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Epidemiology of *Cryptosporidium* infection in different hosts in Nigeria: A meta-analysis

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ABSTRACT

Cryptosporidium is a medical and veterinary significant protozoan parasite that infects all classes of vertebrates. Environmental contamination with infective oocyst increases the risk of transmission to susceptible host. Estimates of *Cryptosporidium* prevalence in humans and animals are lacking in Nigeria, therefore a systematic review and meta-analysis were performed to understand the epidemiology of the disease over a period of 30 years using publications from EMBASE, Ovid MEDLINE, Web of Science, AJOL and Google Scholar databases. Studies that met the inclusion criteria of *Cryptosporidium* infections under the preferred reporting items for systematic reviews and meta-analyses (PRISMA) checklist were analysed. Point estimates prevalence and subgroup analyses based on potential risk factors and diagnostic techniques were evaluated at 95% confidence interval (CI). A total of 64 eligible studies published between 1987 and 2017 were selected for meta-analysis. The prevalence of *Cryptosporidium* infection using quality effects model among human, cattle, sheep, goat, pigs, laboratory animals and birds was estimated as 15.0, 26.1, 16.6, 26.0, 20.1, 9.0 and 7.2%, respectively. The high report of *C. parvum* subtype family IIc indicates the importance of anthroponotic transmission of *Cryptosporidium* in Nigeria. Heterogeneity of subgroup (regions, species) and risk factors (HIV status, age, gender, faecal type) analyses were determined. The pooled prevalence of *Cryptosporidium* spp. in different hosts were high and linked with several risk factors such as environmental contamination and animal contact. There is need for increased awareness on the prevalence of the disease to provide strategies that mitigate the disease in humans and animals.

1. Introduction

Cryptosporidium, a protozoan parasite belonging to the Phylum Apicomplexa and Family Cryptosporiidae, is a common cause of diarrhea in humans, domestic animals and wild vertebrates [1]. Globally, *Cryptosporidium* is rated the second cause of severe diarrhea, and leading cause of mortality in young children, with an estimated 800, 000 deaths annually and approximately three million infections detected, most occurring in sub-Saharan Africa and south Asia [2–6]. *Cryptosporidium* is ranked fifth among the 24 most important foodborne parasites in a global ranking by a joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) expert committee [7]. In terms of Disability-Adjusted Life Years (DALYs), the global burden of cryptosporidiosis is similar to that of tuberculosis [8]. Infections in immunocompromised individuals, especially those with HIV/AIDS can result in severe consequences such as long-term diarrhea followed by dehydration and death [9–12]. In the absence of established interventions (improved water-management and provision of antiretrovirals), the burden of cryptosporidiosis continues to increase in developing countries, where infection is both more ubiquitous and clinically important [9].

Human cryptosporidiosis is often caused by either *C. hominis* or *C. parvum*, of which the latter infects a number of mammals, and therefore responsible for most zoonotic infections [13]. Animals have been suggested to play significant role in the transmission of *Cryptosporidium* parasite to humans. Important species of animal origin reported in humans include *C. felis*, *C. cuniculus*, *C. meleagridis*, *C. andersoni*, *C. canis*, *C. ubiquitum*, *C. muris*, *C. fayeri*, *C. viatorum*, *C. scrofarum*, *C. bovis*, *C. erinacei*, *C. tyzzeri* and *Cryptosporidium* skunk, horse and the chipmunk I genotypes [14–16]. Similarly, *C. hominis* has been also detected in non-human animal species including cattle [17,18], domestic dogs [19] and wild mesocarnivores [20]. The advent of molecular tools and techniques have enhanced the detection and differentiation of *Cryptosporidium* at the species/genotype and subtype family levels [14].

In Nigeria, there are several reports of *Cryptosporidium* species infecting humans and animals [21–23], however most of the survey have been restricted to some regions in the country. Predominantly, reported studies were analysed with microscopy technique which cannot

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distinguish species/genotypes [24,25]. Risk factors of *Cryptosporidium* infection in humans include drinking contaminated water, contact with infected animals or humans (particularly children), absence of toilet facilities, consumption of contaminated food, low socioeconomic status and overcrowded living conditions, malnutrition, recreational use of contaminated water and travel to disease endemic areas [1]. Hence, in relation to One-Health concept, the environment is an important factor to consider in the transmission dynamics of *Cryptosporidium* oocysts.

To the best of our knowledge, there is no available information about the overall prevalence of *Cryptosporidium* infection in humans and animals in Nigeria. Therefore, this systematic review and meta-analysis were performed to determine the prevalence of *Cryptosporidium* species in humans and other animals over the last 30 years using published literature to assess the potential risk factors related to their prevalence.

2. Methods

The study was conducted according to the PRISMA checklist (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) to ensure the inclusion of all relevant information in the analysis [26] (Supplementary file 1).

2.1. Search strategy and criteria

Literature searches conducted for papers published in English on prevalence or epidemiology of *Cryptosporidium* infection in vertebrates across Nigeria between January 1, 1987 to December 31, 2017 were retrieved from various databases (EMBASE, Ovid MEDLINE, Web of Science, AJOL and Google Scholar). This range of years was important to study the epidemiology since the first study was reported. The search terms were “cryptosporid*”, “prevalence”, “epidemiology” and “Nigeria”. The reference list of articles was visually examined to locate omitted or additional studies that were not identified by the databases. The authors compared their findings for all the processes using similar databases. Data extraction was repeated three times for accuracy. None of the authors of original studies were contacted for additional information and no attempt was made to retrieve unpublished articles. The full text of all the included articles were downloaded and obtained through library resources and online mechanisms.

All the selected articles fulfilled the following inclusion criteria: cross-sectional study, study done within Nigeria, vertebrate host, exact total numbers and positive cases were provided, sample size (> 25 for possible statistic calculations) and study year. Studies without these characters were all excluded.

2.2. Study quality

Evaluation of risk of bias on the included studies was done using quality assessment checklist. Items were scaled as yes, no and unsure. The item subjects were as follows: Was the research objective described clearly? Was the period of sampling mentioned? Was the sampling method stated? Was the sampling location indicated? Was the sampling size indicated? Was the host type mentioned? Was the diagnostic technique mentioned? Were the samples genotyped or sub-genotyped? Were examined subjects categorized into different subgroups? Did the authors get ethical approval for the study? These items were represented in a scale of 1–10. Each question represented a scale, and ten-question in all was used in complete assessment of study quality.

2.3. Data extraction

Data were extracted using detailed characters of each study to form pre-designed sub-headings in a data collection excel form. Information recorded was study characteristics (first author's name, year of publication, year of study, location, geopolitical region); study design and methodology (survey method, sampling technique, diagnostic technique used, genotyping and sub-genotyping); characteristics of subjects (type of host, gender and prevalence, category and prevalence, age-group and prevalence, animal or human contacts and prevalence, risk factors such as water and soil contamination and prevalence); sample size, number of positive cases and quality score of each study considered.

2.4. Statistical analysis

METAXL version 3.1, a tool for meta-analysis in Microsoft Excel, was adopted to pool the prevalence from each study [27,28]. Estimated pooled prevalence and 95% confidence intervals (CI) were calculated using quality effects model. This model uses quality scores to weigh studies based on sample size and study quality [27]. Various sub-group analyses were calculated from where prevalence data were extracted. Study heterogeneity (Cochran's Q) was evaluated by I^2 (level of inconsistency). The I^2 values of 25%, 50% and 75% were considered as having low, moderate and high degree of heterogeneity, respectively [28]. Risk of bias was assessed using the funnel plots and doi plots. Luis Frya-Kanamori (LFK) index exceeding ± 2 indicates major asymmetry (publication bias) [27]. The LFK index is obtained by plotting the z-score against double arcsin prevalence of examined publications. The degree of freedom (*df*) expressed in the tables signifies the total number of sampled population (n-1), where “n” is the sample population. Variability was further assessed by several sub-group analyses such as animal subjects, risk factors, and species involved. Chi-square analysis was used to compare status, gender, age, regions and animal contact for human infections using WINIPEPI statistical package (UK, version 11.65). Tukey's *post-hoc* multiple pairwise comparison test of one-way ANOVA was used to compare diagnostic techniques and trend of disease over the years using Graphpad prism (San Diego, USA, version 5). *Cryptosporidium* research study intensity in vertebrate hosts according to states was illustrated in a map developed with GIS (version 2.8.10) tools. Raw data were managed in Microsoft Excel and 95% confidence intervals was used for all descriptive analyses.

