Reviewing Fasciola hepatica transmission in the West Indies and novel perceptions from experimental infections of sympatric vs. allopatric snail/fluke combinations

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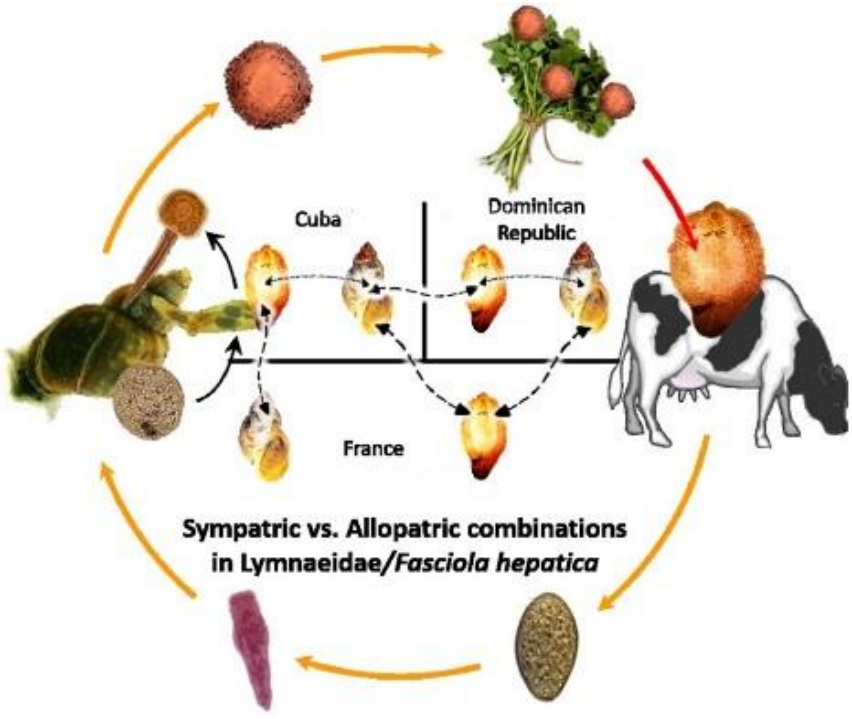
Abstract

Fasciolosis is an important food-borne parasitic disease affecting over two million people worldwide with economic losses related to cattle production of up to US$ 3 billion annually. Despite the long known presence of Fasciola hepatica in the Caribbean islands its transmission is not well known. This study reviews historical and recent data on fasciolosis in the West Indies, revealing for the first time the outcomes of sympatric and allopatric fluke/snail interactions in the area by exploring the susceptibility of four lymnaeid species after exposure to F. hepatica isolates from Cuba, the Dominican Republic and France. Overall, Galba cubensis showed a mean prevalence of 71.8% and appears to be the most suitable intermediate host species irrespective of the isolate used. Sympatric combinations (snail and parasite from the same country) were generally more compatible (higher susceptibility, parasite intensity and snail survival post-exposure) and only the allopatric interaction of French G. truncatula/Cuban F. hepatica attained 100% prevalence and mean intensity over 33 rediae/snail. However, certain Dominican populations of Pseudosuccinea columella showed high parasite intensities (>30 rediae/snail) when infected with Cuban flukes, highlighting the potential risks of biological introductions. Overall, high compatibility in most sympatric combinations compared to low or moderate compatibility in allopatric ones, suggests the existence of local adaptation from a long sustained interaction that has led to high rates of transmission. Interestingly, attempts to infect G. schirazensis with sympatric and allopatric flukes failed and coupled with the lowest survival rates which supposes a low risk of fasciolosis transmission in areas where this is the only snail species. Although there are significant gaps in the actual status of fasciolosis transmission from several islands in the West Indies these results show a permanent risk. We conclude that fasciolosis transmission is high in areas where the local snail, G. cubensis, occurs, and will be even higher in the presence of the invasive P. columella.



Graphical Abstract

Keywords :Fasciola hepatica, Lymnaeidae, , Caribbean, Parasite transmission, Experimental infections

1. Introduction

Fasciolosis is a food-borne disease caused by the parasites Fasciola hepatica and F. gigantica and transmitted by freshwater snails of the family Lymnaeidae (Andrews, 1999). Fasciolosis affects nearly 17 million people worldwide, with up to 180 million at risk of infection (Mas-Coma et al., 2018). The public health risk keeps increasing, linked to the growing problem of fasciolosis in livestock farming and an increase in the frequency of infected animals that are mainly used for meat and milk production (Khan et al., 2013). Worldwide, estimated economic losses of animal fasciolosis reach nearly US $3 billion annually with over 600 million animals infected (Toet et al. 2014).

Liver fluke infection by F. hepatica is considered to be one of the most widespread trematodes (Mas-Coma et al., 2009), due in part, to the fact that around 30 lymnaeid snail species worldwide are recognized as intermediate hosts (Vâzquez et al., 2018). Its introduction into the Western Hemisphere is related to the European colonization of the Americas (1500-1800) by means of non-native infected cattle brought for settlement (Mas-Coma et al., 2009). With a supposed Eurasian origin (Lotfy et al., 2008), F. hepatica has found suitable hosts in the local American lymnaeid species of the genera Galba and Pseudosuccinea (Vâzquez et al., 2018).

