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Impact of species hybridization on the clinical management of schistosomiasis: A prospective study

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ABSTRACT

Background: Species hybridization represents a real concern in terms of parasite transmission, epidemiology and morbidity of schistosomiasis. It is greatly important to better understand the impact of species hybridization for the clinical management.
Methods: A prospective observational study was carried out in sub-Saharan migrants who were diagnosed with confirmed genitourinary schistosomiasis. A tailored protocol was applied, including *Schistosoma* serology, a specific urine LAMP tests for schistosomiasis and an ultrasound examination before treatment with praziquantel. A scheduled follow-up was performed at 3, 6 and 12 months to monitor treatment response, comparing patients carriers of *Schistosoma* hybrids with carriers of only genetically pure forms.
Results: A total of 31 male patients from West Africa were included in the study with a mean age of 26.5 years. Twelve (38.7 %) of the patients were carriers of *Schistosoma* hybrids. As compared with patients infected with *S. haematobium* alone, hybrid carriers had lower haemoglobin levels (13.8 g/dL [SD 1.8] vs 14.8 g/dL [SD 1.4], p = 0.04), a greater frequency of hematuria (100 % *vs* 52.6 %, p = 0.005), a higher ultrasound score (2.64, SD 2.20 vs 0.89, SD 0.99; p = 0.02). However, the presence of hybrids did not result in differences in clinical and analytical responses after treatment.
Conclusions: The presence of *Schistosoma* hybrids seems to cause increased morbidity in infected individuals. However, it does not appear to result in differences in diagnostic tests or in clinical and analytical responses after treatment.

1. Introduction

Human schistosomiasis is a chronic parasitic disease caused by infection with trematodes of the genus *Schistosoma*. It is endemic in more than 78 countries in tropical and subtropical areas, with sub-Saharan Africa having the highest annual incidence, morbidity and mortality. It is estimated that at least 236.6 million people needed preventive treatment worldwide in 2019 [1]. Four parasites that infect humans (*Schistosoma haematobium*, *S. mansoni, S. intercalatum* and *S. guineensis*) are common in Africa, while two (*S. japonicum* and *S. mekongi*) are found in Asia. *S. haematobium* is mainly responsible for genitourinary schistosomiasis, and the remaining species for hepatointestinal

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schistosomiasis [2]. Schistosomiasis is also a disease of major veterinary importance, with 19 species naturally affecting ruminants, primates and small rodents. These include *S. mattheei*, *S. bovis* and *S. curassoni*, which are phylogenetically in the same clade as *S. haematobium* [3].

In Western countries, the significant migratory flow in recent years from African countries to Europe has increased the number of patients diagnosed with schistosomiasis in non-endemic regions, as well as severe complications of the disease [4–6]. This is reason behind the recommendation for the use of serological screening in new migrants coming from endemic countries by European health authorities [7]. Moreover, in recent years, two outbreaks of autochthonous transmission of urinary schistosomiasis have been described in Corsica (France) [8] and Almeria (Spain) [9], favoured by climate change which is enabling the establishment of the freshwater intermediate snail (*Bulinus* spp.) in areas where climatic conditions were not favourable before [10].

Both humans and animals can become infected with one or more of the different schistosome species, which can lead to combined symptoms of disease and comorbidities, and encourage hybridization between different species [3,11,12]. Although the existence of hybrids is well known [3], they have only recently gained special interest following the involvement of *S. haematobium* and *S. bovis* hybrids in the Corsica outbreak [13]. The spread of these hybrid forms has been reported in African countries in humans and in domestic and wild animals, with numbers in some cases exceeding 90 % of the infected population [11, 12,14–17]. Land use change, climate change, the expansion of trade, especially in livestock, migration and mobility are likely responsible for the increase in parasite hybridization as well as zoonotic effects. Therefore, the One Health approach has become a priority for achieving disease control, and it is the line being emphasized by the World Health Organisation [18].