3. Results

3.1. Search results and eligibility studies

A total of 529 published articles through searches in five databases and reference lists of relevant studies were assessed (Fig. 1). A total of 137 studies were excluded from initial screening of titles/and or abstracts out of 257 records of combined searches. A total of 39 studies duplicated were further excluded. The remaining 81 full-text articles were downloaded and read individually, of which 17 studies were excluded from the inclusion criteria. A total of 64 studies were primed for meta-analysis which included 6 articles from the reference lists in this paper (Fig. 1). Studies for meta-analysis include humans ($n = 45$), cattle ($n = 11$), sheep ($n = 5$), goats ($n = 6$), pigs ($n = 5$), laboratory animals ($n = 2$) and birds ($n = 2$). There were combined animal species reports of *Cryptosporidium* in six studies.

3.2. Characteristics of the eligible studies

The study characters include year of study, region, publication year, host involved, status of human infection, gender, animal contact and diagnostic method (Supplementary file 2a). The total number of studies for each diagnostic test for the human infection has been reported. Quality score study characteristics was based on sub-group data, sample size and study design, which were used to assess the integrity of the study. All the included studies had 7 score upwards (Supplementary file 2b).

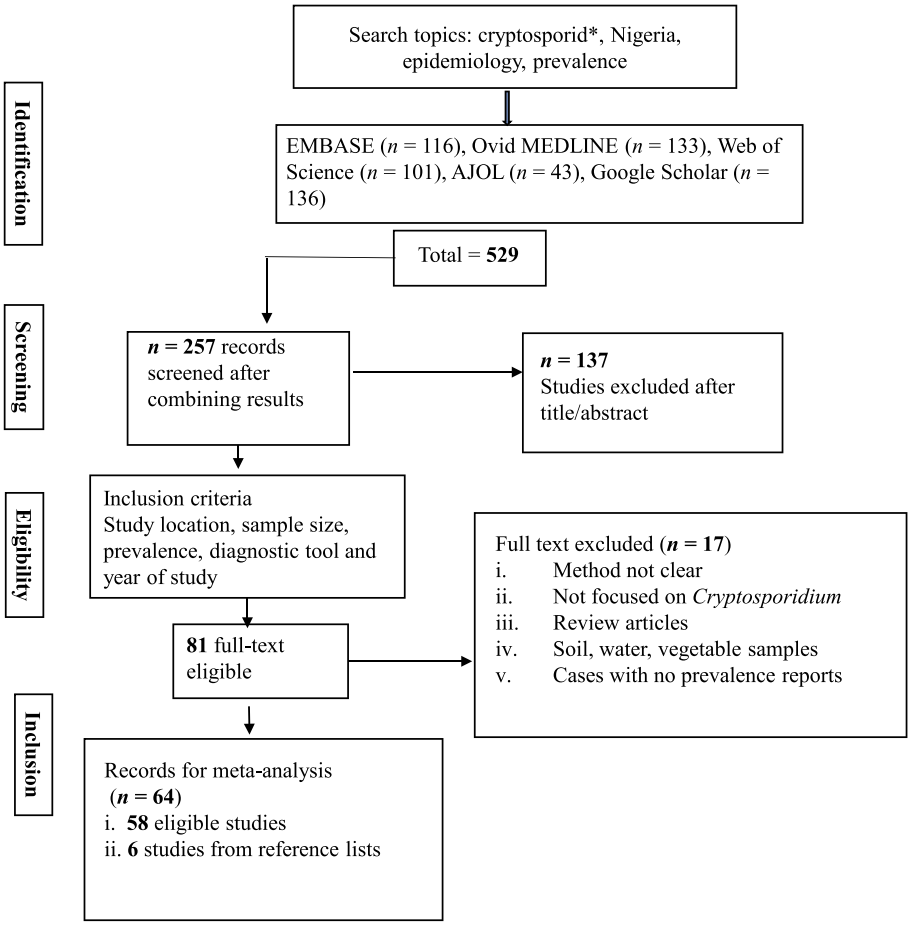

Fig. 1. Flow diagram detailing the selection of eligible studies and excluded studies in a systematic approach for the prevalence of *Cryptosporidium* infections in Nigeria.

3.3. Pooling and heterogeneity analyses of Cryptosporidium in humans

The pooled prevalence estimates of *Cryptosporidium* spp. in humans ($n = 45$) with individual studies on human *Cryptosporidium* infections are shown in a forest plot (Fig. 2). Studies revealed pooled prevalence of 15.0% (8.6–22.7); $Q = 2821$; $I^2 = 98.4$; $df = 44$; $P < .0001$). Only four studies were observed before the new millennium, while the remaining studies were very recent. The prevalence of *Cryptosporidium* of each study varied from 1.2 to 73.3% (median = 17.8%) with substantial heterogeneity among studies. Of all the 44 studies classified into regions, there was no significant difference in investigations carried out in the north ($n = 21$) or south regions ($n = 23$) while a study involved both regions. However, a significantly higher prevalence *Cryptosporidium* infection was observed in northern Nigeria compared to the south ($X^2 = 294.8$, $OR = 1.9$, $P < .0001$). The uneven socio-economic development in Nigeria have resulted to variations in disease prevalence across the country. Hence, prevalence was analysed based on geopolitical regions. For northern regions, more studies have been carried out in northwest region ($n = 10$), while least in northeast ($n = 4$). Pooled prevalence of 13.0, 22.0 and 41.0% for northwest, northcentral and northeast, respectively was observed (Fig. 3). Meanwhile, the southern regions revealed more studies in southwest ($n = 11$) and least in south-south ($n = 4$). Pooled prevalence was 16.0, 7.0 and 9.0% for southwest, southeast and south-south, respectively (Fig. 4).

The potential risk factors are presented as sub-group analyses. Human infection was also classified into two statuses; HIV infected or diarrhoeic patients. There was significant increase of *Cryptosporidium* infection in HIV positive patients ($X^2 = 546.2$; $OR = 13.9$; $P < .0001$) compared to the HIV negative. Even though, the number of examined patients with HIV is lower, yet a high level of heterogeneity was observed. No significance ($P = .548$) in *Cryptosporidium* observed in $HAART^+$ and $HAART^-$ individuals. Based on $CD4^+$ cell counts, the presence of *Cryptosporidium* in HIV^+ patients with $CD4^+$ cell counts < 200 cell/μl was significantly ($P < .001$) higher than those with $CD4^+$ cell counts > 200 cells/μl in the pooled prevalence. Diarrhoeic patients showed high potential of having *Cryptosporidium* compared ($X^2 = 199.6$; $OR = 2.3$; $P < .0001$) to non-diarrhoeic patients. Out of the 17 studies that reported sex prevalence, the pooled prevalence between the male compared with female shows no significant difference ($X^2 = 0.2$; $OR = 1.0$; $P > .05$). The prevalence based on diagnostic technique revealed 40.6%, 19.3%, 13.3% and 12.1% for serology (ELISA), Ziehl Neelsen, Safranin-methylene blue, and PCR methods, respectively. Sub-group analyses based on factors such as age, animal contact for human infections were further analysed. According to age, prevalence was highest in humans with ages ≤5 years at 22.5%, followed by those of ages > 50 years with prevalence of 21.7%. Age groups of > 5–14 years and 15 – ≤ 50 years showed homogenous prevalence of 10.3%. There was no significant difference ($P > .05$) in the prevalence of humans with or without animal contact. All the characterised *Cryptosporidium* species were those from PCR methods. *Cryptosporidium hominis* was mostly reported with 4.1% prevalence, followed by *C. parvum* with 3.8% prevalence in humans, while the lowest prevalence of 0.2% was revealed in *C. canis*. Other *Cryptosporidium* species in humans reported include *C. viatorum*, *C. felis*, *C. meleagridis*, *C. ubiquitum* and *C. cuniculus* (Table 1).

