

Prevalence of Intestinal Parasitism and Associated Risk Factors in Rural and Urban Areas of Southern Algeria: a Four-year Community Based Study

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Research

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Abstract

Background: Intestinal parasitic infections are amongst the most common infections worldwide and have been identified as one of the most significant causes of morbidity and mortality among disadvantaged populations. This community based study was conducted to assess the magnitude and pattern of intestinal parasitism and to identify the key risk factors associated with intestinal parasitic infections in Laghouat province, south Algeria.

Methods: Stool samples were collected from 2277 symptomatic and asymptomatic patients aged 1-89 years old. Structured questionnaire were used to identify environmental, socio demographic and behavioral factors. Stool specimens were collected and examined for intestinal parasitic infection.

Results: More than 33% of participants were found to be infected for at least one protozoan and/or helminth parasite. Of these, the most common parasites were *Blastocystis* spp. (17.79%), followed by *Entamoeba histolytica/dispar* (8.78%), *Giardia intestinalis* (4.22%), *Entamoeba coli* (4.08%) and more rarely *Endolimax nana*, *Cryptosporidium* spp, *Trichomonas intestinalis*, *Enterobius vermicularis*, *Taenia* spp., and a single case of *Trichuris trichiura*. Multivariate analysis revealed significant associations between prevalence and species richness of combined protozoan parasites and contact with animal, living in rural areas and relying primarily on tap water, particularly in young individuals. Analysis of temporal consistency showed a high rate of infection in rural locations in the spring of 2018 and a firm relationship between school children and tap water was also detected.

Conclusions: The high prevalence of intestinal parasitic infections among the Laghouat population, with identification of several risk factors indicates that parasitic infections are important public health problems. Therefore, several strategies are recommended in order to effectively reduce these infections including good animal husbandry practices, health education focused on good personal hygiene practices especially for school children, availability of safe drinking water and adequate sanitation.

Introduction

Intestinal parasitic infections are amongst the most common infections worldwide causing significant morbidity and mortality [1]. It is estimated that some 3.5 billion people are affected annually, and that 450 million develop clinical illness as a result of these infections and of those, at least 50% are school-age children [2]. These infections cause serious public health problems ranging from diarrhea and stunting in children to impaired cognitive development, iron deficiency anemia and other physical and mental health problems [3]. The distribution and prevalence of various species of intestinal parasites differ from country to country and even regionally within countries. However, many studies have revealed high frequencies of parasitic infections in rural areas compared with urban areas [4, 5]. This difference may be linked causally to any, or a combination of factors including, lack of sanitation, poverty, close contact with animals, lack of access to safe water, poor socioeconomic conditions and poor hygiene in rural communities [6].

Common intestinal parasites with direct life cycles, are transmitted mostly by the fecal oral route through direct contact with infected persons or animals, or indirectly through ingestion of contaminated water or food. Giardiasis, caused by *Giardia intestinalis* is a frequent cause of diarrhea, affecting approximately 200 million people worldwide [7], whereas *Blastocystis* spp. is currently estimated to infect up to 1 billion humans worldwide [8]. Among parasitic diseases, deaths attributable to intestinal amebiasis, caused by the protozoan *Entamoeba histolytica*, are only exceeded by those from malaria and schistosomiasis [9]. Approximately 40 million people, worldwide suffer from this disease per year and 40,000 die due to the resulting dysentery and liver abscesses [10]. The opportunistic protozoan, *Cryptosporidium* spp. has also emerged as an important cause of diarrheal illnesses worldwide, particularly in young children and immunocompromised patients, with a prevalence of 4% in developed countries and three to four times more frequent infections in developing countries [11]. These parasites constitute serious health challenges particularly in economically developing countries and are regarded as the most common and important etiological agents for more than 900 waterborne outbreaks of epidemic and endemic human diseases [12, 13].

In this context, surveys carried out in Algeria and neighboring countries have highlighted the presence of protozoan cysts and helminths eggs in treated wastewater used for irrigation in Médéa Algeria [14], and even in water stored in private cisterns in rural areas of Tunisia [15]. These transmission stages of parasites have also been recorded in faecal samples of exposed populations that use wastewater in agriculture in rural areas of Morocco [16]. No major waterborne or foodborne outbreaks of giardiasis, amoebiasis or cryptosporidiosis have been reported so far in Algeria, but the presence of these protozoan genera has been recorded in epidemiological studies of human populations [17–20] and in domestic animals highlighting the high risk of zoonotic transmission [21–23].

To the best of our knowledge, no current information is available on the potential risk factors associated with these protozoan infections for communities living in the Algerian steppe area. Laghouat province is characterized by a diversity of life styles and includes urban, rural and Bedouin communities. It is also known as the province with the second most numerous livestock ownership in Algeria, so this makes Laghouat an interesting area for parasitological research although to date there are no published reports on the extent of prevalence and distribution of human intestinal parasites among people living in this region. Thus, the objective of the current study was to determine the prevalence of intestinal parasites and to identify associated risk factors among the Laghouat population.

Materials And Methods

Study area and study subject

The study was carried out in the province of Laghouat, the province with the second most numerous livestock and one of the important socio-economical spot in Algeria, located in the center of the country at 400 km to the south of the capital Algiers (33° 48' N, 02° 53' E). The province of Laghouat is affected by three types of climates: semi-arid in the north, arid in the center and Saharan in the extreme south of

the province. Daily temperatures average – 5°C in winter months and over 40°C in summer. The average annual rainfall is 151.21 mm per year [24]. Laghouat province is located on the banks of the Mzee valley in the Amour Range of the Saharan Atlas mountains, and constitutes an oasis on the northern edge of the Sahara Desert. This province covers about 25,052 km² and had a population estimated at 603.876 inhabitant in 2016, of which 81.5% lived in an urban environment and 4.9% in rural areas of the province [25]. These two populations live under essentially similar conditions with respect to local infrastructure and provision of drinking water, which is made available through boreholes and taps (supplied by wells or public water delivery systems). However, the rural population is mainly engaged in agricultural activities such as livestock husbandry and breeding (sheep, goats, cattle, horses, camels, chicken) [26]. The remaining 13.6% of the population, the Bedouin (Nomads), live in sparsely populated parts of the province in relatively isolated communities. This population lives without infrastructure, with unsafe water supplies and inadequate levels of sanitation [25].

We carried out a community based study (March 2015 to July 2018) involving symptomatic and asymptomatic patients who attended the laboratory of public hospital (LPH) and a private laboratory for medical analysis (Dr. Debagha) for parasitological examination. Patients were from two municipalities in urban areas; Laghouat (n = 916), Ksar El Hiran (n = 321) and from four municipalities in rural areas; Kheng (n = 408), Sidi Makhoulouf (n = 216), Tadjmout (n = 226) and Hassi Delaa (n = 190) (Fig. 1). In general, parasite assessments are requested by doctors following the presence of digestive disorders and may also be requested for provision of a medical certificate of health status required for job applications and for conscripted soldiers joining the army.