Within the Americas, the study of fasciolosis transmission in the West Indies (islands of the Caribbean Sea) is particularly interesting given its marked insularity. Although usually not separated by more than a few hundred kilometres, each island probably has its own transmission dynamics related to the mollusc species involved, the particularities of their freshwater ecosystems and the type of cattle husbandry in use (Fig. 1). Only three lymnaeid species —G. cubensis, G. schirazensis and P. columella— are reported in the West Indies and each has been suggested to play different roles in the parasite transmission (Bargues et al., 2011; Gutiérrez et al., 2011; Vâzquez et al., 2014).

Even if transmission is not well documented in most of the West Indies, particularly in the Lesser Antilles, the burden of F. hepatica infection in either humans or animals (domestic or wild) is expected to be as high as in neighbouring islands with similar epidemiological characteristics (Table 1). In such an insular model, intermediate hosts may reach very high densities caused by low interspecific competition due to the low numbers of sibling species and, in consequence, the chances of becoming infected are high and simplify the arrival of the parasite to the next host. Therefore, this region is highly suitable for fasciolosis transmission and, given its climate, is ideal for an uninterrupted life-cycle of the parasite (Mas-Coma et al., 2018).

However, within the West Indies, only countries such as Cuba, Haiti, Dominican Republic, Puerto Rico, Jamaica and St. Lucia have reports of infections in definitive hosts (Table 1). Cuba is the only country where significant studies have been carried out, and nine outbreaks have been informed in the human population. According to a 1990's study from the WHO, 2594 human cases were acknowledged worldwide particularly from Europe and Asia but also from Cuba and Puerto Rico (Chen and Mott, 1990). In other countries such as Haiti, there are reports of 60% infected cattle (Gentilini et al., 1964) with a supposed high prevalence in the human population (Rojas et al. 2010). In the case of Lesser Antilles reports are only available for animal fasciolosis in St. Lucia and Martinique (Table 1). In the latter, infected cattle imported from Lorraine (France) were observed by the slaughtering services at Fort-de-France (Grétillat, 1966) but autochthonous transmission has not been assessed.

Regarding the intermediate host, freshwater snails of the family Lymnaeidae occur in the vast majority of the Caribbean (Hubendick, 1951). Galba cubensis and P. columella have been reported in most of the West Indies (Table 1), but so far, G. schirazensis has only been reported in the Dominican Republic (Bargues et al., 2011). However, parasites on the snail hosts Galba cubensis and P. columella, have only been reported in Cuba (Table 1).

Due to the relatively short distances separating each island and the existence of natural biological corridors (e.g. wading birds' migration pathways), the particular transmission scenario of one island may affect transmission on others since snails can easily be introduced (Wesselingh et al., 1999; Cowie and Holland, 2006). Given that snails and/or parasites may migrate (by means of natural or human mediated introduction), more susceptible/infective genotypes could be introduced in the future shaping transmission atterns. Invasive genotypes of the lymnaeid host

P. columella introduced worldwide are supposed to have facilitated the spread of F. hepatica in many regions due to a lack of genetic diversity that may counteract infection (Lounnas et al., 2017). In Cuba, for example, the same effect has been observed and the only infected natural populations of P. columella carried a widespread genotype particularly present in monomorphic populations (Alba et al. 2019).

The study of compatibility between different parasite and snail populations may serve to better understand and predict transmission. To this end, experimental infections are key to explore traits in host-parasite interactions. It is particularly interesting to include G. schirazensis, as to date there is no consensus of its actual role in transmission (Bargues et al., 2011; Dreyfuss et al., 2015; Caron et al., 2017).

This study aims to test the compatibility of several lymnaeid snail populations with sympatric and allopatric fluke isolates. To this end, several F. hepatica isolates and snail species from Cuba (two lymnaeid species coexist) and from the Dominican Republic (three lymnaeid species coexist) served as biological models. Also, given the reports of infected cattle in Martinique brought from France (Grétillat, 1967), an isolate of F. hepatica and a fourth lymnaeid snail (G. truncatula) from France were used to test compatibility over larger allopatric scales. Regarding G. truncatula, this species has not been reported in the West Indies but is present in South America (Jabbour-Zahab et al., 1997) as well as in many other world regions (Kock et al., 2003; Rondelaud et al., 2006; Jones et al., 2015) and is considered the main intermediate host of F. hepatica in Europe (Correa et al., 2017). These results will give insights into the actual players in the host-parasite interaction in the West Indies by exploring snail-parasite combinations from its largest islands.

2. Material and Methods

2.1. Sampling of snail populations and liver fluke isolates

We sampled several field populations (Fig. 2) of G. cubensis (5), G. schirazensis (2), G. truncatula (1) and P. columella (6) from Cuba, the Dominican Republic and France (Table 2). The snails were directly removed from the mud or aquatic vegetation using soft forceps and sieves (up to 100 individuals per locality when possible). The snails were brought to the laboratory and kept isolated in Petri dishes under controlled conditions (i.e. temperature ranging 25-26°C, fed ad libitum with algae; Sânchez et al., 1995) until oviposition to obtain lab-reared colonies for experimental infection trials with F. hepatica isolates.