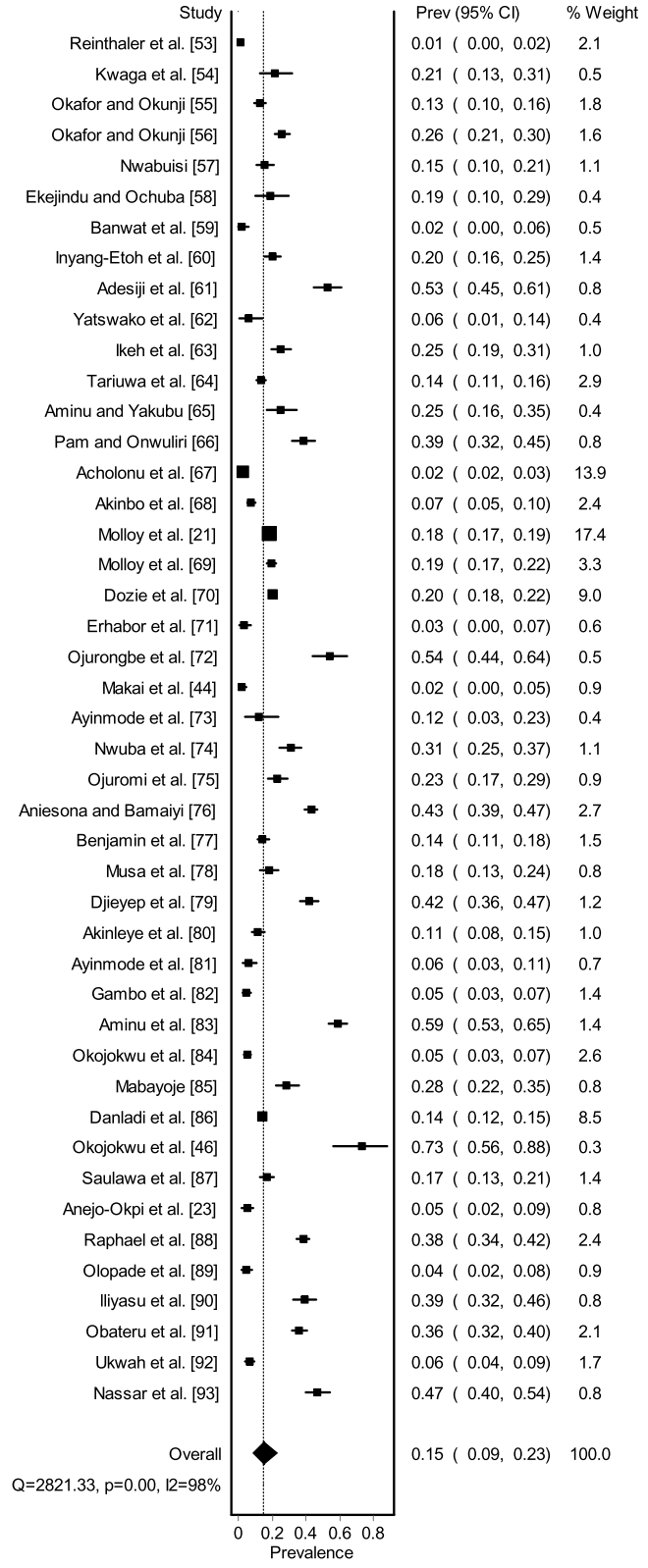

Fig. 2. Forest plot of point estimates of *Cryptosporidium* pooled prevalence of humans in Nigeria between 1987 and 2017. N·B- Squares are the sample sizes (varied square shapes are represented by sample population; bigger and smaller shapes are automatically generated based on the sample sizes). A diamond indicates the pooled estimate of the total studies [21,23,44,46,53–93].

3.4. Meta-analysis and heterogeneity of Cryptosporidium infection in cattle

The prevalence of *Cryptosporidium* in cattle (26.1% (18.5–34.5), $I^2 = 91.79$, $df = 10$, $Q = 121.2$) revealed major publication bias (LFK = 3.08). The range prevalence of *Cryptosporidium* of each study varied from 16 to 78% (median = 31%) (Fig. 5). No significant difference ($X^2 = 11.9$, $OR = 0.6$; $P > .05$) in the prevalence between the male and female. *Cryptosporidium* was mostly observed in calves (< 6 months) compared to weaned (> 6–12 months) and adult cattle (> 12 months). However, multiple comparison test revealed no statistical significance ($F_{2,12}$; $P = .129$) between the groups. Even though diarrhoeic cattle showed slightly higher prevalence 31.2% (26.4–36.4) than non-diarrhoeic 26.3% (23.1–29.8), there is no significant difference ($X^2 = 2.6$, $OR = 1.27$) in their prevalence. Reported genotypes/species from cattle were *C. bovis*, *C. ryanae*, *C. andersoni* and *C. parvum*. Of all the species, *C. bovis* was mostly reported either as a single infection or mixed with *C. andersoni* or *C. ryanae* (Table 2). The reported *C. parvum* were from slaughtered cattle.

3.5. Cryptosporidium infection in sheep

Of the five studies reported on sheep, there was major asymmetry (LFK = 2.29) (Fig. 5). The pooled prevalence of *Cryptosporidium* in sheep (16.6% (0.5–43.5), $Q = 119.1$, $I^2 = 96.6$, $df = 4$) shows heterogeneity (Table 2). The prevalence varies between 1.0 and 51.0% (median = 16.0%). More cases have been reported in female 52.8% (41.2–64.2) compared to male 28.2% (17.6–40.2) with significant difference in prevalence ($X^2 = 8.4$, $OR = 0.4$). No report of characterised *Cryptosporidium* species was recovered.

3.6. Cryptosporidium infection in goats

Of the 526 total cases examined, 156 were reported positive for *Cryptosporidium* infection (Table 2). The pooled prevalence from six studies revealed (26.0% (2.9–58.2), $Q = 187.0$, $I^2 = 97.3$, $df = 5$) major asymmetry (LFK = 2.65). The prevalence varies between 3.0 and 83.0% (median = 26.5%) (Fig. 5). Females show significant higher ($X^2 = 4.8$, $OR = 0.5$) prevalence when compared to male.

3.7. Cryptosporidium infection in pigs

The prevalence of *Cryptosporidium* infection in pigs (20.1% (8.4–34.9), $df = 5$, $Q = 57.0$, $I^2 = 93.0$) revealed substantial heterogeneity with major asymmetry (LFK = 3.57). The prevalence varies between 14.0 and 45.0% (median = 27.0%) (Fig. 5). Further variability on sex sub-group analyses revealed no significant difference ($X^2 = 0.4$, $OR = 1.1$) in the prevalence [29] between male and female (Table 2).

3.8. Cryptosporidium infection in laboratory animals (rat and rabbit)

The prevalence of *Cryptosporidium* infection in laboratory animals (9.0% (0.0–100), $df = 1$, $Q = 117.2$, $I^2 = 99.2$) revealed substantial heterogeneity (Table 2). Due to the limited number of studies [30,31], the publication bias could not be assessed. The rat and rabbit individual prevalence were 1.5 and 92.6%, respectively with PCR and ELISA diagnostic techniques used. Further variability on *Cryptosporidium* genotype/species analyses revealed the presence of *C. andersoni* (0.1%) and *C. rat genotype* II (0.8%).