Sample collection and stool examination

We assessed intestinal parasitic infections among 2.277 hospitalized subjects and outpatients, aged 1–89 years (27.4 ± 18.66) who had been referred to the two laboratories referred to above for routine stool tests in Laghouat province. The objectives of this study were explained to the participants and a standard questionnaire summarizing personal information was completed by each individual or his/her guardian (i.e. age, sex, place of residence (urban or rural), the nature of the water consumed, the presence of digestive disorders, immune status, and possible contact with animals).

Fresh fecal specimens were collected from each subject in 25 ml clean wide-mouth, covered plastic containers and then immediately examined in normal saline (0.9%) and Lugol's iodine preparations, using direct microscopy as part of a routine parasitological examination. After completing the direct stool examination, the formol ether concentration and Willis flotation techniques were performed in order to increase the likelihood of detection of parasites [27]. Additional individual fecal smears were fixed with methanol and stained by the modified Ziehl-Neelsen for detection of intestinal coccidian parasites. Stool samples suspected of containing *Blastocystis* were inoculated into 5ml of modified Boeck and Drbohlav Locke-egg medium supplemented with 10% horse serum and incubated at 37°C. The cultures were checked for the presence of *Blastocystis*, by direct microscopy on the third day after inoculation. We performed also the Graham technique (Scotch test) for detection of pinworm infection in children.

Statistical analysis

Data are summarized as prevalence (percentage of infected subjects \pm 95% confidence limits [CI₉₅]) and as mean protozoan species richness (PSR, mean number of protozoan species harbored per subject \pm standard error of the mean (S.E.M.)). The 95% confidence limits were calculated by bespoke software based on the tables of Rohlf and Sokal (1995) [28]. Analysis of data for each of the protozoan infection measures (prevalence and PSR) was undertaken in two phases. First, for prevalence univariate log-linear models were fitted in IBM-SPSS (version 24) with each factor in turn and infection (combined protozoan infections at 2 levels, present or absent), as described elsewhere [29]. For PSR we fitted non-parametric tests (Mann-Whitney *U* test or Kruskal Wallis test). We fitted in turn the following seven factors SEX, (at two levels, male or female), AGE (11 levels corresponding to the following age limits, level 1 = 1–4 years; level 2 = 5–9; level 3 = 10–14; level 4 = 15–19; level 5 = 20–24; level 6 = 25–29; level 7 = 30–34; level 8 = 35–39; level 9 = 40–44; level 10 = 45–54; level 11 = > 54), AREA (location in which people lived either rural or urban), YEAR (4 levels, 2016–2018), SEASON (4 levels, spring, summer, autumn and winter), WATER (2 levels, subjects using bottled or tap water) and ANIMALS (subjects owned or did not own domestic animals).

In a second phase we fitted multi-factorial models. For analysis of prevalence we used maximum likelihood techniques based on log-linear analysis of contingency tables as described previously [29], beginning with the full factorial model, and then employing the backward selection procedure in SPSS we simplified the model until only significant terms remained. For each level of analysis in turn, beginning with the most complex model, involving all possible main effects and interactions, those combinations that did not contribute significantly to explaining variation in the data were eliminated in a stepwise fashion beginning with the highest level interaction (backward selection procedure). A minimum sufficient model was then obtained, for which the likelihood ratio of chi-square was not significant, indicating that the model was sufficient in explaining the data. The importance of each term in interactions involving infection in the final model was assessed by the probability that its exclusion would alter the model significantly and these values are given in the text, assessed by a likelihood ratio test between nested models with and without each factor of interest.

For analysis of PSR we implemented a multifactorial generalized linear model (GLM), fitted with a Poisson log link in SPSS 24, incorporating all the main effects and all relevant 2-way interactions. As above, the model was simplified in steps beginning with the full factorial model. Model simplification was by backward selection, deleting the least significant interaction in turn at each successive cycle, until only significant 2-way interactions, and relevant main effects remained. Significance was based on the Wald χ^2 output of the minimum sufficient model thus generated.

Relationships between prevalence of infection or PSR, and levels within specific factors that showed a directional trend (meaningful increase across levels e.g. change with host age) were examined by the non-parametric Spearman's test in SPSS 24, and *rs* is given. *P* values less than 0.05 were considered to indicate statistical significance.

Results

The overall prevalence of parasitic infections in the study population was 33.3% [31.40-35.27], the majority of which were attributable to protozoan species (prevalence = 32.5% [30.62–34.47]), helminth infections in this population being relatively rare (prevalence = 0.79% [0.469–1.249]). The prevalence of each of the ten species of parasitic organisms identified is given in Table 1.

Table 1. Prevalence of protozoan and helminth species.

	n	Prevalence (%)	Lwr Lim	Upr Lim
<i>Blastocystis</i> spp.	405	17.79	16.216	19.357
<i>Entamoeba histolytica/dispar</i>	200	8.78	7.621	9.946
<i>Giardia intestinalis</i>	96	4.22	3.415	5.149
<i>Entamoeba coli</i>	93	4.08	3.296	5.004
<i>Endolimax nana</i>	40	1.76	1.255	2.392
<i>Cryptosporidium</i> spp.	06	0.264	0.097	0.574
<i>Trichomonas intestinalis</i>	23	1.01	0.640	1.516
<i>Enterobius vermicularis</i>	13	0.571	0.304	0.976
<i>Taenia</i> spp.	04	0.176	0.048	0.450
<i>Trichuris trichiura</i>	01	0.044	0.001	0.245

The most common species was *Blastocystis* spp. and the rarest was the nematode *T. trichiura*, which was recorded in only one subject. Because helminths were so rare in this group of subjects, further analysis is focused on infections by protozoan species, but it is of interest to note that of the 18 subjects infected with helminths, 16 were under 14 years in age, and 13 of these were attributable to *E. vermicularis*. Only two adult subjects were diagnosed with helminths, both in their 30s and both with infections with *Taenia* spp.

Combined species richness (both protozoa and helminths) was 0.39 ± 0.013 species/host, and mostly attributable to the protozoan species (0.38 ± 0.013). Single species protozoan infections were the most common (27.8% of total population, and 85.6% among the protozoa infected subjects), and multiple species protozoan infections much rarer (two species = 4.1%, three species = 0.57%, and four species 0.44%, of all subjects).

Intrinsic factor affecting prevalence and species richness of combined protozoan parasites

Prevalence of combined protozoan infections varied significantly with host age (Fig. 2A; $\chi^2_{10} = 34.15$, $P < 0.001$), increasing from age class 1 to age class 2 by 11.4% and then falling as host age increased to a low of 23.1% among the oldest age class. Although the fall in prevalence with increasing host age was modest (a reduction of 21.7% from age class 2 to 11), it was nevertheless significant ($r_s = -0.095$, $n = 2.277$, $P < 0.001$). Prevalence was very similar and not significantly different between the sexes (Table 2; $\chi^2_1 = 0.173$, $P = 0.68$).

Protozoan species richness (PSR) also varied significantly between the age classes (Fig. 2B; Kruskal-Wallis test, $H_{10} = 35.42$, $P < 0.001$), and showed declining values with increasing host age ($r_s = -0.099$, $n = 2.277$, $P < 0.001$), much as for prevalence. Interestingly, however despite the lower mean values in subjects in age classes 7–11, corresponding to subjects who were older than 29 years of age, eight of the 13 cases of 3-species infections occurred in these age classes. The single case of a 4-species infection was in a 21-year old male. Mean PSR was very similar in both sexes and not significantly different (Table 2; $z = 0.781$, $P = 0.44$).