The isolates of F. hepatica were retrieved directly from the liver of recently slaughtered cattle in Cuba (Pinar del Río province), the Dominican Republic (Dajabôn province) and France (Tour du Valat, Camargue) (Fig. 2). All adult F. hepatica samples were kindly donated by the veterinary authorities of each slaughterhouse after routine activities. Adult flukes were placed in a saline solution (0.85%) with glucose (9%) to promote egg laying. Eggs were later stored in tubes containing saline solution (0.85%) at 4 °C in the dark until use (Vâzquez et al., 2014).

2.2. Morphological and molecular identification of snail species

Snails were identified using morphological and molecular markers. Pseudosuccinea columella can be distinguished without ambiguity from Galba species due to its shell morphology and internal anatomy (i.e. shell with a short spire; periostracum ornamented with spiral sculptures; ureter with two distant flexures; Pointier, 2008). In the case of G. cubensis, G. schirazensis, and G. truncatula, these species are very similar in shell morphology and anatomical variation which render them undistinguishable, and can be considered as cryptic species (Correa et al., 2011).

In order to identify these three Galba species, up to 30 individuals from each Galba field population were tested using a molecular tool designed to this end by Alda et al. (2018). Previously obtained egg clutches of field-collected snails were used for founding colonies after confirmation of the species using multiplex PCR. DNA was extracted using a Chelex protocol following Estoup and Martin (1996) adapted to 96-well plates. The multiplex PCR (Alda et al. 2018) was based on species-specific primers amplifying microsatellite loci targeting cryptic species and producing band sizes that are specific to the three targeted species (179–200 pb in G. cubensis, 227–232 pb in G. schirazensis, and 111–129 pb in G. truncatula).

2.3. Infection trials

Two-week old snails obtained after two successive lab-reared generations were infected with hatched F. hepatica miracidia. Eggs of F. hepatica were placed in spring water and incubated for a fortnight, at 27 °C, in darkness. Direct light exposure served to induce miracidial hatching. A dose of five miracidia/snail was used to expose 30 individuals from each population following the methodology described in Vâzquez et al. (2014). Briefly, lymnaeid snails were placed in a 96 well plate containing the established dose of free¬swimming miracidia. Snails were forced to remain submerged by filling the wells with spring water and placing glass slides on top. After three hours of exposure, each snail was returned to their rearing Petri dishes previous confirmation that no miracidia remained in the well. After 25 days post-exposure all snails were dissected to verify their infection status. Dissection was carried out under a stereoscope where each snail was carefully crushed and separated from its shell using forceps (Caron et al. 2008). Only living F. hepatica rediae (irrespective of their size or developmental stage) were used to measure parasite intensity. We kept 30 unexposed individuals (experimental controls) of each species/population subjected to the same conditions and procedures. Mortality was recorded daily and the infection status (infected/non-infected) was noted in all dead individuals that died after the first week post-exposure by observing F. hepatica rediae. Individuals dying during the first week post-exposure usually have no traceable clues of infection because early developmental stages are difficult to observe. Therefore, in order to prevent introducing uncontrollable bias they were considered non-infected in our experiment. We explored different infection outcomes in relation to sympatric (same country) and allopatric (different country) combinations within the region.

2.4. Data analysis

All gathered data was used to assess compatibility in each snail/fluke combination. Prevalence (proportion of infected individuals within the host sample) and mean parasite intensity (average of intensity values –rediae/snail– calculated for a sample) with 95% confidence intervals (CI) was recorded for each trial according to Reiczigel et al. (2019). Spearman rank correlations were performed to test associations between parasite prevalence and intensities. Parasite intensities were compared among trials by means of one-way ANOVA after normality and homogeneity of variance were verified by the Shapiro-Wilk and Levene tests. A post hoc Tukey test was performed to obtain significant differences for each particular combination. Statistical differences of survivorship in each trial was assessed through a log-rank test of Kaplan-Meier curves built from day 0 to day 25 post-exposure. All statistical tests were performed in Statistica v.12 (StatSoft. Inc., Tulsa, OK, USA 2014) and all differences were considered statistically significant at values of $P < 0.05$.

3. Results

With the exception of G. schirazensis that was not-infected (no observation of any larval stages) with any of the F. hepatica isolates tested (data not shown), the other three snail species showed a variable degree of susceptibility with prevalence ranging from 0 to 100% (Fig. 3). Overall, G. cubensis showed a higher prevalence (mean 72%, range 27–93%) than P. columella (48%, 0–70%) irrespective of the isolate used. Other than G. schirazensis, only P. columella from El Chico (Cuba) failed to develop infection with the Dominican isolate (allopatric combination, data not shown) but was always infected with its sympatric Cuban flukes (Fig. 3). The French population of G. truncatula showed a 100% infection prevalence with the Cuban F. hepatica (larger allopatric combination) with a mean intensity of 33.5 (95% CI; 27.5–39.6) rediae per snail.

Parasite intensities were also variable and significant differences were found depending on the snail/fluke combination (Fig. 3). Overall, higher parasite intensity was found in sympatric combinations in G. cubensis, but some allopatric P. columella/F. hepatica combinations were also high (e.g. DR.05/Cuba, mean 33.1, 95% CI; 25–41.1). Parasite intensities of 15–20 rediae/snail were observed during infection with the French isolate of F. hepatica but were lower than sympatric combinations. In fact, allopatric combinations such as those of P. columella (DR.06/Cuba, mean 7.5, 95% CI; 4.8–10.7 and CU.04/DomRep., mean 8.2, 95% CI; 5.2–11.9) and G. cubensis (DR.01/Cuba, mean 14.7, 95%CI; 10.8– 19.3 and CU.02/Dom. Rep., mean 14.4, 95% CI; 11–18) showed lower parasite burden. We found no correlation between parasite prevalence and mean intensity in G. cubensis ($r = 0.49$, $P = 0.176$) but a positive significant correlation for P. columella ($r = 0.86$, $P = 0.003$).