3.9. Cryptosporidium infection in birds

Analyses of *Cryptosporidium* infections in birds showed pooled prevalence (7.2% (5.8–87.0), $df = 1$, $Q = 0.3$, $I^2 = 0.0$) with no heterogeneity between studies. However, bird categories within studies revealed variations in prevalence. For instance, wild birds revealed 5.3% (7/132) prevalence across various species, Speckled pigeons (*Columba*

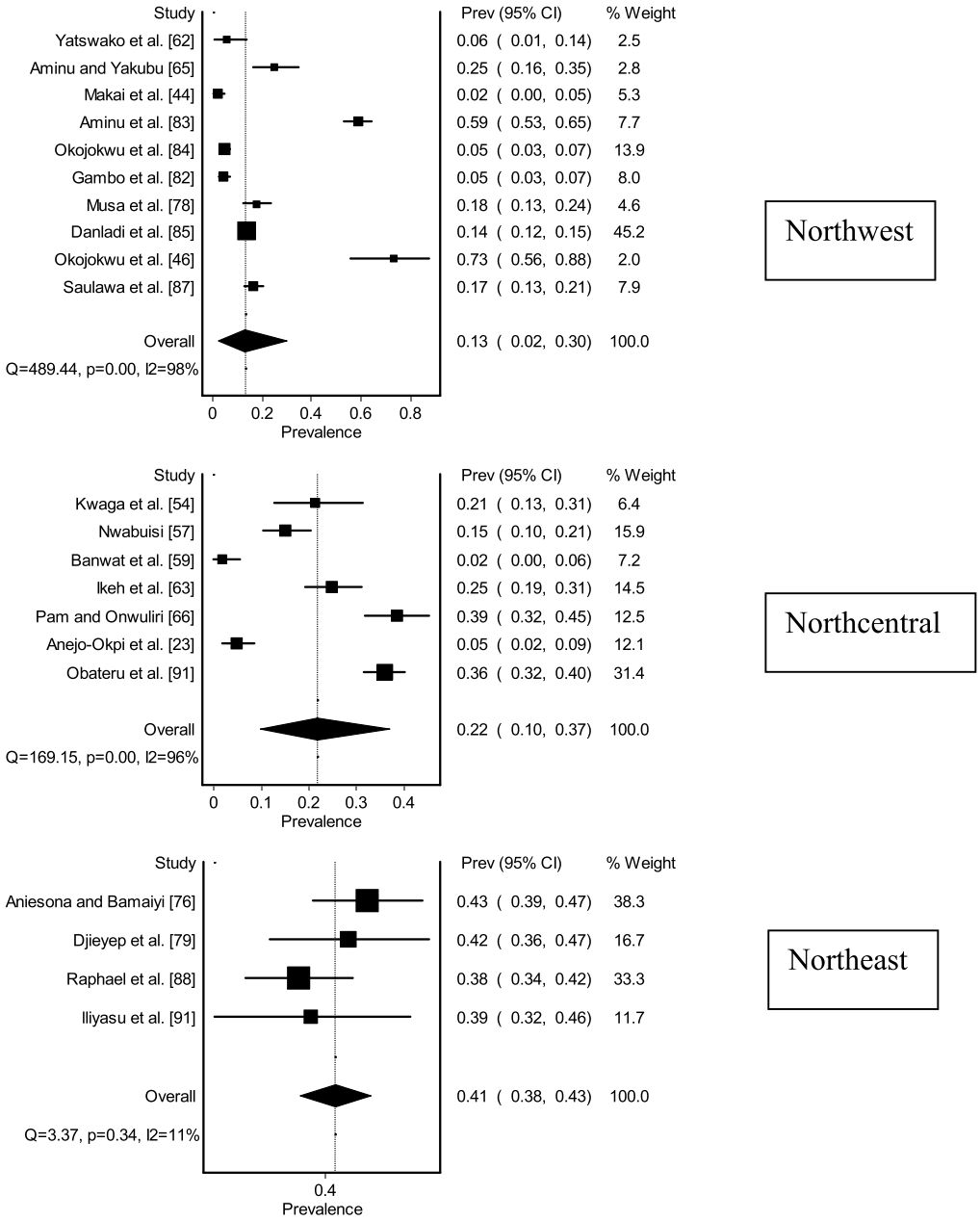

Fig. 3. Forest plot of point estimates of *Cryptosporidium* pooled prevalence of humans in northern geopolitical regions of Nigeria between 1987 and 2017. N·B-Squares are the sample sizes (varied square shapes are represented by sample population; bigger and smaller shapes are automatically generated based on the sample sizes). A diamond indicates the pooled estimate of the total studies [23,44,46,54,57,59,62,63,65,66,76,78,79,82–85,87,88,91].

guinea)-2.4% (1/41), Laughing doves (*Streptopelia senegalensis*)- 5.4% (2/37), Mourning doves (*Streptopelia decipiens*)- 0.0% (0/15), Village weavers (*Ploceus cucullatus*)- 14.3% (4/28), Brown babblers (*Turdoides plebejus*)- 0.0% (0/2), Black crakes (*Limnocorax flavirostra*)- 0.0% (0/3), Red bishops (*Euplectes orix*) - 0.0% (0/4), and Bush fowls (*Francolinus bicalcaratus*)- 0.0% (0/2). Local and Exotic chickens revealed prevalence of 8.2% (46/562) and 6.6% (30/453), respectively. Other poultry groups include Turkeys, Ducks, Guinea fowls and Pigeons at prevalence of 18.8 (3/16), 9.1% (1/11), 0.0% (0/38) and 0.0% (0/8), respectively. Due to the limited number of studies [32,33], the publication bias could not be assessed.

3.10. Environmental sources of Cryptosporidium oocyst in Nigeria

Mean *Cryptosporidium* oocyst concentration detected in surface water in Abakaliki, was 120.6 oocysts per litre [34]. Also, children who are in contact with stream were reported to be highly infected (47.6%) in a study in Ogun state [35]. Other prominent risk factors are food materials consumed especially those from contaminated soil. e.g. vegetables. A study reported *Cryptosporidium* contamination in various vegetables with severity in lettuce (48%) and Fluted pumpkin (44%) in Zaria, Kaduna State [36]. In Ibadan, Oyo State, 0.6% of soil examined in an urban centre were observed to be positive for *Cryptosporidium* oocyst [37]. There is lack of sizeable information on environmental sources and hence the studies could not be included in the final analyses.

3.11. Species variability in Nigeria

Seven studies characterised their subtypes using *gp60* PCR out of the total 10 studies done with PCR for human *Cryptosporidium* infection. Six of these characterised studies had been earlier analysed and reported [38]. The reported species of *gp60* sequences from studies showed that