Seasonal, annual and location-specific effects on prevalence and species richness of combined protozoan parasites

Table 2 shows the prevalence of combined protozoan infections in each year, season and area in which the subjects lived. Analysis of each factor in turn revealed that prevalence differed significantly between subjects living in rural and urban areas ($\chi^2_1 = 26.67$, $P < 0.001$), with prevalence among subjects from rural sites 10.2% higher than that for subjects living in urban sites.

Table 2. Prevalence of combined protozoan infections, by year, season and area.

	n	Prevalence		Species richness	
		%	± 95% C.L.	Mean	± S.E.M.
Sex					
Male	1333	32.0	29.45-34.46	0.37	0.016
Female	944	33.4	29.45-37.51	0.39	0.020
Area					
Urban	1237	27.9	25.39 - 30.39	0.32	0.016
Rural	1040	38.1	35.13 - 41.03	0.46	0.020
Year					
2015	436	28.7	23.15 - 34.84	0.32	0.026
2016	789	33.0	29.43 - 36.69	0.38	0.021
2017	544	35.7	32.63 - 38.81	0.41	0.026
2018	508	31.9	28.03 - 34.89	0.40	0.029
Season					
Spring	679	33.9	30.55 - 37.33	0.41	0.025
Summer	453	30.2	24.53 - 36.57	0.34	0.027
Autumn	641	32.9	29.74 - 36.27	0.36	0.022
Winter	504	32.3	29.48 - 35.34	0.39	0.027
Water					
Bottled	1076	29.6	26.92 - 32.38	0.34	0.018
Tap	1201	35.1	32.44 - 37.84	0.41	0.018
Animal ownership					
No	1632	23.0	21.00 – 25.08	0.26	0.013
Yes	645	56.6	53.12 – 60.00	0.68	0.027

As Table 2 shows, overall prevalence was similar in each of the years of the study varying only between 28.7% (2015) and 35.7% (2017). Prevalence was even more constant when examined by season, varying only from 30.2–33.9%. Neither of these was significant. However, when all three factors were fitted in a multifactorial model, the highest order interaction (YEAR x SEASON x AREA x INFECTION) could not be simplified ($\chi^2_9 = 20.673$, $P = 0.014$), indicating significant interactions at this level. These are illustrated in Fig. 3, which shows that whereas prevalence in most data subsets centred around 30%, unusually low prevalence was detected in 2018 in spring and summer among people living in urban areas and a very high prevalence was seen also in 2018 in the spring in rural areas.

Much the same outcome was seen when PSR was analysed by non-parametric tests: there was a highly significant difference in mean PSR between subjects from urban and rural areas (Mann-Whitney-U test, $z = 5.406$, $P < 0.001$), but none between the seasons (Kruskal-Wallis test, $H_3 = 2.19$, $P = 0.535$) or between years (Kruskal-Wallis test, $H_3 = 5.862$, $P = 0.5118$).

Prevalence and species richness of combined protozoan parasites in relation to sources of drinking water and ownership of animals

Subjects who relied primarily on tap water were more likely to experience infections with protozoan organisms than those who used bottled water ($\chi^2_1 = 4.04$, $P = 0.045$), but the difference in prevalence, while significant, was not marked between these two groups (Table 2). However, prevalence was more than twice as high among those who had animals compared to those who did not (Table 2; $\chi^2_1 = 224.6$, $P < 0.001$).

A similar outcome was found for PSR. The difference between subjects relying on bottled and tap water was significant (Table 2; Mann-Whitney U test, $z = 2.818$, $P = 0.005$). Mean PSR was also significantly higher among those who had animals compared with those who did not (Table 2; $z = 15.52$, $P < 0.001$).

The key factors affecting prevalence of combined protozoan infections

As a final stage to this analysis we fitted a statistical model that incorporated all the key significant factors from stages described above. This included the effects of AGE, AREA, YEAR, WATER, ANIMALS and INFECTION. The minimum sufficient model comprised five terms that included INFECTION. The first term was ANIMALS x AREA X INFECTION ($\chi^2_1 = 4.81$, $P = 0.028$) and this is illustrated in Fig. 4A, where it is clearly apparent that the possession of animals was a key determinant of prevalence. Whether living in an urban or rural area, prevalence was much higher among subjects that had animals. Although in both cases prevalence was higher among the inhabitants of rural areas, the difference between subjects from rural and urban areas was more marked in those without animals. While possession or contact with animals greatly enhanced the likelihood of infection, the difference in prevalence between subjects who worked with animals and those that did not, also varied between years (ANIMALS x YEAR x INFECTION, $\chi^2_3 = 27.232$, $P < 0.001$). However, as Table 3 shows, prevalence was always higher in each year of the study among those with animals, but the effect was not totally consistent and the difference in prevalence between the two groups varied in magnitude. The difference between areas also varied

between years (Table 3; AREA x YEAR x INFECTION, $\chi^2_3 = 38.53$, $P < 0.001$), and whilst higher among rural inhabitants in three years, especially in 2018, there was little difference between subjects from these two areas in 2015, and therefore the effect of where the subjects lived was not consistent from year to year.

Table 3. Temporal variation in prevalence of combined protozoan infections in subjects who owned or looked after animals and those that had no contact with animals, those living in rural and urban regions of the province and those that relied on tap or bottled water.

Year	No animals			With animals		
	n	%	± 95% C.L.	n	%	± 95% C.L.
2015	290	23.4	19.30-28.13	146	39.0	31.34-47.41
2016	588	20.4	17.81-23.22	201	69.7	65.45-73.57
2017	394	27.7	22.55-33.45	150	56.7	48.16-64.83
2018	360	21.9	17.49-27.06	148	56.1	47.64-64.18

Year	Urban			Rural		
	n	%	± 95% C.L.	n	%	± 95% C.L.
2015	235	29.4	25.23-33.84	201	27.9	24.05-31.95
2016	456	31.6	25.72-38.02	333	34.8	29.70-40.32
2017	245	33.9	29.45-38.57	299	37.1	32.15-42/37
2018	301	16.3	12.70-20.55	207	54.6	50.13-59.05

Year	Bottled water			Tap water		
	n	%	± 95% C.L.	n	%	± 95% C.L.
2015	191	22.5	15.31-31.66	245	33.5	29.08-38.16
2016	385	35.3	29.76-41.28	404	30.7	25.33-36.61
2017	234	38.0	33.56-42.72	310	33.9	28.97-39.15
2018	266	19.2	15.54-23.42	242	45.9	41.11-50.63

While overall reliance on tap rather than bottled water resulted in a higher prevalence of protozoan infections, the difference between the two groups was dependent on host age (WATER x AGE x INFECTION, $\chi^2_{10} = 22.83$, $P = 0.011$). As Fig. 4B shows the earlier finding of declining prevalence with age was mostly driven by subjects who relied on tap water and among them there was a significant negative correlation between age and prevalence of infection with combined protozoa ($r_s = -0.176$, $n = 1.201$, $P <$

0.001). Among those that used bottled water, prevalence was much the same in all age classes and did not correlate significantly with host age ($r_s = -0.029$, $n = 1.076$, $P = 0.34$). There was also a marked difference between years in the extent of the difference in prevalence between those that used bottled or tap water (Table 3; WATER x YEAR x INFECTION, $\chi^2_3 = 14.431$, $P = 0.002$). In two years (2015 and 2018) prevalence was higher among those relying on tap water, and in the other two prevalence was higher among bottled water users.

