovis* from cattle, *C. suis* and *C. scrofarum* from pigs, and *C. xiaoi* from sheep and goats.[1,2]

GloWPa-Crypto L1 does not distinguish between *Cryptosporidium* species, for three main reasons: (1) comprehensive quantitative data on the relative occurrence of the various species in different livestock worldwide are not available, (2) *Cryptosporidium* species denomination has changed over the years and is subject to disagreement,[3] and (3) observational data on *Cryptosporidium* oocysts in the environment usually do not differentiate between species either, meaning that it would be near impossible to validate a species-specific model. However, if the outcome of GloWPa-Crypto L1 were to be used as input for risk assessment for human disease, data or assumptions are needed on the prevalence of *Cryptosporidium spp.* in livestock that are infectious for humans (mainly *C. parvum* and *C. hominis*) or only the input from the most relevant livestock species (cattle) should be incorporated.

GloWPa-Crypto H1[56] is the human counterpart of GloWPa-Crypto L1. It calculates global human *Cryptosporidium* emissions to surface water to be 1.6×10^{17} oocysts/year.[56] GlowPa-Crypto L1 calculates a much higher total global oocyst load from animal manure of 3.2×10^{23} oocysts/year. However, it should be noted that this is the load to land, not to surface water. Rainfall and subsequent runoff will transport only a small part of manure to surface waters, and in the meantime oocysts will also decay. Besides, as mentioned before, not all livestock *Cryptosporidium* is infectious for humans. A comparison of the relative importance of human and animal *Cryptosporidium* for waterborne disease is therefore, at this point, only speculative. However, our model suggests that the contribution from livestock should definitely not be ignored.

3.6. Outlook for *Cryptosporidium* Modeling. Gaining insight into the environmental pathways of *Cryptosporidium* is important in the context of managing human and animal cryptosporidiosis. Facing scarcity of observational data of *Cryptosporidium* in the environment, process-based modeling and scenario analysis can help to provide insight in handling options, such as the reduction potential from manure storage and treatment. More detailed scenario analyses could investigate the effects of different types of manure treatments, to answer specific management questions.

A next step is to go toward the exposure pathways that determine risk of contracting cryptosporidiosis, such as water- and foodborne pathways. A model of surface water oocyst concentrations can be constructed when the outcomes of GloWPa-Crypto L1 are combined with estimates on the survival of oocysts in manure on fields, transport with runoff to surface waters, hydrological information, and the outcomes of GloWPa-Crypto H1. Together with information on the share of *Cryptosporidium* spp. that are pathogenic for humans, such a model can provide a basis for risk assessments. In addition, GloWPa-Crypto L1 could be further refined to operate on a smaller time step or for specific regions. A model at a smaller time step could look into birthing seasons and herd structure development. This would require more detailed input data sets.

This paper provides a first spatially explicit assessment of *Cryptosporidium* spp. oocysts from livestock manure to land. The total global load is large (3.2×10^{23} oocysts per year) and should not be ignored in risk studies. Spatial differences are linked to animal spatial distributions. The GloWPa-Crypto L1 model is most sensitive to oocyst excretion rates, due to large variation reported in literature. Scenarios that include manure treatment (especially thermophilic anaerobic digestion) strongly reduce the loads to land (up to 4.6 log units). Manure treatment could be important to improve microbial environmental quality.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b00452.

S1. Distribution of livestock over the world; S2. manure production; S3. fraction young animals; S4. prevalence

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Environ. Sci. Technol. 2017, 51, 8663−8671

and excretion rates; S5. data on intensive and extensive farming and manure storage systems; S6. temperature-dependent survival during manure storage; and S7. sensitivity analysis (PDF)

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Notes

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REFERENCES

(1) Robertson, L. J.; Björkman, C.; Axén, C.; Fayer, R. Cryptosporidiosis in Farmed Animals. In *Cryptosporidium: Parasite and Disease*; Cacciò, S. M., Widmer, G., Eds.; Springer: New York, 2014; pp 149−235.