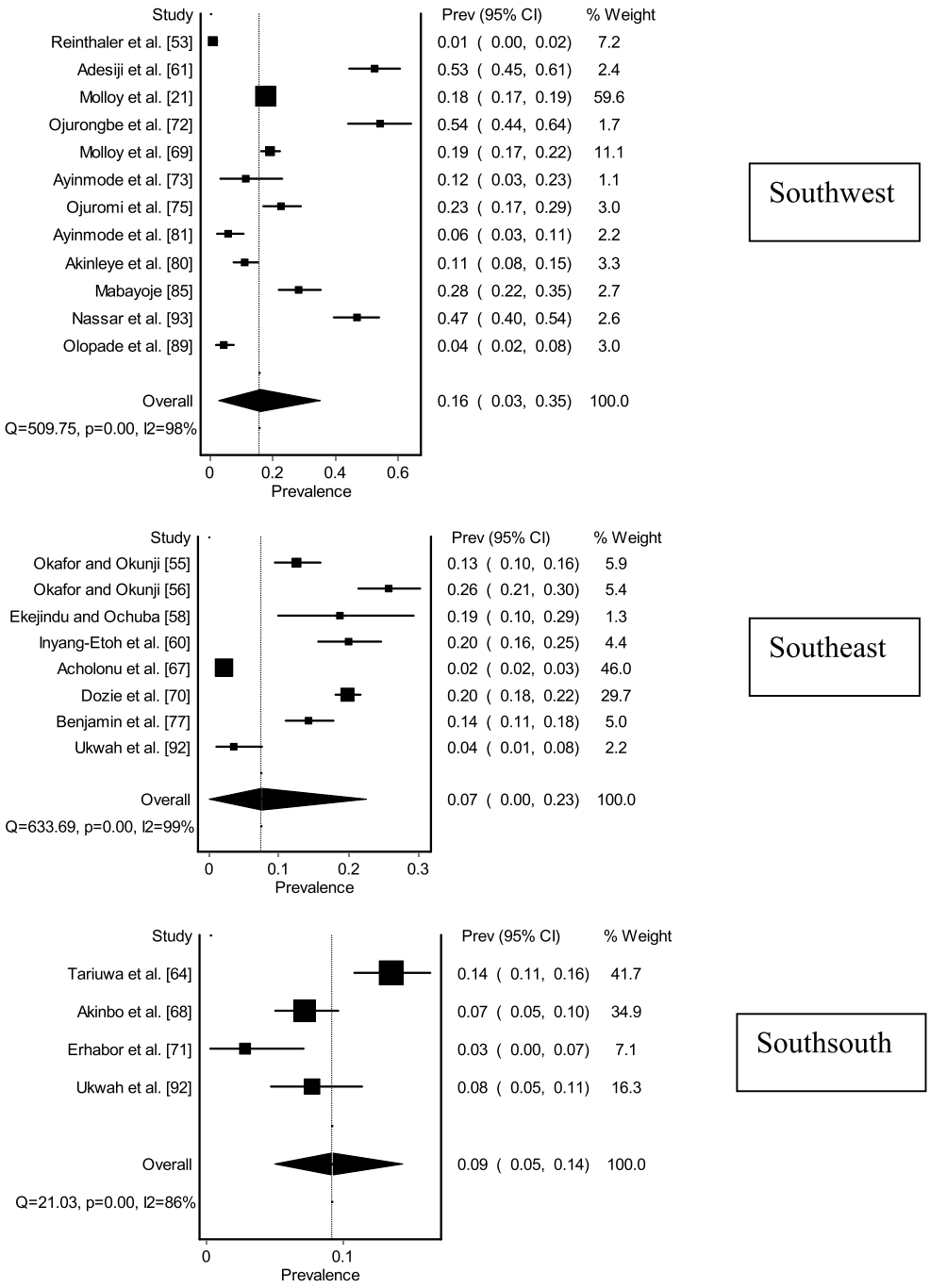

Fig. 4. Forest plot of point estimates of *Cryptosporidium* pooled prevalence of humans in southern geopolitical regions of Nigeria between 1987 and 2017. N·B-Squares are the sample sizes (varied square shapes are represented by sample population; bigger and smaller shapes are automatically generated based on the sample sizes). A diamond indicates the pooled estimate of the total studies [21,53,55,56,58,60,61,64,67–73,75,77,80,81,85,89,92,93].

five subtype families of *C. hominis* (Ia, Ib, Id, Ie and Ih) and six sub-families of *C. parvum* (IIa, IIc, IId, IIe, IIi, and IIm) have been reported thus far in humans in Nigeria (Table 3). Out of the total 1558 examined for either *C. hominis* or *C. parvum* in humans, a prevalence of 5.5% (4.4–6.7) and 4.3% (3.4–5.4), respectively have been characterised using *gp60* sequences (Table 3). The most commonly sub-families of *C. parvum* were IIa and IIc while that of *C. hominis* were Ia and Ie. There were three and one studies recorded for PCR characterisation in cattle and laboratory animals, respectively. Of 300 cattle examined for *gp60* sequences, only *C. parvum* was positive with prevalence of 1.3% (0.5–3.4). No study characterisation for other examined animals. *Cryptosporidium* infection analysis from the databases revealed many states in the country with little or no active screening, while only four states showed areas of active on-going research work on the causal organisms (Fig. 6). More studies have been done identifying the organisms with staining methods, with only few studies using molecular detection.

3.12. Sensitivity tests and bias

The reliability and stability of the analysed results were examined from the sensitivity tests of each analysis conducted in the MetaXL. Further assessment of the funnel plot within the 95% confidence interval and doi plot (LFK index = 1.29) ruled out significant bias risk of

Table 1
Total report of cryptosporidiosis in humans and associated species between 1987 and 2017.

	Sample size	No positive	Pooled Prev.	95% CI	Cochran's Q	I^2	*df*	$\alpha_{0.05}$	Chi^2, OR/ anova
Human infection	22,321	3872	15.0	8.6–22.7	2821.3	98.4	44	< 0.0001	
Region									
Northern	7477	1744	20.8[a]	11.1–32.5	1144.4	98.3	20	< 0.0001	X^2 = 294.8; OR = 1.9
Southern	14,642	2066	11.9	5.0–21.1	1365.8	98.4	22	< 0.0001	
HIV status									
HIV$^+$	2370	503	18.2[a]	6.9–32.8	514.7	97.9	11	< 0.0001	X^2 = 546.2; OR = 13.9
HIV$^-$	3112	59	1.5	0.0–13.8	113.6	96.5	4	< 0.0001	
HAART status and *Cryptosporidium* presence									
HAART$^+$	628	58	8.3	2.7–16.2	25.2	88.1	3	< 0.0001	X^2 = 0.362;
HAART$^-$	275	22	8.2	4.8–12.4	3.6	16.4	3	0.3090	OR = 1.2
CD4$^+$cell counts/μl in HIV$^+$ patients									
< 200	422	174	37.2[a]	1.4–82.8	128.2	96.8	4	< 0.0001	X^2 = 16.0
> 200	913	275	24.8	2.7–56.3	299.5	98.7	4	< 0.0001	OR = 1.6
Faecal consistency									
Diarrhoeic	4853	898	14.3[a]	4.3–28.3	1147.6	98.3	20	< 0.0001	X^2 = 199.6; OR = 2.3
Non-diarrhoeic	5635	511	6.8	1.2–15.5	400.1	97.0	12	< 0.0001	
Sex									
Male	3345	667	17.7[b]	7.5–30.7	471.4	96.6	16	< 0.0001	X^2 = 0.2; OR = 1.0
Female	3601	702	17.1	8.5–27.8	521.6	96.9	16	< 0.0001	
Age									
≤ 5 years	2117	549	22.5	8.3–40.4	361.6	97.5	9	< 0.0001	
> 5–14 years	1392	151	10.3	5.5–16.2	39.3	89.8	4	< 0.0001	$F_{3,17}$; P = .7911
15 – ≤ 50 years	1942	269	10.3	0.0–30.5	150.5	98.0	3	< 0.0001	
> 50 years	69	17	21.7	0.0–64.1	13.0	92.3	1	< 0.0001	
Diagnostic technique									
ZN	6453	1316	19.3	10.6–29.8	402.8	96.3	15	< 0.0001	
SMB	488	68	13.3	5.5–23.7	3.7	73.1	1	0.054	$F_{3,29}$; P = .0744
ELISA	1228	499	40.6	22.2–60.4	170.7	97.7	4	< 0.0001	
PCR	2715	365	12.1	5.7–20.4	193.8	95.4	9	< 0.0001	
Animal contact									
Yes	728	95	12.3	4.8–22.3	36.2	89.0	4	< 0.0001	X^2 = 2.7; OR = 0.8
No	1144	181	12.9[b]	1.5–31.0	145.2	97.2	4	< 0.0001	
Species in humans									
C. parvum	2783	123	3.8	1.3–7.2	100.1	91.0	9	< 0.0001	
C. hominis	2406	110	4.1	1.8–7.3	56.4	85.8	8	< 0.0001	
C. viatorum	526	2	0.5	0.0–1.3	0.8	0.0	1	0.3800	
C. felis	894	3	0.4	0.1–0.9	0.1	0.0	1	0.7760	
C. canis	1192	2	0.2	0.0–0.6	0.1	0.0	1	0.7830	
C. meleagridis	994	9	0.8	0.1–2.1	2.5	60.0	1	0.1140	
C. ubiquitum	994	4	0.4	0.0–1.4	3.2	68.6	1	0.0740	
C. cuniculus	994	7	0.7	0.0–2.2	4.8	79.0	1	0.0290	
C. parvum/C. hominis	994	8	0.8	0.2–1.7	1.4	30.8	1	0.2290	

Abbreviations: I^2 = level of inconsistency, *df* = degree of freedom, $\alpha_{0.05}$ = level of significance, OR = odd ratio, CI = confidence interval, HIV$^+$ = human immunodeficiency virus positive, HIV$^-$ = human immunodeficiency virus negative, HAART$^+$ = highly active antiretroviral therapy positive, HAART$^-$ = highly active antiretroviral therapy negative, CD4 cell = a glycoprotein predominantly found on the surface of helper T cells, *a* = shows significance at P < .05, *b* = no significance at P > .05.

the analysed studies for human *Cryptosporidium* infection. The minor asymmetry observed indicates considerable heterogeneity. For subgroup evaluation of regional publication bias, the LFK index reveals 1.70, −2.03, −1.22, 1.18, 3.46, and −1.24 for northwest, north-central, northeast, southwest, southeast and south-south, respectively. This indicates minor regional publication bias except for northcentral and southeast showing major asymmetry. Publication bias evaluation in animals revealed LFK index of 3.08, 2.29, 2.65, 3.57, in cattle, sheep, goats and pigs, respectively. There is major asymmetry of the examined publications for the animals.