Hybridization represents a real concern in terms of parasite transmission, epidemiology and morbidity of schistosomiasis. It can complicate disease prevention programmes, diagnosis, effective control and treatment, as hybrid forms may exhibit greater virulence and/or resistance to treatment than their parent species [17]. At present, very few studies refer to possible differences in the clinical presentation of schistosomiasis depending on the presence of hybrid or pure forms. In non-endemic areas they are limited to cases of acute schistosomiasis in travellers [19,20] or reports of isolated cases or small series of cases [13, 21–23]. In endemic regions, the few studies seem to indicate increased hepatic but decreased urogenital morbidity, and reduced improvement after treatment with praziquantel (PZQ), in those patients infected by zoonotic hybrids as compared to non-hybrids [24].

Therefore, it is highly important to better understand the implications of hybridization for the clinical management of patients with schistosomiasis. The aim of our study was to compare the epidemiological characteristics, sensitivity of diagnostic methods, clinical profile and response to treatment of sub-Saharan migrants with urinary schistosomiasis caused by pure forms of *S. haematobium* with others with hybrid schistosome infections.

2. Materials and methods

2.1. Population and study design

A prospective observational study was carried out in patients who attended the Tropical Medicine Unit (TMU) of the Hospital Universitario Poniente (El Ejido, Almería, Spain) from January 2020 to November 2023.

The patients included in the study were sub-Saharan migrants over 14 years old, attended consecutively at the TMU, who were diagnosed with confirmed genitourinary schistosomiasis after the detection of *Schistosoma* eggs in an urine sample, and who agreed to participate in the study and signed the informed consent form. A tailored protocol for other infectious diseases was systematically applied, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), *Schistosoma* and *Strongyloides* serologies, stool parasites (three concentrated stool samples), and Knott and/or saponin tests for microfilariae. Chest and abdomen X-rays were also routinely performed. Patients with HIV infection were excluded. The study was completed with a molecular urine loop-mediated isothermal amplification (LAMP) test specific for schistosomiasis (*Schisto*-LAMP) and an ultrasound examination before treatment with PZQ. Ultrasound data were collected and categorized following the World Health Organization-Niamey score for the assessment of schistosomiasis-related morbidity [25]. The researchers who carried out the ultrasound studies did not know at any time whether the patients presented pure or hybrid schistosome infections. Patients were treated with PZQ at the usual dose (40 mg/kg for 1 day). Praziquantel was provided free of charge to all patients to take on their own, and they were subsequently asked about taking the medication correctly and whether they had experienced any adverse effects.

Subsequent revisions were scheduled at 3, 6 and 12 months after PZQ administration (Fig. 1). A complete blood count, biochemistry with IgE, systematic urinalysis, study of parasites in urine, *Schistosoma* serology and LAMP tests were also performed. The ultrasound study was repeated only for patients who had a previous pathological ultrasound. When schistosome eggs were again detected in the urine, a viability test was performed.

At any post-treatment visit, the presence of viable schistosome eggs in the urine indicated retreatment. At the 6-month visit, retreatment was also indicated in cases of persistent eosinophilia without another justification, persistent bladder lesions or positive *Schisto*-LAMP results. At the 12-month visit, the treatment with PZQ was repeated in cases of the presence of viable schistosome eggs in the urine, persistent eosinophilia without another justification or persistent bladder lesions, but for 3 days.

Cystoscopy was indicated for patients in whom ultrasound lesions of the bladder had not disappeared or had not improved significantly at the 3-month follow-up.

This study was approved by the Ethics Committee of the Coordinating Site (Almería, Spain) with the code PI_19_30.

2.2. Urine samples and microscopy

The urine samples were collected between 9 a.m. and 12 a.m. and processed on the same day. After centrifugation and concentration, each urine sample was placed on a labelled slide and examined microscopically (100 ×) for schistosome eggs. Hematuria was defined as the presence of 5 or more red blood cells per high power field of centrifuged urine samples. Aliquots of fresh urine samples were sent to the Health Parasitology Unit of the University of Valencia for molecular analysis and genotyping. Other aliquots were reserved and stored at −80 °C until shipment to the laboratory at the IBSAL-CIETUS (Salamanca, Spain) for further DNA extraction for LAMP testing.