Survival of snails after 25 days post-exposure varied in relation to the lymnaeid species and the isolate of F. hepatica, but the most frequent peaks of mortality were observed within the first seven days post-exposure (Fig. 4). Overall, G. truncatula (survival 100%) and G. cubensis (survival ranged from 73–100%) showed the highest survival rate following infection. Notably, G. schirazensis showed a very low survival rate during the 25 days post-exposure (survival ranged from 3.3–13.3%) particularly when exposed to the Cuban isolate (allopatric combination). In this case, this species showed a sustained mortality throughout the experiment even when no infection was observed in dead snails. Overall, lower survival rates were attained with the allopatric French isolate in G. cubensis and P. columella. Control groups (unexposed individuals, n = 30) of any species/population showed zero mortality during the time of the experiment (data not shown). Also, no correlation between the effects of snail survival and parasite prevalence ($r = -0.12$, $P = 0.3$) or intensity ($r = 0.2$, $P = 0.18$) was observed.

4. Discussion

The results of testing different snail/fluke combinations revealed a range of competence for the different snail species, from high to very low suitability as hosts in terms of prevalence, parasite burden and snail survival. Overall, G. cubensis and G. truncatula were more compatible than P. columella, while G. schirazensis did not show any sign of compatibility (at least the populations tested). In particular, these results endorse G. cubensis as playing the major role of parasite transmission in the region. This is the case in Cuba where G. cubensis shows high compatibility with F. hepatica both in the laboratory (Vâzquez et al., 2014) and in the field (Vâzquez et al., 2015; Alba et al., 2016) compared to P. columella (Gutiérrez et al. 2011). According to Hubendick (1951), G. cubensis has acircum-Caribbean distribution, occurring in most of the West Indies. Moreover, the present study indicates the potential risks of transmission via G. truncatula if this species is introduced into the West Indies. This risk is not negligible as this lymnaeid not only showed high compatibility with a local F. hepatica isolate (Cuba) but is known to be invasive elsewhere (Meunier et al., 2001).

The understanding of transmission patterns in these countries could be vital to comprehend the overall scenario in the West Indies. According to Mas-Coma et al. (2018), this area is characterized by the 'Caribbean insular pattern' characterized by small and frequent outbreaks in human hypoendemic areas. This is consistent with the scenario observed in Cuba where fasciolosis is not yet considered a severe public health problem but outbreaks occur (Rojas et al., 2010). On the contrary, the country is characterized by a hyper endemic situation at the veterinary level with prevalence ranging from 70-100% in cattle (Vâzquez et al. 2016). The assessment of the different roles of each intermediate host snail species in the region is important to characterize the epidemiological context and to anticipate it under varying conditions. For this, experimental infections are a valuable approach to investigate differences in compatibility at a phenotypical level since they allow all variables to be managed and controlled (e.g. the combination of snails and parasites tested, the infective parasite dose, etc.), all of which could significantly influence the infection outcome (Sorensen and Minchella, 2001).

Little is known about the immunobiology and the particular mechanism involved in the F. hepatica – snail interaction, but variation in infection outcomes, depending on the parasite isolate/snail population combination, are reported here and elsewhere (Rondelaud et al., 2013; Vâzquez et al., 2014; Alba et al., 2018). In the widely-studied model of Biomphalaria glabrata – Schistosoma mansoni, an infectivity mosaic that follows the model of 'matching alleles' is also observed (Théron and Coustau, 2005) and is identified as a polymorphism of compatibility. Overall, the F. hepatica-lymnaeid snail interplay is apparently characterized by different compatibility that will ultimately depend on the particular host-parasite (genotype) combination (Vâzquez et al., 2014; Alba et al., 2018). From the results observed here, the latter seems to be associated with local adaptation as there was high compatibility in most sympatric and low-to-moderate compatibility in most allopatric combinations for G. cubensis and P. columella. In this sense, it can be hypothesized that local adaptation has rendered sympatric combinations more compatible. The concept of local adaptation is highlighted when we tested the French F. hepatica with West Indian snails. This isolate appeared as 'highly virulent' and drastically decreased the survival of snails. However, certain allopatric combinations in P. columella also showed higher values of prevalence, intensity and survival compared to sympatric combinations. From these findings one could speculate that a random encounter of highly compatible snail/fluke genotypes in this species may trigger the probability of infection if other conditions are met (e.g. ecological, epidemiological, anthropological, etc.).

The case of G. schirazensis is particularly interesting given the current results. This species failed to develop the parasite regardless of the F. hepatica isolate used. Similarly, a previous study of a one-time series of experimental infections using laboratory reared F. hepatica (from which genetic diversity is expected to be reduced) also reported non-infection results for this species when populations derived from Egypt and Spain were challenged (Bargues et al., 2011). Based on this result, Bargues et al. (2011) labelled G. schirazensis as a species with no role in the transmission of fasciolosis (refractory to F. hepatica). However, while one may agree that the overall infection risk of this snail is considerably lower in relation to other lymnaeid species (G. cubensis or P. columella), this species should be proposed as highly incompatible to F. hepatica (at least the combination tested). The results from the surviva particularly if the parasite strain is highly virulent for the host, could explain the overall impairment of survival observed in the allopatric combination.