4. Discussion

This meta-analysis aimed to evaluate the prevalence of *Cryptosporidium* infection among humans and animals with associated risk factors in Nigeria. The majority of characterised *Cryptosporidium* spp. from human were mostly positive for *C. hominis* followed by *C. parvum* and other genotypes/species (Table 1). Pooled prevalence in humans (15%) from this study is comparable to the report of the overall high (1.3–31.5%) *Cryptosporidium* prevalence in developing countries [39], Squire and Ryan).

The higher prevalence of *Cryptosporidium* in northern regions despite lower total cases examined when compared to the south is significant. The dry vegetational belt of northern Nigeria could increase the contamination spread to water-bodies. Notably, the northern region being home to nomadic Fulani herdsmen, where most animals are raised in Nigeria, could lead to increased animal contact, thereby, predisposing humans to zoonotic *Cryptosporidium* infections. HIV positive patients showed significantly increased pooled prevalence compared to HIV negative (Table 1). This is due to the immunodeficiencies associated with HIV disease which affects important body organs [40,41]. Heterogeneity observed from HIV patients was due to the group surveyed, such as children or adult, highly active antiretroviral therapy (HAART) or non-HAART patients. The reduction in viral load by antiretroviral drugs and simultaneous increase in the CD4 counts have been observed to reduce oocyst shedding and clinical symptoms

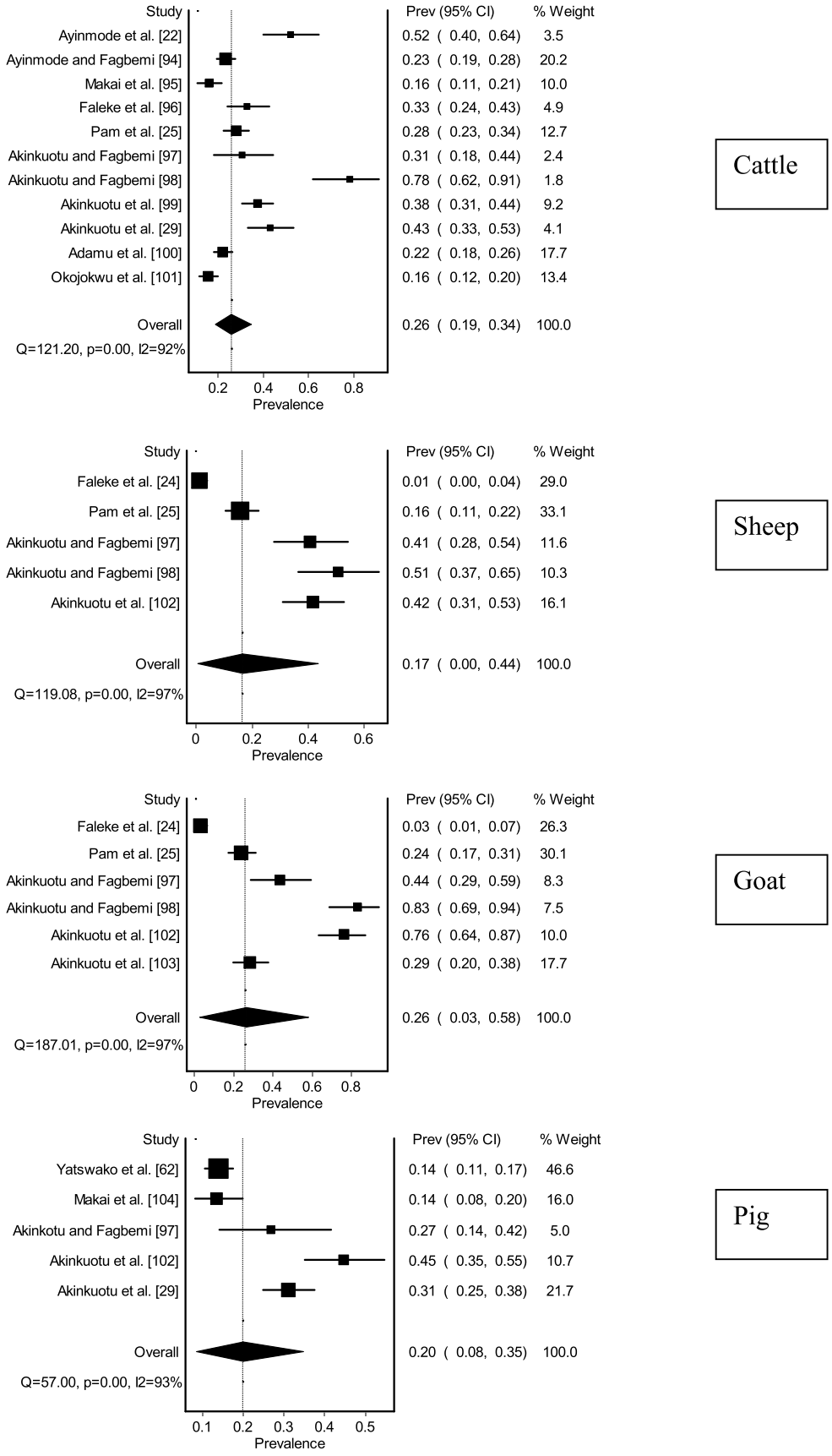

Fig. 5. Forest plot of livestock (cattle, sheep, goats and pigs) *Cryptosporidium* infection in Nigeria using quality effects model between 1987 and 2017. N·B- Squares are the sample sizes (varied square shapes are represented by sample population; bigger and smaller shapes are automatically generated based on the sample sizes). A diamond indicates the pooled estimate of the total studies [22,24,25,29,62,94–104].

Table 2
Report on cryptosporidiosis in animals and associated *Cryptosporidium* species between 1987 and 2017.

	Sample size	No positive	Pooled prev.	95% CI	Cochran's Q	I^2	*df*	$\alpha_{0.05}$	Chi^2, OR/ anova
Cattle	2091	555	26.1	18.5–34.5	121.2	91.7	10	< 0.0001	
Sex									
Male	755	159	20.6	13.7–28.5	17.6	71.6	5	< 0.0001	X^2 = 11.9; OR = 0.6
Female	489	145	28.9[A]	19.9–38.8	23.3	78.5	5	< 0.0001	
Age									
Calves (≤ 6 months)	561	202	35.7	19.2–54.0	105.7	93.4	8	< 0.0001	$F_{2,12}$; P = .129
> 6–12 months	198	41	19.5	6.1–37.4	11.6	82.8	2	0.003	
> 12 months	689	164	23.7	19.0–28.7	5.8	48.6	3	0.120	
Faecal consistency									
Diarrhoeic	327	102	31.2	26.4–36.4	46.9	89.3	5	< 0.0001	X^2 = 2.6; OR = 1.27
Non-diarrhoeic	654	172	26.3	23.1–29.8	21.1	76.3	5	0.001	
Species									
C. bovis	559	40	6.7	0.0–18.4	33.3	94.0	2	< 0.0001	
C. ryanae	559	15	2.6	0.0–7.1	12.3	83.7	2	0.0020	
C. andersoni	194	5	2.6	1.1–5.9	–	–	–		
C. parvum	300	4	1.3	0.5–3.4	–	–	–		
C. bovis/C. andersoni	194	1	0.5	0.1–2.9	–	–	–		
C. bovis/C. ryanae	259	14	0.9	0.0–2.5	0.8	0.0	1	0.3810	
Sheep	480	105	16.6	0.5–43.5	119.1	96.6	4	< 0.0001	
Sex									
Male	61	17	28.2	17.6–40.2	0.1	0.0	1	0.716	X^2 = 8.4; OR = 0.4
Female	72	38	52.8[A]	41.2–64.2	0.4	0.0	1	0.524	
Goat	526	156	26.0	2.9–58.2	187.0	97.3	5	< 0.0001	
Sex									
Male	95	35	35.4	8.0–68.4	13.9	85.6	2	< 0.0001	X^2 = 4.8; OR = 0.5
Female	95	50	53.4[A]	21.6–84.0	16.9	88.2	2	< 0.0001	
Pig	802	194	20.1	8.4–34.9	57.0	93.0	4	< 0.0001	
Sex									
Male	260	78	29.1[B]	18.6–40.7	9.9	69.6	3	0.02	X^2 = 0.4; OR = 1.1
Female	220	60	22.8	5.9–45.4	89.3	89.3	3	< 0.0001	
Laboratory animal	161	27	9.0	0.0–100	117.2	99.2	1	< 0.0001	
Species									
C. andersoni	194	1	0.1	0.1–2.9	–	–	–	–	
C. rat genotype II	134	1	0.8	0.0–4.1	–	–	–	–	
Birds	1220	87	7.2	5.8–87.0	0.3	0.0	1	0.5600	

Abbreviations: I^2 = level of inconsistency, *df* = degree of freedom, $\alpha_{0.05}$ = level of significance, OR = odd ratio, CI = confidence interval, Chi^2 – chi-square.