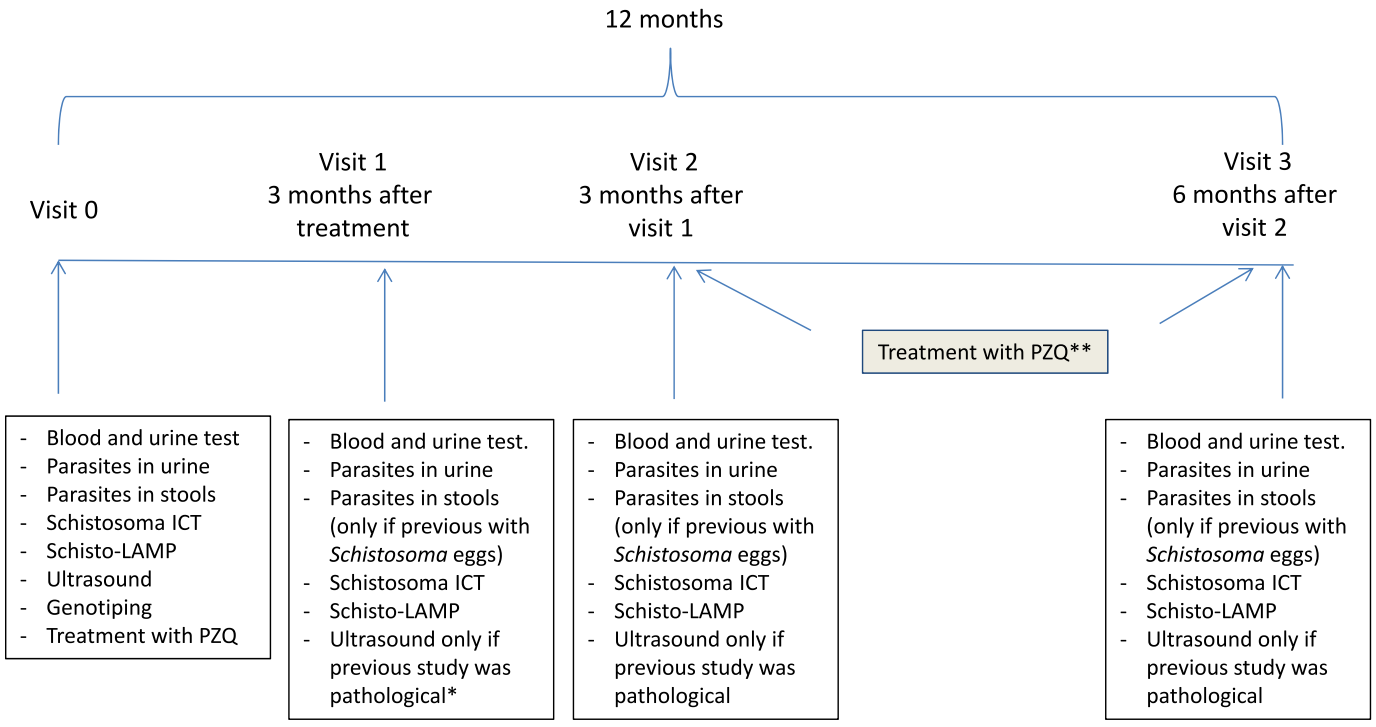
2.3. Schistosoma serology and LAMP testing

The serum samples were tested for antibodies against *Schistosoma* spp. using the rapid immunochromatographic test Schistosoma ICT IgG-IgM (LDBIO Diagnostics, Lyon, France) according to the manufacturer's instructions.

The LAMP tests were performed in combination with three different LAMP assays (hereafter, *Schisto*-LAMP assays) previously described for the specific detection of *S. mansoni* (Sm-LAMP) and *S. haematobium* (Sh-LAMP), and for the detection of the genus *Schistosoma* (*Schistosoma*-LAMP) [26] (Appendix 1).