(2) Ryan, U.; Fayer, R.; Xiao, L. Cryptosporidium species in humans and animals: current understanding and research needs. *Parasitology* **2014**, *141* (13), 1667−1685.

(3) Šlapeta, J. Cryptosporidiosis and Cryptosporidium species in animals and humans: A thirty colour rainbow? *Int. J. Parasitol.* **2013**, *43* (12−13), 957−970.

(4) Naghavi, M.; Wang, H.; Lozano, R.; Davis, A.; Liang, X.; Zhou, M.; Vollset, S. E.; Ozgoren, A. A.; Abdalla, S.; Abd-Allah, F.; et al. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990−2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2015**, *385* (9963), 117−171.

(5) Liu, J.; Platts-Mills, J. A.; Juma, J.; Kabir, F.; Nkeze, J.; Okoi, C.; Operario, D. J.; Uddin, J.; Ahmed, S.; Alonso, P. L.; et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. *Lancet* **2016**, *388* (10051), 1291−1301.

(6) Zambrano, L. D.; Levy, K.; Menezes, N. P.; Freeman, M. C. Human diarrhea infections associated with domestic animal husbandry: A systematic review and meta-analysis. *Trans. R. Soc. Trop. Med. Hyg.* **2014**, *108* (6), 313−325.

(7) Shirley, D.-A. T.; Moonah, S. N.; Kotloff, K. L. Burden of disease from cryptosporidiosis. *Curr. Opin. Infect. Dis.* **2012**, *25* (5), 555−563.

(8) Dixon, B. R. Parasitic illnesses associated with the consumption of fresh produce—an emerging issue in developed countries. *Curr. Opin. Food Sci.* **2016**, *8*, 104−109.

(9) Sweeny, J. P. A.; Ryan, U. M.; Robertson, I. D.; Jacobson, C. Cryptosporidium and Giardia associated with reduced lamb carcase productivity. *Vet. Parasitol.* **2011**, *182* (2−4), 127−139.

(10) Shafiq, M. A. B.; Maqbool, A.; Khan, U. J.; Lateef, M.; Ijaz, M. Prevalence, Water Borne Transmission and Chemotherapy of Cryptosporidiosis in Small Ruminants. *Pak. J. Zool.* **2015**, *47* (6), 1715−1721.

(11) Maurya, P. S.; Rakesh, R. L.; Pradeep, B.; Kumar, S.; Kundu, K.; Garg, R.; Ram, H.; Kumar, A.; Banerjee, P. S. Prevalence and risk factors associated with *Cryptosporidium* spp. infection in young domestic livestock in India. *Trop. Anim. Health Prod.* **2013**, *45* (4), 941−946.

(12) Maddox-Hyttel, C.; Langkjær, R. B.; Enemark, H. L.; Vigre, H. Cryptosporidium and Giardia in different age groups of Danish cattle and pigs-Occurrence and management associated risk factors. *Vet. Parasitol.* **2006**, *141* (1−2), 48−59.

(13) Smith, R. P.; Clifton-Hadley, F. A.; Cheney, T.; Giles, M. Prevalence and molecular typing of Cryptosporidium in dairy cattle in England and Wales and examination of potential on-farm transmission routes. *Vet. Parasitol.* **2014**, *204* (3−4), 111−119.

(14) Hutchison, M. L.; Walters, L. D.; Moore, A.; Avery, S. M. Declines of zoonotic agents in liquid livestock wastes stored in batches on-farm. *J. Appl. Microbiol.* **2005**, *99* (1), 58−65.

(15) Jenkins, M. B.; Liotta, J. L.; Bowman, D. D. Inactivation kinetics of *Cryptosporidium parvum* oocysts in a swine waste lagoon and spray field. *J. Parasitol.* **2013**, *99* (2), 337−342.

(16) Garcés, G.; Effenberger, M.; Najdrowski, M.; Wackwitz, C.; Gronauer, A.; Wilderer, P. A.; Lebuhn, M. Quantification of *Cryptosporidium parvum* in anaerobic digesters treating manure by (reverse-transcription) quantitative real-time PCR, infectivity and excystation tests. *Water Sci. Technol.* **2006**, *53* (8), 195−202.