To understand this problem, a comparison with a different infection related phenotype of resistant snails in this system should be carried out. Some Cuban populations of P. columella, that are considered to be resistant to F. hepatica infection (Gutiérrez et al., 2003; Alba et al., 2018), show an unusually higher survival following exposure (>70%, Gutiérrez et al., 2002; >80%, Alba et al., 2018) in relation to susceptible individuals. Also, the long-term maintenance in laboratory conditions has no effect on the stability of the phenotype. In these populations, resistance is associated to an effective immune encapsulation of the parasite (Gutiérrez et al., 2003) and it is not restricted to a host genotype – parasite genotype interaction (Gutiérrez et al., 2003; Alba et al., 2018). In fact, no reversion of the phenotype has occurred after different experimental challenges, even with geographically distant parasites (Vâzquez et al., 2014; Alba et al., 2018).

In contrast, G. schirazensis has been found infected in the field (6% prevalence) in Ecuador (Caron et al., 2017) and Bargues et al. (2011) found parasitic development in 2 out of 10 exposed snails when challenged with a Polish F. hepatica isolate. Also, populations of G. schirazensis were able to be infected and develop F. hepatica rediae and free cercariae after several laboratory reared generations and experimental exposures, pointing to an eventual increase of its compatibility in relation to the selective pressure of particular parasite genotypes (Dreyfuss et al., 2015). Thus, the role of this species as a potential intermediate host of F. hepatica should not be totally disregarded, particularly if local adaptation occurs. For instance, G. schirazensis has been the only lymnaeid species found after a series of samplings in La Trampa (Venezuela) where F. hepatica is prevalent in the bovine population maintained under anthelmintic treatment every six months (Unpublished results).

Further studies are needed to better describe what occurs in G. schirazensis after parasitic exposure and which particular conditions/factors (snail and parasite diversity, densities, varying compatibility according to matching genotypes, etc.) might facilitate transmission in the field. Moreover, it should also be considered that the presence of G. schirazensis inl curves observed here (<20%, this study) and elsewhere (<35%, Dreyfuss et al., 2015) provided some insight on this issue as higher survival should be expected after parasitic challenge in a refractory host (Ibrahim and Trpis, 1987; Abdel-Hamid et al., 2006). The high mortality observed in exposed G. schirazensis might be related to a “hypersensitivity” phenomenon occurring in the host in association with the parasitic infection that results in an unsuitable environment for F. hepatica development. A highly significant hyper pathological/physiological reaction, the Americas has been largely overlooked and is only relatively recently acknowledged (Correa et al., 2010; Bargues et al., 2011). This species may co-occur with other Galba species (in the present study we found G. schirazensis co-occurring with G. cubensis in Auyamas, Dominican Republic) and since they cannot be accurately differentiated at the morphological level, assuming an intermediate host role by either of the cryptic Galba, and particularly by G. schirazensis could be difficult without molecular discrimination (Alda et al., 2018).

Finally, while in the West Indies F. hepatica is probably highly prevalent, more research is needed to give insights into the particular scenarios of islands with unavailable data. Concerning the intermediate hosts, we can predict that the risk of transmission is higher in areas where the local snail G. cubensis occurs, and even higher if the invasive P. columella co-occurs. Low to very low probability is expected in areas with only G. schirazensis unless compatibility increases through local adaptation with the circulating parasite strain.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Competing Interests None

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Tables

Species	**Country**	**Locality**	**Code**	**Coordinates**	**Habitat**
Galba cubensis	Cuba	Arroyo Dolores	CU.01	22°26’27.6”N 79°25’51.6W	brook
		Aurora	CU.02	23°4’44.4”N 81°55’4.8”W	pond
		Playa Jibacoa	CU.03	23°8’38.4”N	brook
	Dominican Republic	Auyamas	DR.01	18°53'34.8''N 70°44'45.599''W	ditch
		La Victoria	DR.02	18° 34' 22.8''N 69°50'52.799''W	pnd
Galba schirazensis	Dominican Republic	Auyamas	DR.03	18°53'31.2''N 70°44'45.599''W	ditch
		Rosa Linda	DR.04	18°58'44.4'' N 70°38'20.399''W	ditch
Galba truncatula	France	Saint Guilhem	FR.01	43°44'2.4''N 3°32'56.399''E	ditch
Pseudosuccinea columella	Cuba	Aurora	CU.04	23°4'44.4''N81°55'4.8''°W	pond
		El Chico	CU.05	22°57'28.8''N82°27'54''W	channel
	Dominican Republic	Jarabacoa	DR.05	19°6'21.6''N 70°37'44.4''W	watercress beds
		Río Isbe	DR.06	18°30'57.6''N 69°57'7.199''W	Rive
	Pseudosuccinea columella				
St. Lucia	Galba cubensis	NK		Malek (1980)	
		NK	10-23%	Barnish et al. (1980)	

Table 1. Past and recent reports of Fasciola hepaticca transmission and snail host occurrence in the West Indies.NK: not known

Table 2. Sampling details of parental lymnaeid snails used to establish laboratory populations for infection trials (each code refers to country.locality).