2.4. Molecular study of schistosome eggs/miracidia and genotyping

Individual eggs/miracidia were molecularly characterized by analysis of mitochondrial *cox*1 and nuclear internal transcribed spacer 2 (ITS



*Cystoscopy was indicated in those patients in whom the bladder ultrasound lesions had not disappeared or had not significantly improved at the 3-month review.
** Treatment with PZQ in cases of presence of viable eggs in urine, persistence of eosinophilia without other justification, persistence of bladder lesions or positive Schisto-LAMP. In visit 2: PZQ 40 mg/kg, x 1 day; in visit 3: PZQ 40 mg/kg, x 3 days.

Fig. 1. Protocol schedule.

2) and 18S rDNA regions. The information provided by the maternally inherited mtDNA combined with the rDNA was used to determine species and identify hybrids [14,15].

PCR amplification and sequencing were performed independently on each of egg/miracidia and sequence polymorphisms between *S. haematobium* and *S. bovis* or *S. curassoni* were carefully analysed to identify the presence of possible heterozygosity [27,28] (Appendix 2).

2.5. Statistical analysis

For the descriptive analysis of the sociodemographic characteristics, quantitative variables were expressed as mean and standard deviation or median and interquartile range, and qualitative variables as frequency and percentage. Quantitative variables were tested for normality using the Kolmogorov-Smirnov or Shapiro-Wilk test. A bivariate analysis was carried out to determine whether there were significant differences in the different clinical and sociodemographic variables according to the presence or absence of hybrid Schistosoma eggs. For comparisons of quantitative variables, Student's t-test and Mann-Whitney *U* test were performed based on previous results of normality. For the comparison of qualitative variables, the Fisher or Chi-square tests were applied. All tests were performed with 95 % confidence intervals.

The statistical software used was R Statistical Software (v4.1.2; R Core Team 2021) and SPSS version 26 (IBM Inc., Armonk, NY, USA).

3. Results

A total of 31 patients were included in the study. All of them were male with a mean age of 26.5 years. The most frequent countries of origin were Mali, Senegal and Mauritania (Table 1). Thirty (96.8 %) were non-VFR (visiting friends and relatives) migrants and had an average period of residence in Europe of 16.5 months. None of them travelled outside Europe during the entire follow-up period. Medical visits were timed according to the protocol, with an interval of days between each visit (median, IQR) of: visit 0-visit 1: 94.0 days (90–106); visit 1-visit 2: 101.0 days (92.5–112.2); and visit 2-visit 3: 190.0 days (177.5–232.5). Twenty-four patients (77.4 %) reached the end of the study at the 12-month visit.

3.1. Genotyping and hybridization

In 12/31 (38.7 %) of the patients analysed, it was genetically confirmed that they were carriers of *Schistosoma* hybrids, in all cases coexisting with terminal-spined eggs belonging to the genetic profile of pure *S. haematobium* (*Sh*) (Table 2).

The most common hybrid genetic profile observed was *Schistosoma haematobium x S. bovis* (*ShxSb*), found in 6/12 (50 %) of the patients with hybrids. *S. haematobium x S. mansoni* (*ShxSm*) was detected in 4/12 (33.3 %) of patients, while *S. haematobium x S. curassoni* (*ShxSc*) was found in 2/12 (16.6 %) of the cases. One patient (8.3 %) from the Ivory Coast carried both *ShxSm* and *ShxSc* hybrids.

No differences were found between the two groups of patients (hybrid carriers or not) in terms of age or average length of stay in Spain. Regarding the migratory route, many cases patients crossed through countries other than their own that are also endemic for schistosomiasis, where they could have acquired the infection or been reinfected.

3.2. Analytical data and co-infections

Patients in both groups only showed differences in their analytical parameters at baseline (Table 1). When compared with those infected with *S. haematobium* alone, hybrid carriers had lower haemoglobin

Table 1
Epidemiological characteristics of the patients studied and their analytical and radiological results depending on whether or not they were carriers of *Schistosoma* hybrids.