The key factors affecting protozoan species richness

As a final step in exploring the factors that influenced PSR, we fitted a GLM (with Poisson errors, main effects and all 2-way interactions) with all the factors (SEX, AGE, AREA, SEASON, YEAR, WATER and ANIMALS). This confirmed the significance of differences in mean PSR between age classes, although with all other factors and their 2-way interactions taken into account significance was only marginal (Wald $\chi^2_{10} = 18.11$, $P = 0.05$). The difference between areas in which subjects lived was also significant (Wald $\chi^2_1 = 19.11$, $P < 0.001$), as was the hugely significant effect of ownership of animals (Wald $\chi^2_1 = 119.25$, $P < 0.001$). As in the earlier analysis the difference between the sexes was not significant (Wald $\chi^2_1 = 0.051$, $P = 0.82$), nor was that between seasons (Wald $\chi^2_3 = 3.34$, $P = 0.34$). However, with all other factors and their 2-way interactions taken into account there was no independent effect of quality of water (Wald $\chi^2_1 = 1.58$, $P = 0.21$), and the difference between years gained significance (Wald $\chi^2_3 = 11.912$, $P = 0.008$).

This analysis identified five significant interactions, which are illustrated in Fig. 5. The most marked interaction was that between AREA and YEAR (Wald $\chi^2_3 = 26.212$, $P < 0.001$). While mean PSR was generally higher among subjects living in rural compared with urban areas, when the data were broken down by year of the study, it became apparent that this effect was not consistent from year to year (Fig. 5A). In 2015 there was no difference, in 2016 and 2017, there was only a marginal difference while in 2018 there was a huge difference with mean PSR being almost four times higher among subjects in rural areas. Ownership of animals (Fig. 5C) was another key factor. While mean PSR was considerably higher in all four years of the study among subjects that owned animals, compared to those that did not, the extent of the difference between the two groups varied from year to year (Wald $\chi^2_3 = 17.960$, $P < 0.001$), but was always in the same direction. Therefore, the effect of ownership of animals showed some temporal consistency and was clearly an important risk factor predisposing people to infection with protozoan parasites. While the earlier univariate analysis indicated that people who relied on tap water had a higher mean PSR than those who used bottled water (see above), when other factors had been taken into account the difference was no longer significant, but there was a significant interaction between WATER and YEAR (Wald $\chi^2_3 = 16.498$, $P = 0.001$; Fig. 5B). Subjects relying on tap water had a higher mean PSR in 2015 and there was a marked difference 2018, but not in 2016 and 2017, so the difference between people relying on tap versus bottled water was not consistent from year to year. The two remaining significant interactions were weaker (Figs. 5D and 5E). Although there was no overall significant difference between the sexes, and this was particularly the case in summer and autumn, in

spring mean PSR was higher in female subjects while in winter it was higher in males (Wald $\chi^2_3 = 11.727$, $P = 0.008$). Finally, the difference in mean PSR between subjects who lived in urban versus rural areas was greater when they relied on bottled water compared with tap water (Wald $\chi^2_1 = 6.361$, $P = 0.012$).

Prevalence and species richness of combined protozoan parasites in relation to symptoms experienced by the subjects

Perhaps as expected, prevalence of combined protozoan infections was considerably higher among subjects who showed some symptoms (79.5% [76.55–82.12]; $\chi^2_1 = 849.3$, $P < 0.001$) compared to asymptomatic subjects (14.0% [13.16–16.59]). Among the 623 subjects with symptoms, no evidence of protozoan infection was found in 128, but 18 of these subjects harboured helminth infections.

Table 4 shows the breakdown of symptoms reported by the subjects and/or confirmed at clinical inspection, and the associated prevalence of combined protozoan infections. In all symptomatic categories prevalence was much higher than in symptomless subjects, although sample size was small in some cases.

Table 4. Prevalence of combined protozoan infection among asymptomatic subjects and those showing a range of symptoms.

	n	%	CL ₉₅
Asymptomatic	1654	14.0	13.16 – 16.59
Abdominal pain	343	78.7	73.76 – 83.05
Abdominal pain + nausea/vomiting	16	68.8	43.62 – 86.78
Abdominal pain + diarrhoea	72	83.3	72.79 – 90.59
Nausea	18	66.7	41.41 – 84.36
Vomiting	29	86.2	69.20 – 95.14
Diarrhoea	103	86.4	80.88 – 90.67
Constipation	08	75.0	36.47 – 95.36
Anal pruritus	19	36.8	17.56 – 60.80
Anorexia	05	100	50.00 - 100
Fever	08	100	63.54 - 100
Urticaria	02	100	22.37 – 100

Discussion

In this community based study we performed an analysis of intestinal parasitosis among symptomatic and asymptomatic patients who presented at medical health laboratories for parasitological examination, and then evaluated the results according to the demographic, spatio-temporal, behavioral and occupational factors that had been recorded. Risk factors (age, patient's provenance, clinical symptoms, drinking water resource and contact with animals) were considered for the first time in an Algeria population to identify the key drivers affecting the spread of intestinal parasites in the country.

The mean prevalence of intestinal parasitic infections was high in Laghouat province (33.33%) with one or more intestinal parasites. The overall prevalence found in this study was much lower than had been reported earlier in Blida, north Algeria (60.61%) [18] and was slightly higher compared to those reported in Oran west Algeria (19.96%) [17], in Tunisia (26.6%) [30] in Morocco (14.15%) [31], in Italy (11.1%) [32] and in Spain (13.7%) [33]. However, overall prevalence in our study was comparable to other community-based studies conducted in Saudi Arabia (32.2%) [34], in Qatar (33.9%) [35] and in Morocco (34.5%) [36]. These differences in overall prevalence among North African countries may be attributable to variation in the sampling techniques used, differences in the quality of drinking water, personal and community hygiene, and variation in the environmental conditions in the different study locations. Furthermore, our results also showed that protozoan infections were more common compared to helminth infections, with the majority attributable to *Blastocystis* spp., *E. histolytica/dispar* and *G. intestinalis* and these were the most common enteric parasites associated with symptoms in the study population. This finding is in agreement with studies conducted in Algeria [17, 18], in North Africa [5, 30, 31] and in Europe [32, 33] but differs from studies carried out further south in Africa and elsewhere in the tropics, in which soil-transmitted helminthiasis have been reported to be the dominant parasites [4, 37]. The explanation for this contrast in the species of intestinal parasites involved lies in the geoclimatic differences between North Africa and further south, notably the extreme aridity of northern regions (high ambient temperatures, low humidity and infrequent poor rainfall) compared to elsewhere. Other factors, that also contribute, most likely include soil textures and farming ecosystems. Human intestinal helminths, the soil-transmitted species, cannot easily complete their life cycles in the absence of an appropriate soil environment or under the extremely arid environmental conditions in Laghouat province [24]. Nevertheless, the overall pattern of the prevalence of combined protozoan infections in our study was clearly influenced by the pattern of prevalence of *Blastocystis* spp., *E. histolytica/dispar* and *G. intestinalis*. The high prevalence of *Blastocystis* spp. in this study confirms the results of a previous survey in the same study area in which a prevalence of 32.1% was recorded [19]. Today, *Blastocystis* spp. is considered an under-reported parasite, but has a worldwide distribution. The highest prevalence recorded to date is 100%, which was recorded in Senegalese children [38]. However, the infection rates of *E. histolytica/dispar* were significantly higher than observed in Blida and Oran, Algeria (1.67%-4.83%) respectively [17, 18], while the incidence of *G. intestinalis* is considerably lower than that in other provinces in Algeria (15.32%-41,67%) respectively [17, 18] and in North African countries with similar standards of living, e.g. Tunisia (17%) [30] and Morocco (22.71%) [31]. The higher prevalence of *G. intestinalis* in this provinces could be due to higher rainfall in this areas compared to Laghouat province.