[42]. The opportunistic capability of *Cryptosporidium* complicates HIV/AIDS patients and a < 200/ml CD4 T-cell counts reported individuals exposed to *C. parvum* for instance invariably develop prolonged infection with persistent diarrhea [43]. Furthermore, it has been suggested that HIV protease inhibitors have anti-parasitic potential [38], which could make these individuals to be highly susceptible to cryptosporidiosis.

Children under the age of 5 years had 18.2% prevalence from the pooled estimates (Table 1). Remarkably, infection was reported in children before the age of one [44]. Hence, cryptosporidiosis is one of the leading causes of diarrhea among children and the immunosuppressed [1,4]. Diarrhoeic immunocompromised patients (children < 5 years, aged, HIV patients) have been observed to be significantly susceptible group with increased prevalence when compared to non-diarrhoeic patients in Iran [39]. This suggest that these groups need to be monitored for cryptosporidiosis in Nigeria. Factors responsible for infection among teeming populations include accidental ingestion of the oocyst from contaminated environment, contact with infected humans and animals, travelling to endemic countries and seasonal factors [45]. Generally, the only available drug (nitazoxanide) against *Cryptosporidium* is ineffective in HIV and malnourished individuals [38].

The diagnostic methods used to identify *Cryptosporidium* spp. infection differed among studies. The advent of genotyping tools has greatly improved identification of genotypes/species. Hence, more studies are warranted using recent molecular tools for proper identification of genotypes/species. The use of small subunit (*ssu*) rRNA-based genotyping tools revealed the presence of *C. viatorum*, *C. cuniculus*, *C. ubiquitum*, *C. canis*, *C. felis* and *C. meleagridis* in humans, in addition to the commonly identified *C. parvum* and *C. hominis*. This same occurrence has been reported in Kenya, Switzerland and United States [14]. Of the *C. parvum* assessed, IIa, IIc, IId, IIe, IIi and IIm were reported. Common IIa subtype family, especially the IIaA15G2R1 observed in humans and cattle from this study are also the dominant *C. parvum* subtype family in humans from Netherlands, Spain, Italy, Slovenia, Belgium, Portugal and others [13]. Although, in Nigeria, the zoonotic IIa subtype family was not the most predominant, instead the anthroponotic IIc subtypes family predominate. The IIc subtype family were only found in the examined HIV positive patients. This is because transmission in humans in developing countries are largely anthroponotic [9]. Four IId subtype family were reported from diarrhoeic patients [46], which is a less common zoonotic subtype family. Other *C. parvum* subtype families observed were IIb, IIe, IIi and IIm, respectively in humans. The IIb and IIe subtype families were widely reported in other African countries such as Malawi, Kenya and Uganda [13]. The IIi and IIm subtype families were less reported. *C. hominis* subtype families observed from this study include Ia, Ib, Id, Ie and Ih. Though, the latter seems to be less common and only reported in one study. The *C. hominis* Ia subtype family was the most prevalent from this analysis. *C. hominis* Ia and Id subtype families were observed in the northern region while all the subtype families were found in the southern region. Though, geographic variations in distribution have been reported, the limitation of the subtype families in northern region could be due to limited studies on genotyping. The high heterogeneity of *C. hominis* could be attributed to stable and prevalent *Cryptosporidium* transmission in Nigeria. Subtype families Ih for *C. hominis* and IIi and IIm for *C. parvum*

Table 3
Reported genotypes of *C. hominis* and *C. parvum* in Nigeria from molecular characterisation and sequencing of *gp60*.

Species/ genotype	Host	Total cases	No positive/ percentage	Subfamily	Subtype	References
Total	Human	1558	162 (10.4%)			
C. hominis		162	85 (52.5%)			
		85	32 (37.6%)	Ia	IaA14R3, IaA16R3, IaA24R3, IaA25R3	Akinbo et al. [68]
					IaA18R2, IaA22R2, IaA24R2, IaA25R2, IaA28R2, IaA21R1	Molloy et al. [21]
					IaA24R3	Ayinmode et al. [73]
					IaA23R3, IaA25R3	Maikai et al. [44]
					IaA14R3, IaA24R3, IaA27R3	Ayinmode et al. [81]
					IaA14R6, IaA15R3	Okojokwu et al. [46]
					IaA27R4, IaA18R3, IaA25R3, IaA29R3	Ukwah et al. [92]
		85	17 (20.0%)	Ib	IbA13G3	Akinbo et al. [68]
					IbA10G2, IbA13G3	Molloy et al. [21]
					IbA13G3	Ayinmode et al. [73]
					IbA10G4	Ayinmode et al. [81]
					IbA13G3, IbA10G2	Ukwah et al. [92]
		85	8 (9.4%)	Id	IdA11, IdA17	Molloy et al. [21]
					IdA10G2, IdA10	Okojokwu et al. [46]
		85	13 (15.3%)	Ie	IeA11G3T3	Akinbo et al. [68]
					IeA11G3T3	Molloy et al. [21]
					IeA11G3T3, ***	Ayinmode et al. [81]
					IeA11G3T3	Okojokwu et al. [46]
					IeA11G3T3, ***	Ukwah et al. [92]
		85	1 (1.2%)	Ih	IhA14G1	Molloy et al. [21]
C. parvum		162	67 (41.4%)			
		67	9 (13.4%)	IIa	IIaA15G2R1, IIaA16G1R1	Molloy et al. [21]
					IIaA15G2R1, IIaA16G2R1	Okojokwu et al. [46]
		67	36 (53.7%)	IIc	IIcA5G3a, IIcA5G3h, ***, ***	Akinbo et al. [68]
					IIcA5G3a, IIcA5G3b	Molloy et al. [21]
					IIcA5G3k	Ayinmode et al. [73]
					IIcA5G3a	Okojokwu et al. [46]
					IIcA5G3b, IIcA5G3k	Ukwah et al. [92]
		67	4 (6.0%)	IId	IIdA15G1R1	Okojokwu et al. [46]
		67	4 (6.0%)	IIe	IIeA10G1	Maikai et al. [44]
					IIeA10G1	Ukwah et al. [92]
		67	2 (3.0%)	IIi	IIiA11	Molloy et al. [21]
		67	2 (3.0%)	IIm	IImA14G1	Molloy et al. [21]
	Cattle	300	4 (1.3%)			
C. parvum		4	2 (50.0%)	IIa	IIaA15G2R1$^{\phi}$ (2)	Okojokwu et al. [101]
		4	2 (50.0%)	IId	IIdA15G1$^{\phi}$ (2)	Okojokwu et al. [101]

Abbreviations: GP = glycoprotein, *** = unknown subtype in the study, ϕ = recovered from cattle samples.

have only been reported once in humans [21,47]. PCR-based *Cryptosporidium* detection is often done with the multi-copy *ssu* rRNA gene marker that provides higher diagnostic sensitivity. The low prevalence attributed to *Cryptosporidium* species observed in sub-genotyping was because the *gp60* gene is more suited for higher molecular diversity at the nucleotide level, and it is only a single-copy gene with lower diagnostic sensitivity when compared to the *ssu* rRNA gene [47].