Species	Country	Locality	Code	Coordinates	Habitat
Galba cubensis	Cuba	Arroyo Dolore	CU.01	22°26’27.6”N 79025’51.6W	brook
		Aurora	CU.02	$23^0$4’44.4”N 81°55’4.8”W	pond
		Playa Jibacoa	CU.03	23°8’38.4”N 81°49’15.6”W	brook
	Dominican Republic	Auyamas	DR.01	18°53'34.8"N 70°44'45.599"W	ditch
		La Victoria	DR.02	18° 34' 22.8"N 69°50'52.799"W	pond
Galba schirazensis	Dominican Republic	Auyamas	DR.03	18°18°53'31.2"N 70°44'45.599 W	ditch
		Rosa Linda	DR.04	18°58'44.4" N 70°38'20.399"W	ditch
Galba truncatula	France	Saint Guilhem	FR.01	43°44'2.4"N 3°32'56.399"E	ditch
Pseudosuccinea columella	Cuba	Aurora	CU.04	23°4'44.4"N81°55'4.8"°W	pond
		El Chico	CU.05 DR.05	22°57'28.8"N82°27'54"W	channel
	Dominican Republic	Jarabacoa		19°6'21.6"N 70°37'44.4"W	watercress beds
		Río Isabela	DR.06	18°30'57.6"N 69°57'7.199"W	river

Figure captions

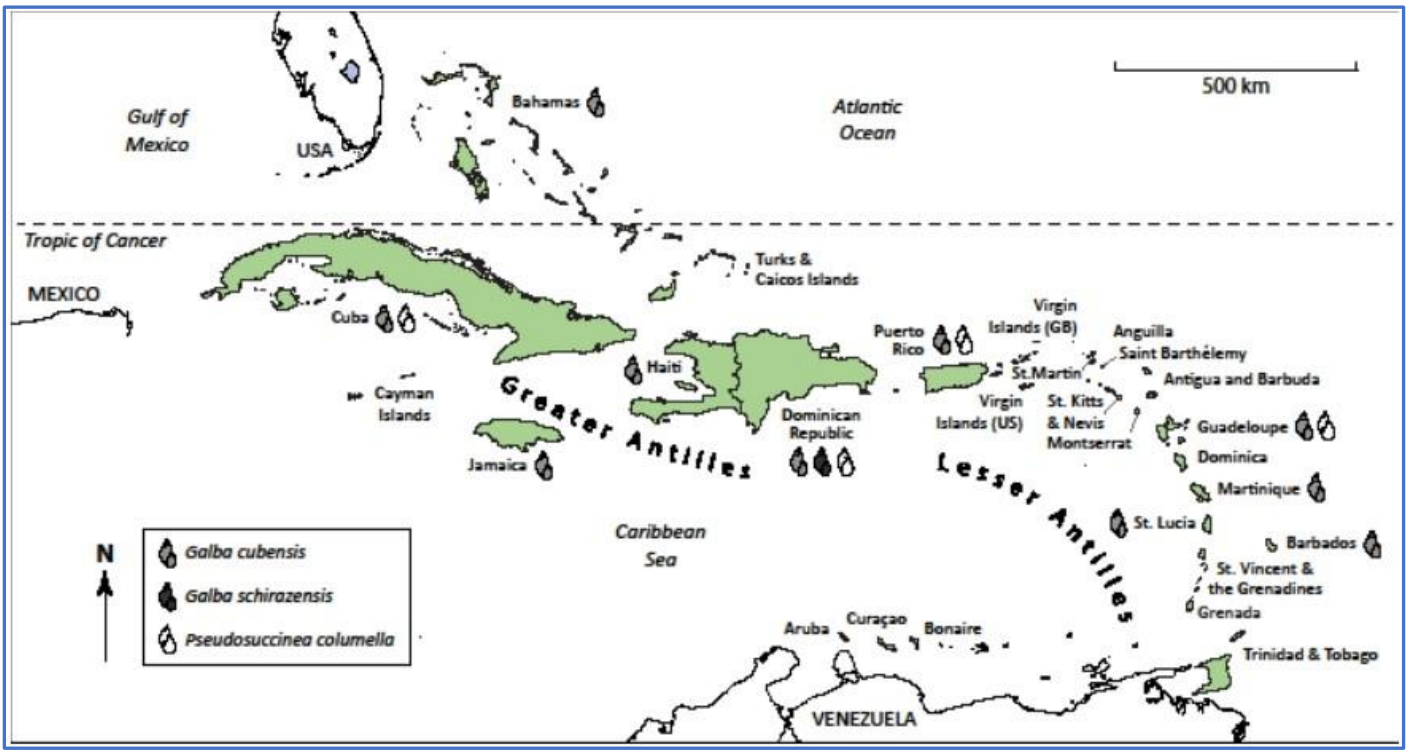

Figure 1. Map of the West Indies indicating the Greater and Lesser Antilles with data on the lymnaeid species reported in each island