Although at the whole population level there was relative stability in the prevalence of combined protozoan infections and PSR from year to year, and virtually no differences between the values of both parameters for the four seasons, more in depth analysis revealed that there were some marked consistent trends and some unexpected differences at the data subset level. The strongest and most consistent influence on both prevalence of infection and PSR was ownership of animals. Subjects who owned or looked after animals had a markedly higher prevalence of infection and harbored more species of protozoan parasites (i.e. had a higher value for PSR). The higher values of these two parameters in those

with animals were evident in each year of the study, and whether people lived in urban or rural locations. As emphasized earlier, Laghouat province is known as the province with the second most numerous livestock ownership in Algeria [24] and farms are located always in rural areas with a high diversity of animals species (sheep, goat, bovine, horse, camel and chicken). Additionally, nomadic and pastoral populations live close to their animals and this close contact increases the risk of zoonotic transmission. Livestock, have often been implicated as a major source of environmental contamination and potential reservoirs for zoonotic infection [21, 22]. In Algeria *G. intestinalis* and *C. parvum* have been reported in domesticated animal species [21–23]. These parasites are potentially zoonotic pathogens and close contact with animals is believed to be the major risk factor for human infections.

The next influence on prevalence and PSR in terms of the magnitude of its effect was the location in which subjects lived. Overall data showed that those living in rural locations had both higher prevalence of protozoan infections and higher PSR, but the magnitude of this effect was not nearly as marked as that of ownership of animals. Similar finding in Morocco showed a high intestinal infection in rural area (53.3%) compared to urban area (29.5%) [36]. This may be explained by the precarious hygienic conditions in rural areas where access to adequate sanitation and the supply of drinking water is difficult, and the most important factor is close contact with livestock. Indeed in our study the relatively small difference in prevalence between people living in rural and urban areas contrasts with the much larger discrepancy in prevalence between those who owned animals and those that did not. Regarding the seasonality there were significant inconsistencies with the inhabitants of rural locations showing an expectedly high prevalence in the spring of 2018 compared to springs in particular, but also seasons in other years. Those living in urban locations showed particularly low prevalence in both spring and summer of 2018. There were also inconsistencies in the magnitude of the difference in PSR between rural and urban inhabitants between the years of the study. In 2015 the values for PSR were almost identical for these two areas, in 2016 and 2017, PSR was marginally higher among rural inhabitants, but the biggest difference occurred in 2018, when there was a huge difference between these two data subsets. The overall difference in PSR between the inhabitants of rural and urban areas was therefore driven largely by the hugely skewed results of 2018, and was not consistent across the years of the study. These within-year fluctuations in prevalence are most likely explained by seasonal changes in eating habits in particular years, with an increase in the consumption of water and raw foods during the hot seasons and on variation in climatic conditions which favour the embryonation and development of the infective stages in ova and oocysts during their external phase. Another pertinent factor centres around agricultural practices in the spring in the Algerian steppe. This is the best time for pasture improvement and in this period livestock owners and breeders are in close contact with their livestock, because this is the season when goat kids and lambs are born and when sheep are sheared. Transhumance of animals in this period is very frequent in search of fresh pastures on which to graze and this close contact between livestock and pastoralists increases the risk of zoonotic transmission in spring and in rural area, in contrast to urban area where pets are not moved seasonally to different locations and are frequently monitored medically.

There was no significant overall variation in the prevalence of combined protozoan infections between years in the present study. Our results therefore contrast with other studies which have reported significant temporal changes in the prevalence of protozoan infections. In Morocco, for example prevalence was found to have fallen from 10.18% in 2001 to 4.50% in 2005 [31], and in Qatar, studies on immigrants revealed a significant decrease in prevalence over a decade, from 7.98% during 2005–2008 [39], 5.13% during 2009–2011 [40], to 4.89% during 2012–2014 [41]. Such changes in prevalence of protozoan infections are most likely attributable to improvements in health and social conditions of the relevant populations. However, the more stable overall annual prevalence in this study suggests that the subjects in the current work did not experience any major changes/improvements in their health and social conditions. Nevertheless, annual prevalence data may hide underlying fluctuations in subsets of the population in the study and in the current work these were revealed by multifactorial statistical models.

As expected our data showed variation in both prevalence of protozoan infections and PSR with age. The prevalence of combined protozoan infection was observed to be significantly higher in children in the age range 6–10 years, which is in line with studies performed elsewhere [5, 30, 37]. Nevertheless, multivariate analysis showed that there was a strong association between the quality of water consumed and age with prevalence of combined protozoan infections declining with increasing host age among those who relied on tap water. Such high rates in school children could be explained by the fact that children in this age are more susceptible to intestinal infectious diseases than adults because of their poor hygiene habits, poor health education, consumption of school tap water, immature immunity of young children and close personal contact in crowded classrooms [37]. The majority of houses and schools in Laghouat province are supplied by public water delivery systems from boreholes and then stored in water tanks and provided via taps. In Algeria, this tap water is treated by advanced physico-chemical techniques to make it suitable for human consumption, with fixed limits for the chemical, physical, organoleptic, radionuclide and bacteriological parameters. However, to the best of our knowledge, it is not subjected to parasitological inspection and relevant treatment, as has been reported in some Arab countries [15, 42]. According to the World Health Organization statistical series for Algeria, 16% of the population does not have access to improved sources of drinking water and 5% of the population does not have access to improved sanitation [43]. It is interesting that the mean PSR was higher in subjects living in rural areas whether they relied on bottled or tap water, compared to those living in urban areas, among whom reliance on bottled water did confer some modest advantage in terms of lower PSR (Fig. 5E, This result is consistent with our finding that ownership of animals in Laghouat province is a far more important risk factor predisposing people to infection with protozoan parasites.

In our study, we noticed that the sex of subjects did not influence the prevalence of the intestinal parasites, this may be due to the fact that people are exposed to the same source of infection regardless of their sex and this finding is in agreement with some other studies [17, 31], although it contrasts with a previous study reported in Morocco [36] in which a significant association between sex and infection was found. However, when examined at a subset level, our data did in fact provide some support for variation in prevalence between the sexes. Thus in spring the mean PSR was higher in female subjects, while in

winter it was higher in males, and this difference between the sexes may have arisen because in winter, men tend to eat fast food, while in spring women are more frequently in contact with raw vegetables of the season than men, when preparing food.