The development of a Mboll RFLP procedure to differentiate the commonly found three intestinal *Cryptosporidium* spp. (*C. bovis*, *C. ryanae*, *C. andersoni*) has facilitated studies on bovine cryptosporidiosis over the years [48]. However, researchers in Nigeria just recently began to use molecular tools to identify *Cryptosporidium* spp. in animals. There was no reported study on animal cryptosporidiosis until the mid-2000s in Nigeria. Several species have now been reported in cattle, while few have been reported from laboratory animals in Nigeria (Tables 1 and 2).

Cattle are established source of zoonotic cryptosporidiosis [49] and the reported *C. parvum* in slaughtered cattle [46], indicates that abattoir workers could be exposed to the zoonotic genotype of *Cryptosporidium* in Nigeria [14]. Although, no significant difference between diarrhoeic and non-diarrhoeic cattle, the higher prevalence in diarrhoeic cattle makes it a substantial risk factor. A clear pattern of age-related infection by different *Cryptosporidium* species has been reported in cattle, with *C. parvum* primarily causing symptomatic infections in calves and *C. andersoni* and *C. bovis* causing symptomatic infections in older animals [50]. Although, the report from this study showed higher prevalence in calves compared to adult cattle, but there seems to be no significance between the pooled prevalence. The presence of zoonotic *C. parvum* (IIa and IId) in cattle indicates that human contact with infected calves could lead to cryptosporidiosis outbreaks among veterinarians, students, abattoir workers and research technicians [14]. The observed *C. parvum*, *C. bovis*, *C. ryanae* and *C. andersoni* have been identified worldwide as the four major species in cattle [13]. Mixed infection of *C. bovis / C. ryanae*, and *C. bovis / C. andersoni* was also reported. This indicates the high prevalence of *C. bovis* in Nigerian cattle. More studies are required on sheep, goats, pigs and other mammals to reduce the publication bias from limited studies.

Socioenvironmental predicators could serve as potential risk factors. Information on *Cryptosporidium* from soil and water sources in different environment including urban, rural, sub-urban, animal-holding areas such as abattoirs, lairage, slaughter slabs, and industrial areas could improve the understanding of the transmission dynamics and the spread of the organism among several host species. Tourist centres in Africa such as game reserves and national parks have recovered *Cryptosporidium* oocyst and genotypes from wildlife [51]. Meanwhile, our database revealed dearth of information on *Cryptosporidium* species from the environment, hence further studies are required in a One-Health approach. *Cryptosporidium* oocysts have been identified in Mollusc collected from Tiga Lake in Kano State, Nigeria [52]. Hence studies on co-infection of trematodes and *Cryptosporidium* in animals could be a possibility. The use of fresh manures as fertilizer in agricultural farmlands could pose public health risk due to the presence of *Cryptosporidium* in soil. Similarly, poor sanitation and hygiene such as uncontrolled human defaecation in agricultural land could increase the spread of *Cryptosporidium* oocyst in the environment.

The limitation from this study is that the numbers of studies in subgroup analyses are quite small, hence predictive values and estimates of

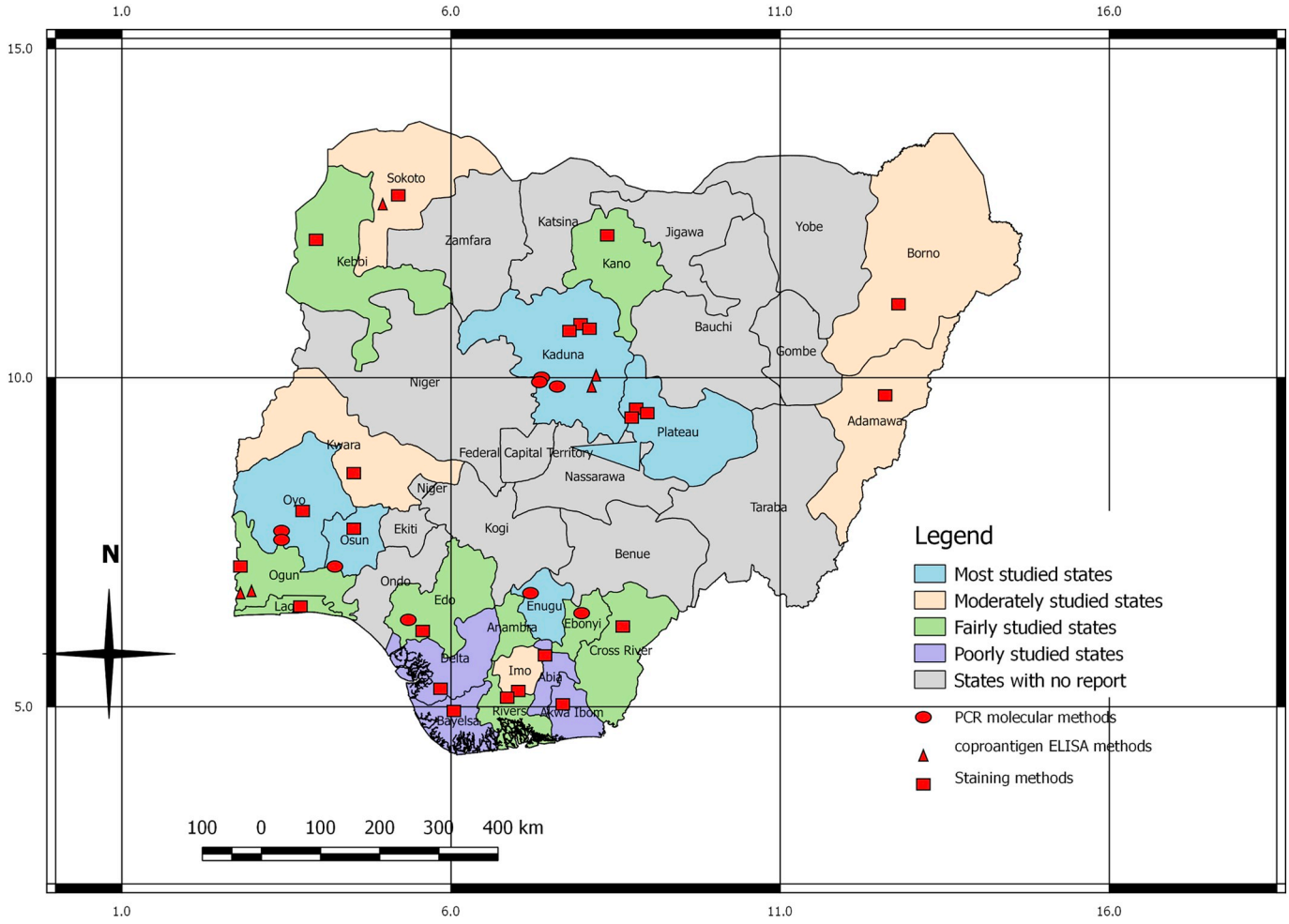

Fig. 6. Map showing study states, the intensity of studies and diagnostic tools used between 1987 and 2017.

the risk factors in some instance (e.g. some regional analysis, SMB diagnostic technique, aged patients and environmental sources of infection) need to be assessed accordingly. However, this is the first study that provides an understanding into the epidemiology of *Cryptosporidium* infection in Nigeria which can be applied to effectively implement control strategy.

5. Conclusions

The prevalence of *Cryptosporidium* infection in human, cattle, sheep, goat and pigs in Nigeria was high (15.0, 26.1, 16.6, 26.0 and 20.1%, respectively) and associated with risk factors from human activities. However, due to low perception of the disease coupled with poor hygiene, this prevalence might significantly expose humans especially the immunocompromised patients and children. Therefore, there is a need for continuous assessment of animals including avian species and prompt reporting of zoonotic genotypes identified. Strict sanitation by proper sewage and animal dung disposal is required to prevent the continuous spread of anthroponotic transmission. Good management practices are recommended to prevent further increase in its prevalence. This study will serve as a reference for national epidemiological studies in identifying best approach in management, treatment and control of cryptosporidiosis across various regions in Nigeria.

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Ethical approval

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Informed consent

There is no informed consent on this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2019.04.007.

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