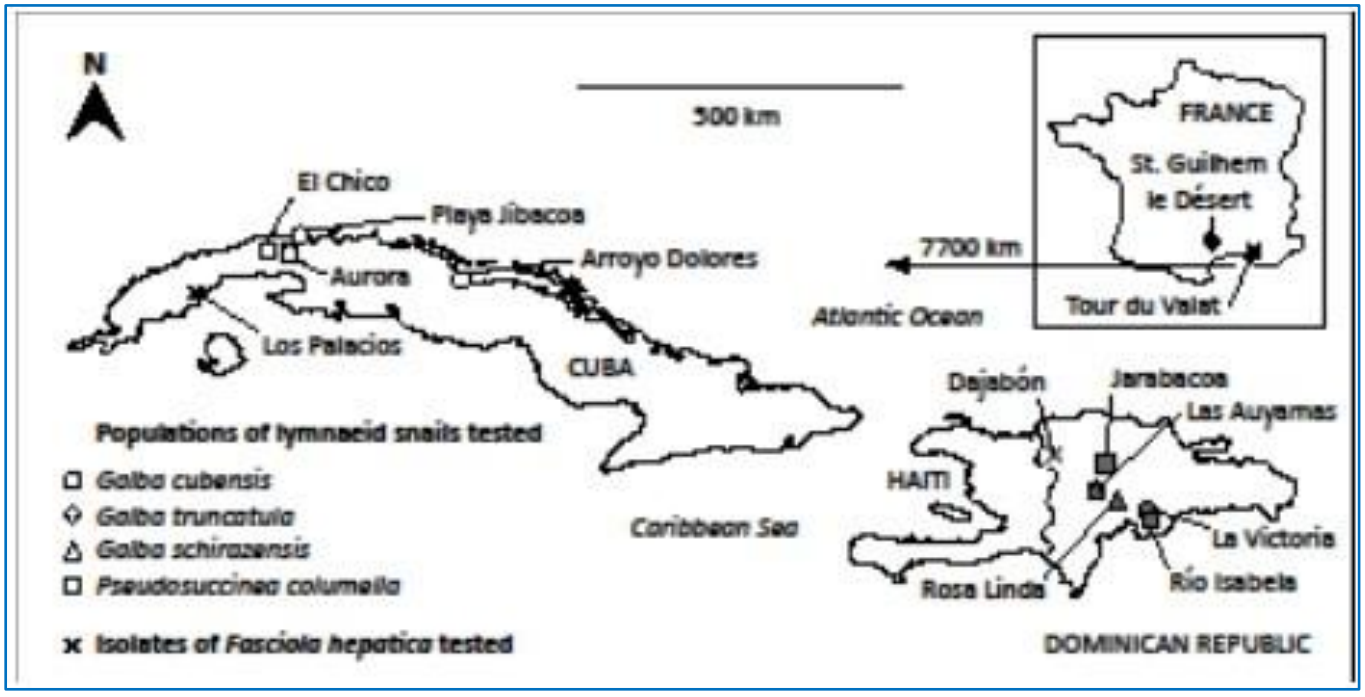

Figure 2. Populations of lymnaeid snails and liver fluke isolates used in the experimental infection trials.