Our application of multivariate analyses in the present study established that contact with animals was the most important risk factor and thus predictor of intestinal protozoan parasitic infections in Laghouat province. This analysis also revealed that living in rural rather urban areas, reliance on tap water and age, whilst showing less temporal stability, were nevertheless additional secondary risk factors that also need to be taken into consideration. Further molecular investigations of the exact genotypes of each of the intestinal parasites recorded here in humans, and extended additionally to animals and tap water samples, are required to confirm these conjectures.

We have demonstrated in our study that the most important risk factor for infection with intestinal protozoan parasites among the subjects that were referred for tests was ownership of and work with animals. While those living in rural versus urban environments and those relying primarily on tap versus bottled water, also showed higher prevalence of intestinal protozoan infections overall, these effects were not consistent from year to year. Our study emphasises the need to examine closely variation in parameters within subsets of data and not to rely just on global statistics that do not control for other influences.

Abbreviations

PSR: protozoan species richness; S.E.M: standard error of the mean; GLM: generalized linear model; CL: confidence limits.

Declarations

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Ethics approval and consent to participate:

The study was ethically approved by the Faculty of Science of Nature and Life, Djelfa University, Algeria (Ref:AT04/E.V.E.S) and informed consent was obtained from each patient or his guardian.

Consent for publication:

Not applicable.

Availability of data and materials:

Not applicable.

Competing interests:

The authors have declared that no competing interests exist.

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Authors' contributions:

Conceptualization: SS, AH, MAAM; Data Curation: SS, JMB, MAAM; Formal Analysis: JMB, MAAM; Funding Acquisition: MAAM; Investigation: SS, DB; Methodology: SS, MAAM; Project Administration: SS, AH, MAAM; Resources: SS, DB, AH; Software: MAAM, JMB; Supervision: SS, AH, MAAM; Validation: MAAM, JMB; Visualization: SS, JMB, MAAM; Writing – Original Draft Preparation: SS, JMB, MAAM; Writing – Review & Editing: SS, JMB, MAAM.

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Figures

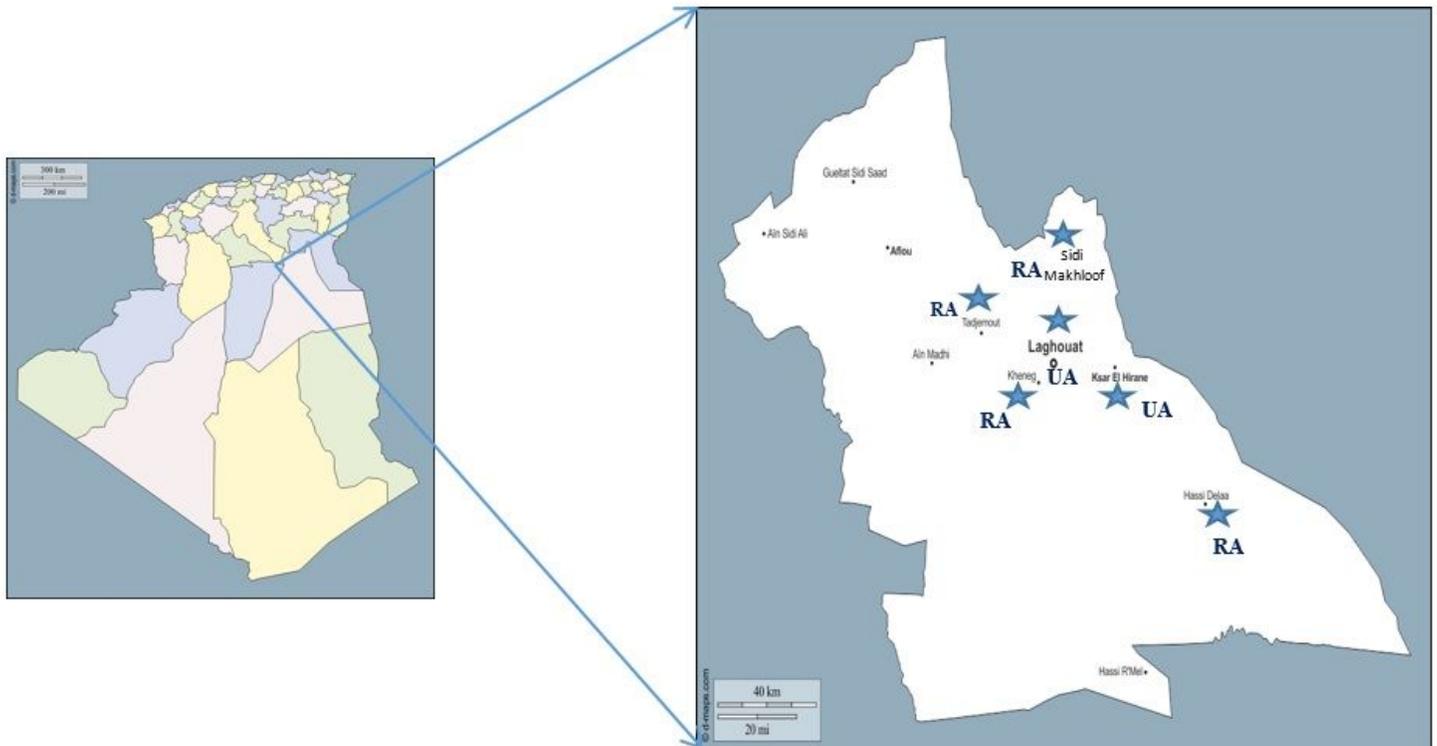


Figure 1

(A): Geographic map of Algeria, (B): Geographic map of Laghouat province showing the origin of the patients (UA: Urban areas, RA: Rural areas). Maps source: https://d-maps.com/carte.php?num_car=189308&lang=fr Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