(17) R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2016.

(18) Robinson, T. P.; Wint, G. R. W.; Conchedda, G.; Van Boeckel, T. P.; Ercoli, V.; Palamara, E.; Cinardi, G.; D'Aietti, L.; Hay, S. I.; Gilbert, M. Mapping the global distribution of livestock. *PLoS One* **2014**, *9* (5), e96084.

(19) Hofstra, N.; Vermeulen, L. C.; Benders, J.; Medema, G. J.Cryptosporidium Prevalence and Oocyst Concentrations in Livestock Manure: A Review *Prep.* **2017**.

(20) IPCC. *2006 IPCC Guidelines for National Greenhouse Gas Inventories, vol. 4 ch. 10*; 2006.

(21) Safley, L. M.; Casada, M. E.; Woodbury, J. W.; Roos, K. F. *Global Methane Emissions from Livestock and Poultry Manure*; USEPA: Washington, D. C., 1992.

(22) Bouwman, L.; Goldewijk, K. K.; Van Der Hoek, K. W.; Beusen, A. H. W.; Van Vuuren, D. P.; Willems, J.; Rufino, M. C.; Stehfest, E. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900−2050 period. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (52), 20882−20887.

(23) Weedon, G. P.; Gomes, S.; Viterbo, P.; Shuttleworth, W. J.; Blyth, E.; Österle, H.; Adam, J. C.; Bellouin, N.; Boucher, O.; Best, M. Creation of the WATCH Forcing Data and Its Use to Assess Global and Regional Reference Crop Evaporation over Land during the Twentieth Century. *J. Hydrometeorol.* **2011**, *12* (5), 823−848.

(24) Hofstra, N.; Bouwman, A. F.; Beusen, A. H. W.; Medema, G. J. Exploring global Cryptosporidium emissions to surface water. *Sci. Total Environ.* **2013**, *442*, 10−19.

(25) FAO. FAOSTAT database collections http://faostat.fao.org (accessed Aug 11, 2015).

(26) FAO. *Global Livestock Environmental Assessment Model—Model description*, Version 1.0.; Food and Agriculture Organization of the United Nations, 2016.

(27) Collick, A. S.; Fogarty, E. A.; Ziegler, P. E.; Walter, M. T.; Bowman, D. D.; Steenhuis, T. S. Survival of *Cryptosporidium parvum* oocysts in calf housing facilities in the New York City watersheds. *J. Environ. Qual.* **2006**, *35* (2), 680−687.

(28) Jenkins, M. B.; Walker, M. J.; Bowman, D. D.; Anthony, L. C.; Ghiorse, W. C. Use of a sentinel system for field measurements of *Cryptosporidium parvum* oocyst inactivation in soil and animal waste. *Appl. Environ. Microbiol.* **1999**, *65* (5), 1998−2005.

(29) Kinyua, M. N.; Trimmer, J.; Izurieta, R.; Cunningham, J.; Ergas, S. J. Viability and Fate of *Cryptosporidium parvum* and *Giardia lamblia* in Tubular Anaerobic Digesters. *Sci. Total Environ.* **2016**, *554−555*, 167−177.

(30) Olson, M. E.; Goh, J.; Phillips, M.; Guselle, N.; McAllister, T. A. Giardia Cyst and Cryptosporidium Oocyst Survival in Water, Soil, and Cattle Feces. *J. Environ. Qual.* **1999**, *28* (6), 1991.

(31) Labatut, R. A.; Angenent, L. T.; Scott, N. R. Conventional Mesophilic vs. Thermophilic Anaerobic Digestion: A Trade-Off between Performance and Stability? *Water Res.* **2014**, *53*, 249−258.