	Total patients (N = 31)	Infected individuals with *Schistosoma haematobium* (N = 19, 61.3 %)	Infected individuals with hybrid schistosomes (N = 12, 38.7 %)	p
Age (years) (mean, SD)	26.5 (8.3)	26.9 (7.6)	26.0 (9.7)	0.36
Gender (Males, %)	31 (100 %)	19 (100 %)	12 (100 %)	
Migrant category (Number,%):				
Non VFR migrants	30 (96.8)			
VFR migrants	1 (3.2)			
Migrants' mean stay in Europe, in months (median, IQR)				
Non VFR migrants	19.5 (12.2–38.0)	26 (15.5–39.5)	13 (11.5–16.5)	0.106
VFR migrants [a]				
Country of birth (Number,%)				
Mali	12 (38.7)	9 (47.4)	3 (25.0)	
Senegal	8 (25.8)	4 (21.1)	4 (33.3)	
Mauritania	7 (22.6)	4 (21.1)	3 (25.0)	
Guinea Bissau	2 (6.5)	1 (5.2)	1 (8.3)	
Gambia	1 (3.2)	1 (5.2)	0	
Ivory Coast	1 (3.2)	0	1 (8.3)	
Hb (mean, SD) [b]				
Visit 0 (31, 100 %)	14.4 (1.6)	14.8 (1.4)	13.8 (1.8)	**0.04**
Visit 1 (23, 74.2 %)	14.4 (1.5)	14.3 (1.7)	14.6 (1.1)	0.78
Visit 2 (27, 87.1 %)	14.6 (1.5)	14.3 (1.7)	15.0 (1.2)	0.29
Visit 3 (19, 61.3 %)	14.8 (1.7)	14.8 (1.9)	14.7 (1.3)	0.74
Eo/mm^3 (mean, SD)				
Visit 0 (31, 100 %)	606.9 (533.8)	503.7 (503.2)	770.4 (561.4)	0.08
Visit 1 (22, 74.2 %)	287.2 (186.8)	292.9 (188.1)	278.3 (195.8)	0.86
Visit 2 (27, 87.1 %)	347.1 (251.2)	338.1 (240.8)	360.3 (277.1)	0.86
Visit 3 (19, 61.3 %)	316.3 (260.7)	349.1 (312.1)	271.2 (177.7)	0.90
IgE (UI/mL) (mean, SD)				
Visit 0 (30, 96.8 %)	2296.1 (3144.1)	2391.3 (3624.2)	2131.6 (2231.0)	0.87
Visit 1 (21, 67.7 %)	3248.7 (4155.1)	3487.9 (4632.3)	2860.0 (3502.0)	0.92
Visit 2 (27, 87.1 %)	1596.3 (2235.7)	1967.1 (2608.8)	1057.0 (1499.2)	0.21
Visit 3 (19, 61.3 %)	1212.4 (1606.6)	1297.7 (1937.0)	1095.1 (1118.0)	0.90
Hematuria (number, %)				
Visit 0 (31, 100 %)	22 (71.0)	10 (52.6)	12 (100)	**0.005**
Visit 1 (20, 64.5 %)	3 (15.0)	2 (16.7)	1 (12.5)	1
Visit 2 (26, 83.9 %)	3 (11.5)	2 (12.5)	1 (10.0)	1
Visit 3 (24, 77.4 %)	2 (8.3)	2 (14.3)	0	0.49
Detection of eggs in urine (number of patients, %)				
Visit 0 (31, 100 %)	31 (100)	19 (100)	12 (100)	1
Visit 1 (22, 71.0 %)	0	0	0	1
Visit 2 (28, 90.3 %)	2 (7.1)	1 (5.9)	1 (9.1)	1
Visit 3 (23, 74.2 %)	2 (8.7)	1 (7.7)	1 (10)	
ICT positive (number, %)				
Visit 0 (31, 100 %)	31 (100)	19 (100)	12 (100)	1
Visit 1 (21, 67.7 %)	21 (100)	13 (100)	8 (100)	1
Visit 2 (26, 83.9 %)	25 (96.2)	15 (93.8)	10 (100)	1
Visit 3 (19, 61.3 %)	19 (100)	11 (100)	8 (100)	1
Schisto-LAMP positive (number, %)				
Visit 0 (31, 100 %)	21 (67.7)	12 (63.2)	9 (75.0)	0.70
Visit 1 (22, 71.0 %)	10 (45.5)	6 (46.2)	4 (44.4)	1
Visit 2 (24, 77.4 %)	12 (50.0)	7 (46.7)	5 (55.6)	1
Visit 3 (24, 77.4 %)	10 (41.7)	5 (35.7)	5 (50.0)	0.68
Urogenital score (mean, SD)				
Visit 0 (30, 96.8 %)	1.53 (1.74)	0.89 (0.99)	2.64 (2.20)	**0.02**
Visit 1 (15, 48.4 %)	0.27 (0.7)	0.29 (0.76)	0.25 (0.71)	1
Visit 2 (5, 16.1 %)	1 (1.41)	0.67 (1.15)	1.50 (2.12)	0.80
Visit 3 (7, 22.6 %)	0.57 (1.51)	0	1 (2)	0.63