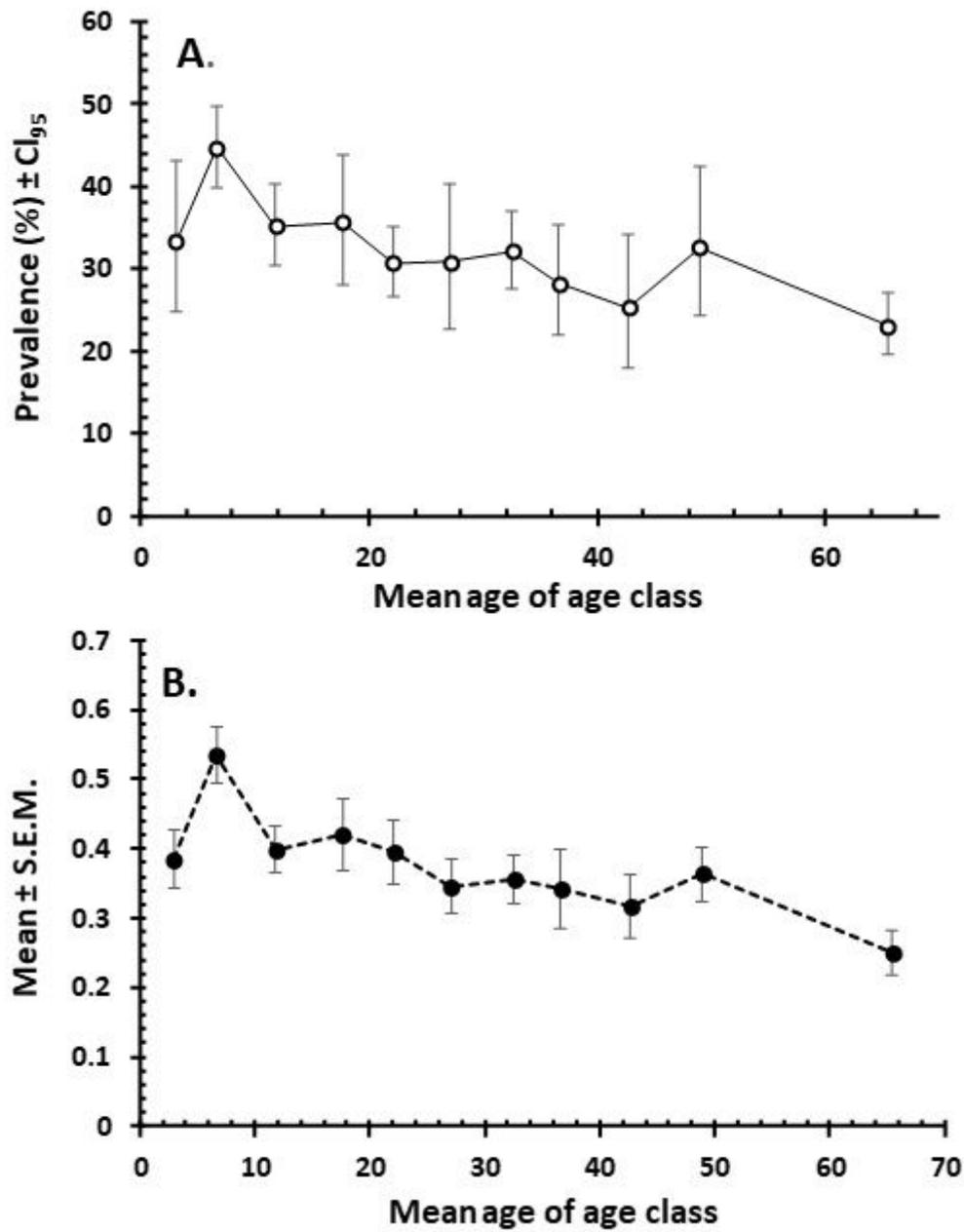


Figure 2

Age-related variation in prevalence (A) and species richness (B) of combined species, protozoan infections.

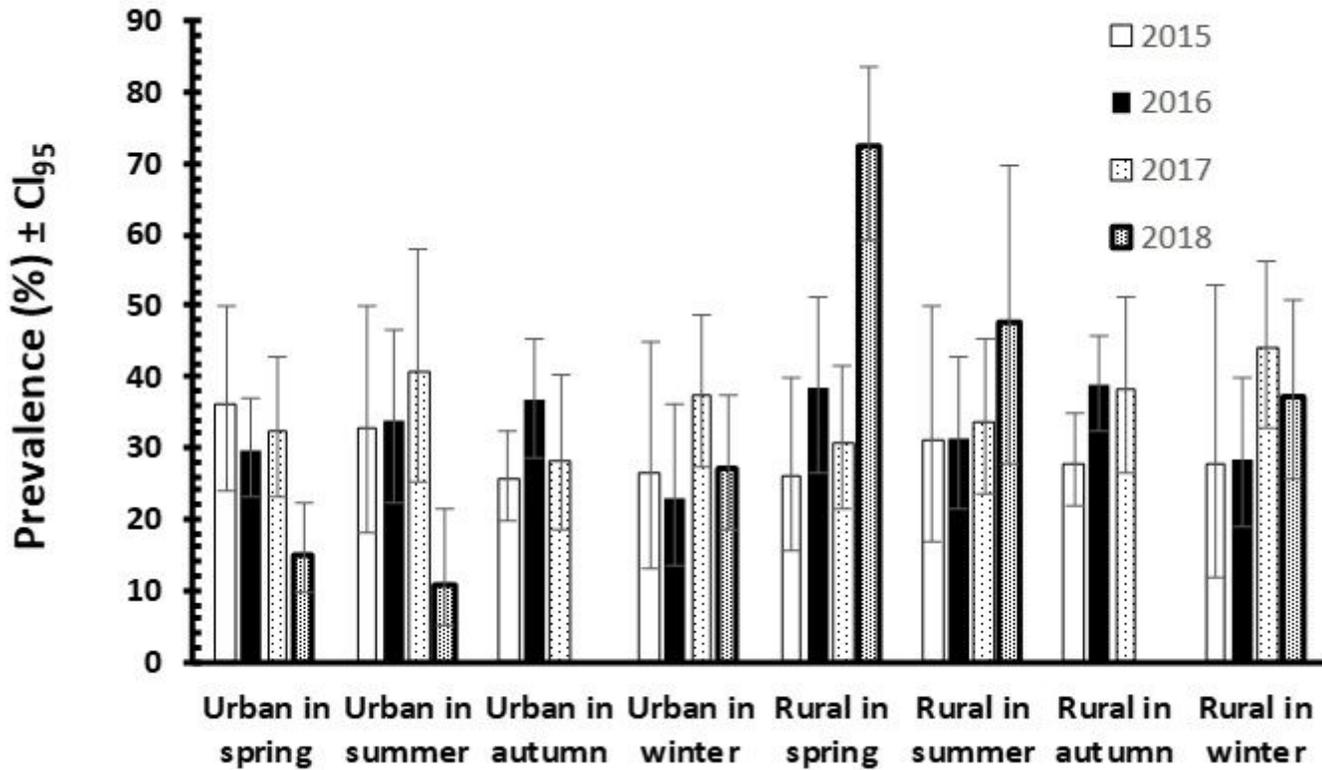


Figure 3

Prevalence of combined protozoan infection by season, year and area in which the subjects lived. Note that no data were available in 2018 for subjects from urban areas in the autumn and rural areas in the summer. For statistical analysis, see text.