(32) Atwill, E. R.; Pereira, M. D. G. C.; Alonso, L. H.; Elmi, C.; Epperson, W. B.; Smith, R.; Riggs, W.; Carpenter, L. V.; Dargatz, D. A.; Hoar, B. Environmental load of *Cryptosporidium parvum* Oocysts

DOI: 10.1021/acs.est.7b00452
Environ. Sci. Technol. 2017, 51, 8663−8671

from Cattle Manure in Feedlots from the Central and Western United States. *J. Environ. Qual.* **2006**, *35* (1), 200−206.

(33) Starkey, S. R.; White, M. E.; Mohammed, H. O. Mohammed, H. O. Cryptosporidium and dairy cattle in the Catskill/Delaware watershed: A quantitative risk assessment. *Risk Anal.* **2007**, *27* (6), 1469−1485.

(34) Duranti, A.; Cacci, S. M.; Pozio, E.; Di Egidio, A.; De Curtis, M.; Battisti, A.; Scaramozzino, P. Risk Factors Associated with *Cryptosporidium parvum* Infection in Cattle. *Zoonoses Public Health* **2009**, *56* (4), 176−182.

(35) Muhid, A.; Robertson, I.; Ng, J.; Ryan, U. Prevalence of and Management Factors Contributing to *Cryptosporidium* sp. Infection in Pre-Weaned and Post-Weaned Calves in Johor, Malaysia. *Exp. Parasitol.* **2011**, *127* (2), 534−538.

(36) Castro-Hermida, J. A.; González-Losada, Y. A.; Ares-Mazás, E. Prevalence of and Risk Factors Involved in the Spread of Neonatal Bovine Cryptosporidiosis in Galicia (NW Spain). *Vet. Parasitol.* **2002**, *106* (1), 1−10.

(37) Maikai, B. V.; Umoh, J. U.; Kwaga, J. K. P.; Lawal, I. A.; Maikai, V. A.; Cama, V.; Xiao, L. Molecular Characterization of *Cryptosporidium* spp. in Native Breeds of Cattle in Kaduna State, Nigeria. *Vet. Parasitol.* **2011**, *178* (3−4), 241−245.

(38) Lai, M.; Zhou, R. Q.; Huang, H. C.; Hu, S. J. Prevalence and Risk Factors Associated with Intestinal Parasites in Pigs in Chongqing, China. *Res. Vet. Sci.* **2011**, *91* (3), 121−124.

(39) Silverlås, C.; Blanco-Penedo, I. *Cryptosporidium* spp. in Calves and Cows from Organic and Conventional Dairy Herds. *Epidemiol. Infect.* **2013**, *141* (3), 529−539.

(40) Geurden, T.; Goma, F. Y.; Siwila, J.; Phiri, I. G. K.; Mwanza, A. M.; Gabriel, S.; Claerebout, E.; Vercruysse, J. Prevalence and genotyping of Cryptosporidium in Three Cattle Husbandry Systems in Zambia. *Vet. Parasitol.* **2006**, *138* (3−4), 217−222.

(41) Qi, M. Z.; Fang, Y. Q.; Wang, X. T.; Du, S. Z.; Guo, Y. X.; Jia, Y. Q.; Yao, L.; Liu, Q. D.; Zhao, G. H.; Zhang, L. X.; et al. Molecular characterization of *Cryptosporidium* spp. in Pre-Weaned Calves in Shaanxi Province, North-Western China. *J. Med. Microbiol.* **2015**, *64* (1), 111−116.

(42) Kváč, M.; Kouba, M.; Vítovec, J. Age-related and housing-dependence of Cryptosporidium infection of calves from dairy and beef herds in South Bohemia, Czech Republic. *Vet. Parasitol.* **2006**, *137* (3−4), 202−209.

(43) Geurden, T.; Berkvens, D.; Martens, C.; Casaert, S.; Vercruysse, J.; Claerebout, E. Molecular epidemiology with subtype analysis of Cryptosporidium in calves in Belgium. *Parasitology* **2007**, *134* (14), 1981−1987.