[a] The VFR patient had been resident in Europe for 19 years, and had returned from the last visit in his home country (Senegal) 10 months prior to inclusion in the study.

[b] At each visit, the number and percentage of patients analysed are indicated. Hb: haemoglobin. Eo: eosinophils. ICT: immunochromatographic test. IgE: Immunoglobulin E.

levels (13.8 g/dL [SD 1.8] vs 14.8 g/dL [SD 1.4], p = 0.04), and a greater frequency of hematuria (12 patients, 100 % vs 10 patients, 52.6 %, p = 0.005) respectively. No differences were found in baseline IgE or eosinophil levels, although there was a clear trend towards higher values of eosinophils in patients with hybrid schistosomes. After PZQ treatment, haemoglobin levels were equal in both groups and the disappearance of hematuria was similar. There were also no differences in changes in eosinophil or IgE levels (Fig. 2).

Only two patients were retreated at 6 months based exclusively on the presence of eosinophilia. In one case, eosinophilia disappeared at the 12-month visit. The other patient had persistent mild eosinophilia at the 12-month visit but refused to continue with the study.

Regarding the co-infections, four patients had chronic HBV infection. *Blastocystis hominis* was isolated from eleven patients, *Giardia lamblia* from one patient, and *Schistosoma mansoni* from one patient. In the latter case, although the patient was coinfected with *S. haematobium*, none of the eggs analysed in urine were genetically hybrids.

Table 2
Baseline characteristics of individuals infected with *Schistosoma* hybrids.

Patient number in the study	Country of birth	Age (years)	Migratory route	*Schistosoma* genetic profile	Eosinophils/mL	Systematic urinalysis	Number of eggs/mL of urine	Urogenital score
3	Guinea Bissau	21	Senegal, Mali, Burkina Faso, Algeria, Morocco, Spain	*Sh + Sh x Sm*	410	Hematuria	0.93	5
6	Senegal	19	Mali, Burkina Faso, Niger, Libya, Italy, Spain	*Sh + Sh x Sm*	1230	Hematuria	0.70	2
7	Mauritania	28	Morocco, Spain	*Sh + Sh x Sc*	384	Hematuria	1.36	0
8	Mali	24	Mauritania, Morocco, Spain	*Sh + Sh x Sb*	370	Hematuria	0.28	Unrealized
10	Mauritania	38	Morocco, Spain	*Sh + Sh x Sb*	110	Hematuria	1.05	2
11	Ivory Coast	23	Burkina Faso, Niger, Algeria, Morocco, Spain	*Sh + Sh x Sm + Sh x Sc*	1140	Hematuria	0.87	2
12	Senegal	23	Morocco, Spain	*Sh + Sh x Sm*	796	Hematuria	2.56	2
16	Senegal	52	VFR patient[a]	*Sh + Sh x Sb*	1470	Hematuria	Not available	3
18	Mali	24	Mauritania, Spain	*Sh + Sh x Sb*	1970	Hematuria	1.04	5
19	Mauritania	18	Direct to Spain (Canary Islands)	*Sh + Sh x Sc*	530	Hematuria	0.29	1
27	Senegal	20	Direct to Spain (visa)	*Sh + Sh x Sb*	480	Hematuria	0.68	0
28	Mali	22	Algeria, Spain	*Sh + Sh x Sb*	360	Hematuria	0.80	7