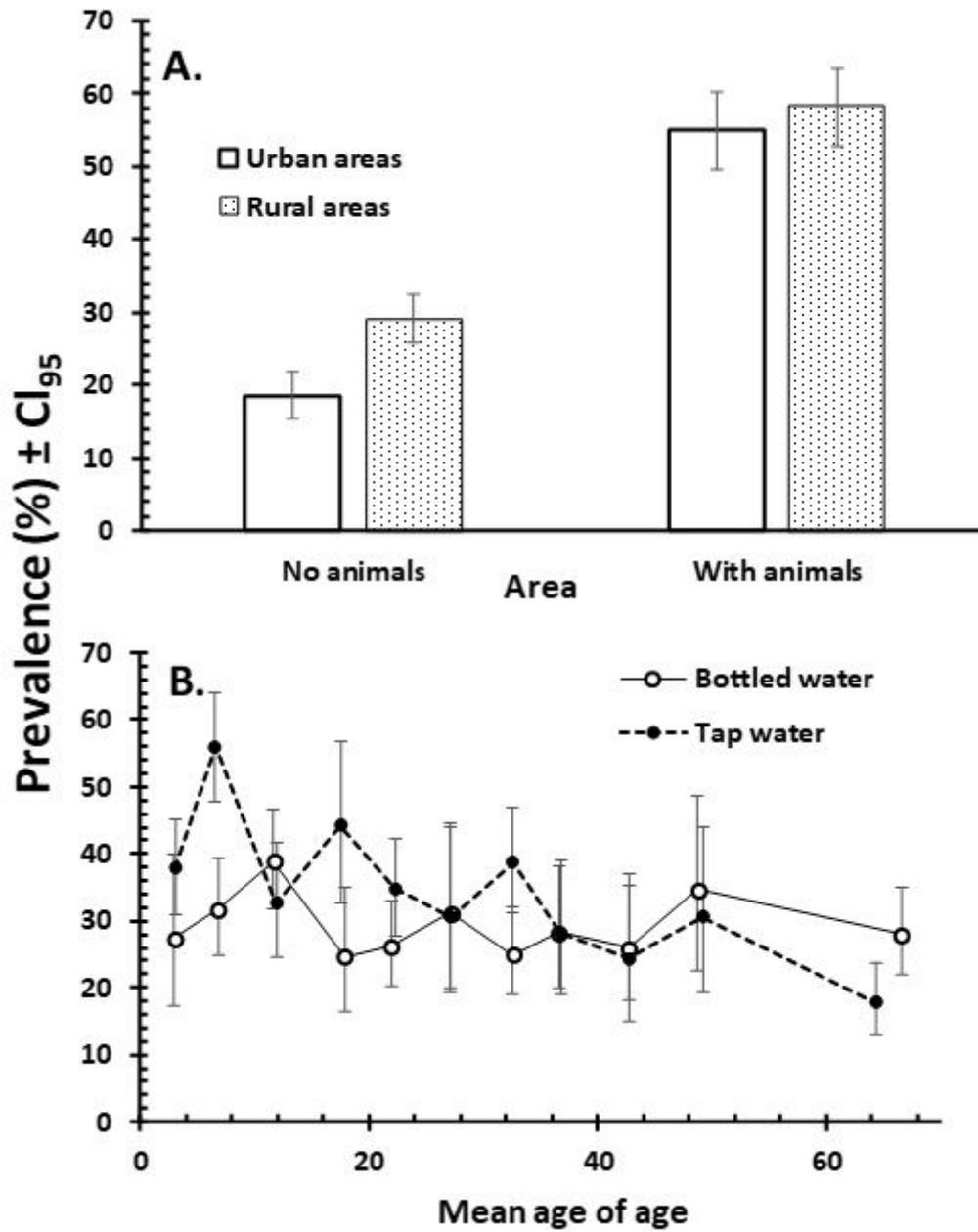


Figure 4

Prevalence of combined protozoan infection in subjects with animals or without animals, living in urban or rural areas (A) and in subjects of different age relying on tap or bottled water. For statistical analysis see text.

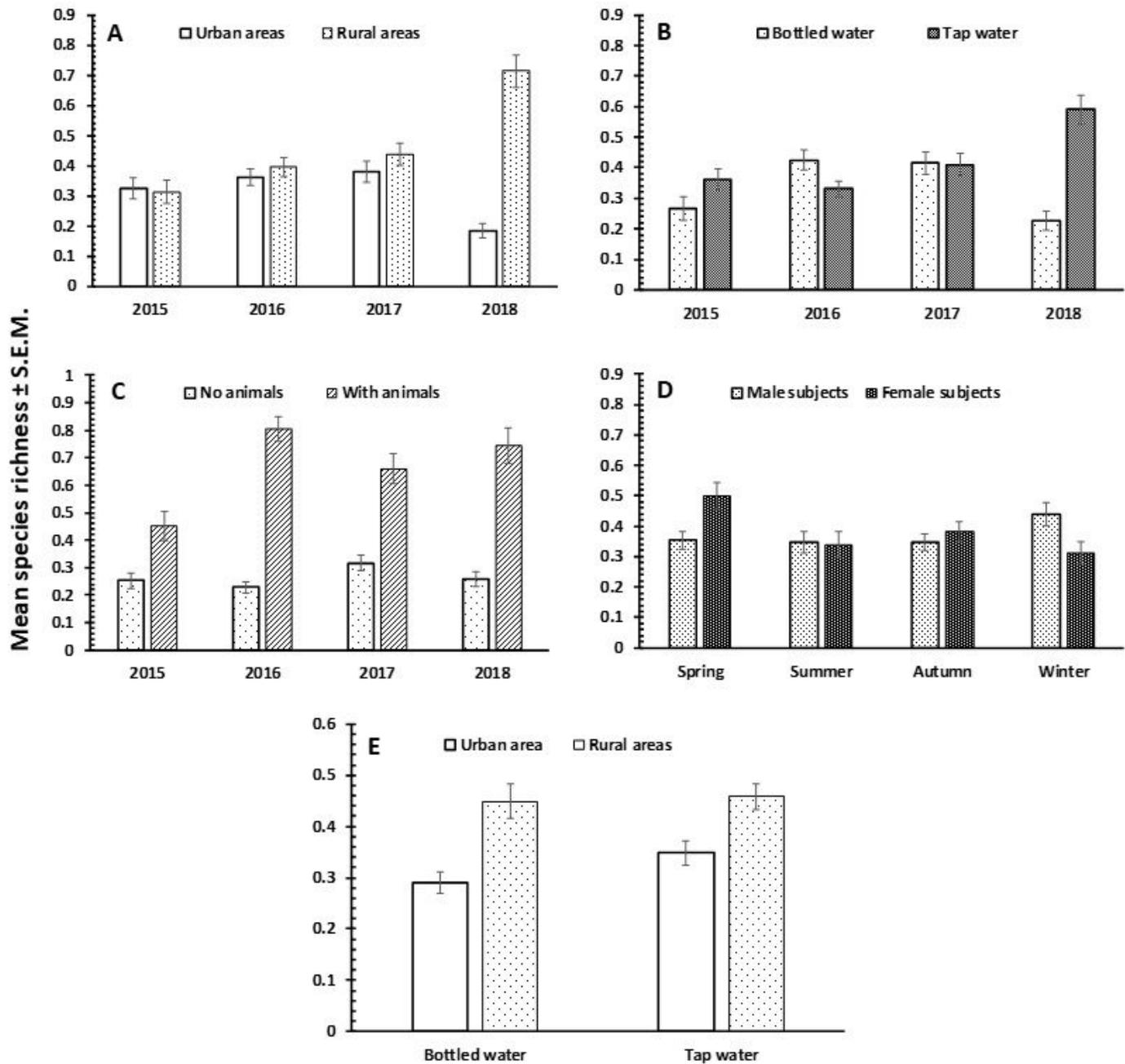


Figure 5

Variation in protozoan species richness. Variation between subjects living in urban and rural areas in different years of the study (A); variation between subjects utilizing bottled or tap water in different years of the study (B); variation between subjects with or without animals in different years of the study (C); variation between the sexes in four seasons (D); variation between subjects living in urban and rural areas and according to whether they utilized bottled or tap water (E).

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