(44) Zhao, W.; Wang, R.; Zhang, W.; Liu, A.; Cao, J.; Shen, Y.; Yang, F.; Zhang, L. MLST subtypes and population genetic structure of *Cryptosporidium andersoni* from dairy cattle and beef cattle in Northeastern China's Heilongjiang Province. *PLoS One* **2014**, *9* (7), e10200610.1371/journal.pone.0102006

(45) Chuah, C. J.; Mukhaidin, N.; Choy, S. H.; Smith, G. J. D.; Mendenhall, I. H.; Lim, Y. A. L.; Ziegler, A. D. Prevalence of Cryptosporidium and Giardia in the water resources of the Kuang River catchment, Northern Thailand. *Sci. Total Environ.* **2016**, *562*, 701−713.

(46) Dixon, B.; Parrington, L.; Cook, A.; Pintar, K.; Pollari, F.; Kelton, D.; Farber, J. The Potential for Zoonotic Transmission of *Giardia duodenalis* and *Cryptosporidium* spp. from Beef and Dairy Cattle in Ontario, Canada. *Vet. Parasitol.* **2011**, *175* (1−2), 20−26.

(47) Augusiak, J.; Van den Brink, P. J.; Grimm, V. Merging Validation and Evaluation of Ecological Models to "Evaludation": A Review of Terminology and a Practical Approach. *Ecol. Modell.* **2014**, *280*, 117−128.

(48) Kuczynska, E.; Shelton, D. R. Method for Detection and Enumeration of *Cryptosporidium parvum* Oocysts in Feces. *Manures, and Soils* **1999**, *65* (7), 2820−2826.

(49) Rhee, J. K.; Jang, B. G.; Park, B. K. Oocyst Production and Immunogenicity of *Cryptosporidium baileyi* in Chickens and Mallards. *Korean J. Parasitol.* **1995**, *33* (1), 45−54.

(50) Zambriski, J. A.; Nydam, D. V.; Bowman, D. D.; Bellosa, M. L.; Burton, A. J.; Linden, T. C.; Liotta, J. L.; Ollivett, T. L.; Tondello-Martins, L.; Mohammed, H. O. Description of Fecal Shedding of *Cryptosporidium parvum* Oocysts in Experimentally Challenged Dairy Calves. *Parasitol. Res.* **2013**, *112* (3), 1247−1254.

(51) Zambriski, J. A.; Nydam, D. V.; Wilcox, Z. J.; Bowman, D. D.; Mohammed, H. O.; Liotta, J. L. *Cryptosporidium parvum*: Determination of ID50 and the Dose-Response Relationship in Experimentally Challenged Dairy Calves. *Vet. Parasitol.* **2013**, *197* (1−2), 104−112.

(52) Sréter, T.; Varga, I.; Békési, L. Age-Dependent Resistance to *Cryptosporidium baileyi* Infection in Chickens. *J. Parasitol.* **1995**, *81* (5), 827−829.

(53) Tůmová, E.; Skřivan, M.; Marounek, M.; Pavlásek, I.; Ledvinka, Z. Performance and Oocyst Shedding in Broiler Chickens Orally Infected with *Cryptosporidium baileyi* and *Cryptosporidium meleagridis*. *Avian Dis.* **2002**, *46* (1), 203−207.

(54) Venu, R.; Latha, B. R.; Basith, S. A.; Sreekumar, C.; Raj, G. D.; Raman, M. Comparative Evaluation of Conventional Diagnostic Methods for Detection of Bovine Cryptosporidiosis. *Indian J. Anim. Sci.* **2013**, *83* (2), 124−128.

(55) Ryan, U. M.; Bath, C.; Robertson, I.; Read, C.; Elliot, A.; Mcinnes, L.; Traub, R.; Besier, B. Sheep May Not Be An Important Zoonotic Reservoir For Cryptosporidium And Giardia Parasites. *Appl. Environ. Microbiol.* **2005**, *71* (9), 4992−4997.

(56) Hofstra, N.; Vermeulen, L. C. Impacts of Population Growth, Urbanisation and Sanitation Changes on Global Human Cryptosporidium Emissions to Surface Water. *Int. J. Hyg. Environ. Health* **2016**, *219* (7), 599−605.

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Environ. Sci. Technol. 2017, 51, 8663−8671