[a] Original migratory route: Gabon, France, Spain. Genetic profile of pure or single species indicated as Sh = *Schistosoma haematobium*. Genetic profile hybrid, indicated by the combination of two *Schistosoma* species involved: *Sh x Sb* = *S. haematobium* x *S. bovis* hybrids; *Sh x Sm* = *S. haematobium* x *S. mansoni* hybrids; *Sh x Sc* = *S. haematobium* x *S. curassoni* hybrids.

3.3. Persistence of eggs in urine and treatment with PZQ

Schistosoma eggs were not detected in the urine of any of the patients studied at visit 1 (3 months). At visit 2 (6 months), viable eggs were detected in two patients, one in the hybrid group (patient number 12, Table 2), and one in the *S. haematobium* alone group. Both patients were retreated with PZQ at 40 mg/kg for 1 day.

At 12 months, patient 12 had already tested negative for parasites in urine, but viable eggs were isolated in patient 8. He was treated with PZQ at 40 mg/kg for 3 days, with a subsequent negative microscopic result after 3 months.

The patient with viable *S. haematobium* eggs at visit 2 had viable eggs again at 12 months. He insisted that he had taken the medication correctly. He was also treated with PZQ at 40 mg/kg for 3 days. After this last dose, two subsequent controls showed persistence in the elimination of eggs although these were no longer viable, and finally resulting in the negativization of the eggs in urine.

According to the study protocol, a total of 11 patients were treated with PZQ at 6 months for the presence of urine eggs or ultrasound lesions, the existence of a positive LAMP test or the persistence of eosinophilia without an alternative diagnosis.

3.4. Serology and LAMP tests

The Schistosoma ICT IgG-IgM test was positive in all patients initially and at all follow-up visits in both groups except at visit 2 for one patient infected exclusively with *S. haematobium*. At visit 3 it was again positive. The LAMP tests showed similar positivity rates at baseline and throughout follow-up in patients with and without the presence of hybrid schistosomes (Table 1).

3.5. Ultrasound findings

Patients with the presence of hybrid schistosomes had higher a pretreatment score (2.64, SD 2.20 vs. 0.89, SD 0.99; p = 0.02) (Table 1). 9/11 patients (81.8 %) with a baseline study had a pathological ultrasound, including 7/11 (63.6 %) with pseudopolyps, with three presenting multiple lesions (2–5 lesions per individual). Other findings in this group were focal wall thickening in four patients (33.3 %), multifocal lesions in two of them, and diffuse bladder thickening in one patient.

In patients infected exclusively with *S. haematobium*, ultrasonography revealed pathological findings in nine of them (47.4 %), with seven patients (36.8 %) with pseudopolyps (all patients with single lesions), and two patients (10.5 %) with focal wall thickening, one of whom had multiple lesions.

After treatment, at medical visit 1, in 15/18 (83.3 %) patients with initial pathological ultrasound (nine in each group) was possible to perform the control ultrasound. Only two patients, one in each group, still had bladder lesions, but in both cases they had improved from the initial examination.

At 6 months, two patients in the hybrid group (one of whom did not undergo ultrasound at 3 months) and one in the *S. haematobium* group still had bladder lesions, two of whom showed improvement as compared with previous studies. Only one of them, patient number 12 (Table 2), presented *Schistosoma* eggs in urine. All patients were retreated with standard doses of PZQ. At the 12-month visit, the bladder lesions had completely disappeared in all three patients.

We found a particular case in patient number 8 (Table 2). The first ultrasound scan was performed 6 months after treatment and was normal. However, at visit 3, when he again presented viable *Schistosoma* eggs in urine, a new ultrasound was requested, which revealed 2 pseudopolyps. The patient denied having travelled to his country during this period. This patient was retreated with PZQ at 40 mg/kg for 3 days, and the pseudopolyps disappeared 3 months later.