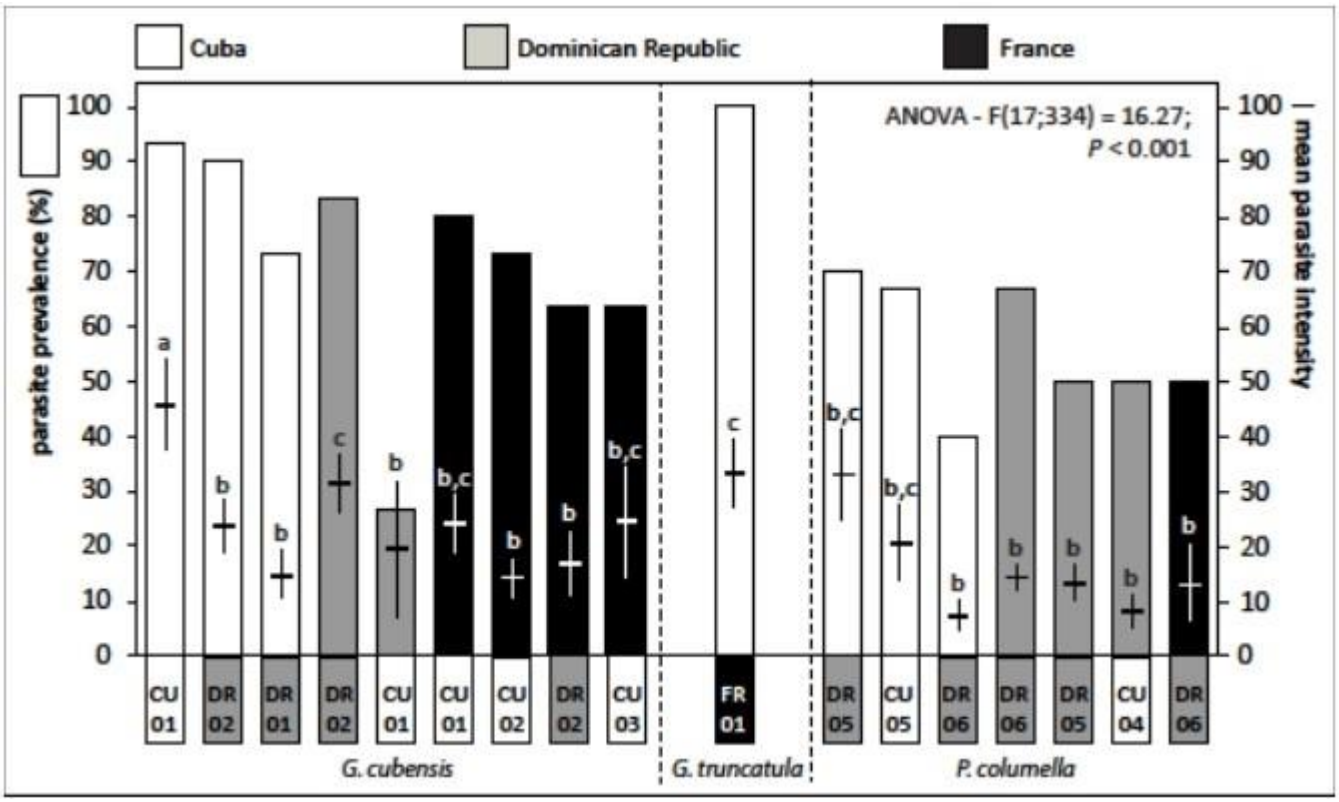

Figure 3. Prevalence and mean intensities (with 95% confidence intervals vertical bars) of Fasciola hepatica in relation to the combination with a particular population of lymnaeid snail originating from Cuba, the Dominican Republic and France. Intensities are compared by means of a one-way ANOVA, different letters mean significant differences between the means according to the post hoc Tukey test.

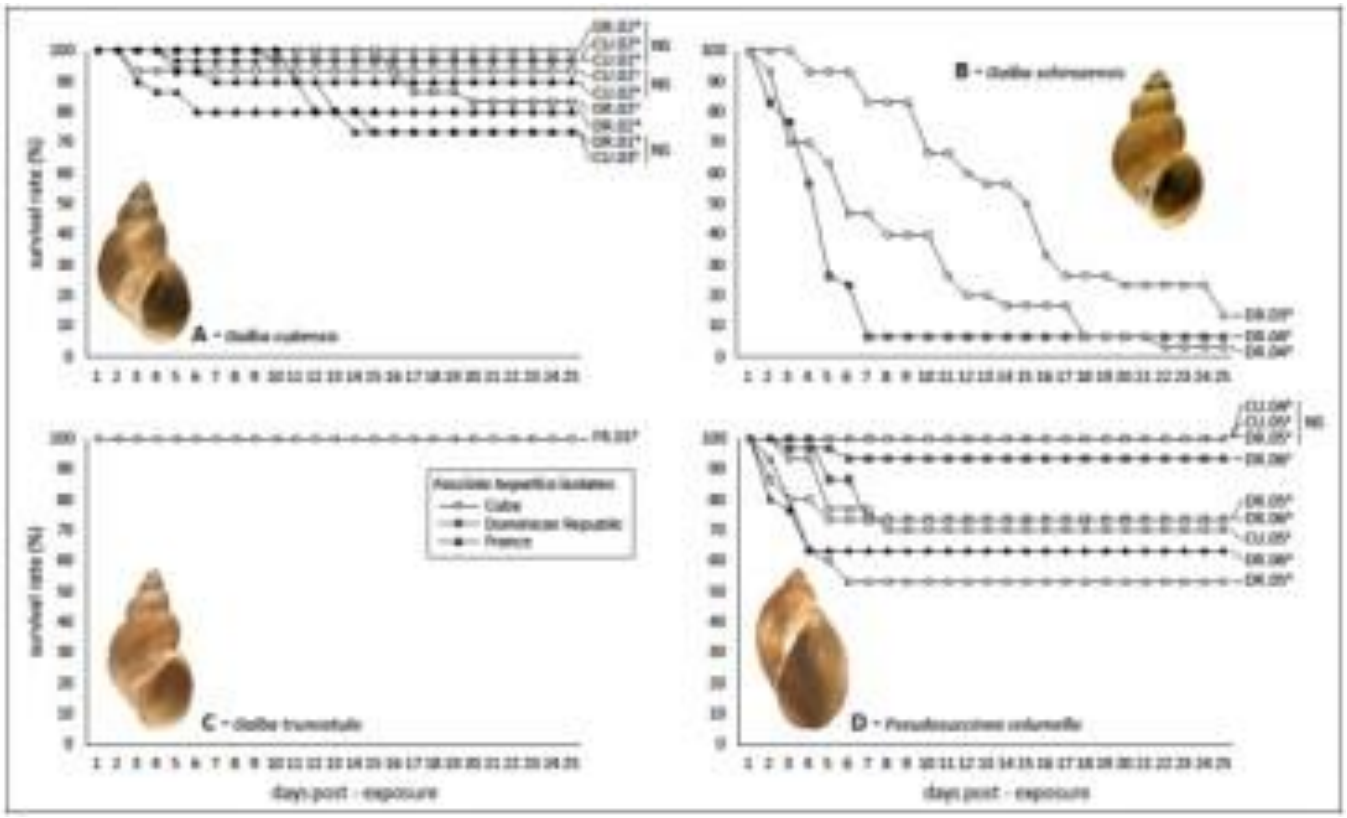

Figure 4. Survival curves of each lymnaeid population when exposed to a particular Fasciola hepatica isolate (A: Galba cubensis, B: Galba schirazensis, C: Galba truncatula, D: Pseudosuccinea columella; population codes are in