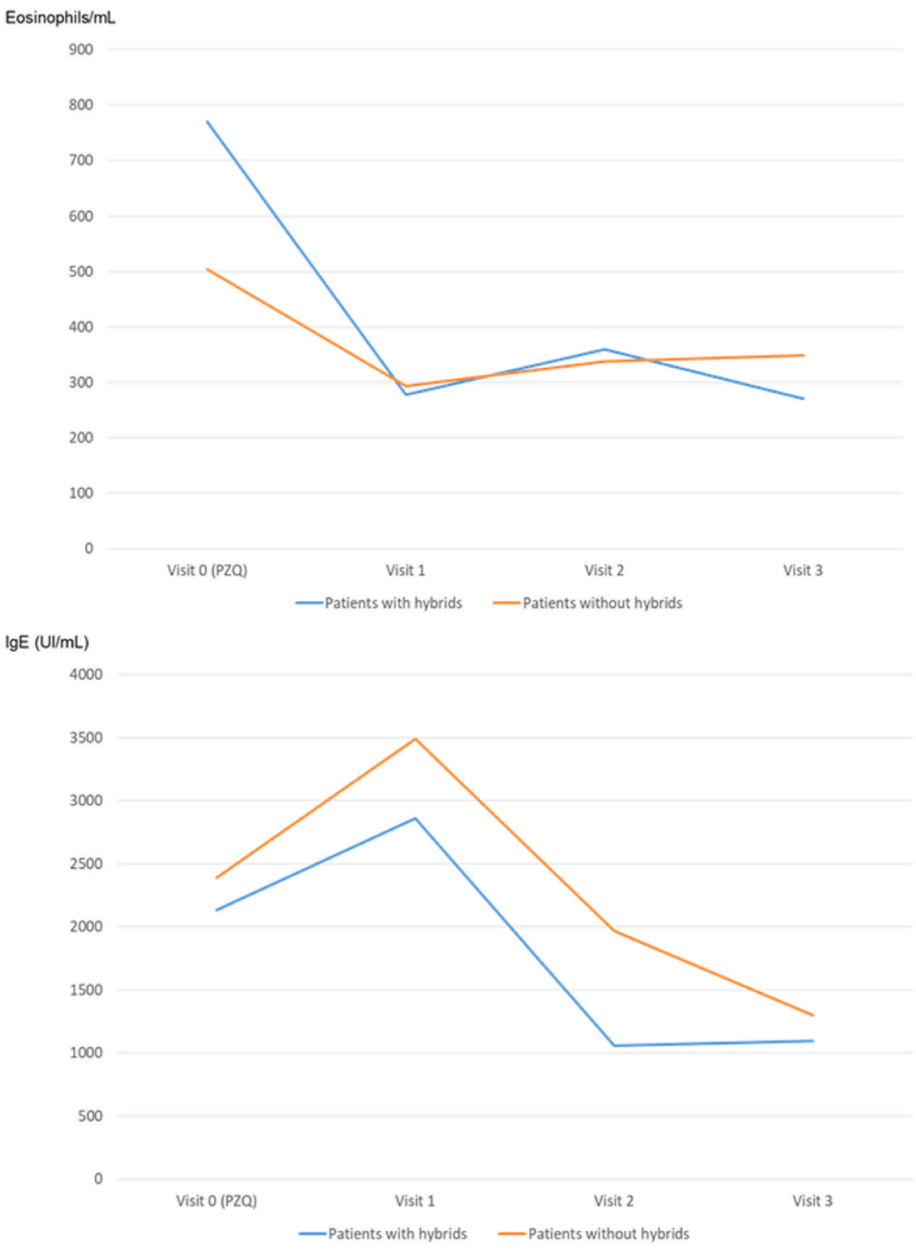


Fig. 2. Trends in eosinophil and IgE levels after treatment.

4. Discussion

Considering the results of our study, we can state that hybridization of *S. haematobium* with other human intestinal species (*S. mansoni*) and zoonotic species (*S. bovis, S. curassoni*) is frequent in migrant patients from West Africa with urogenital schistosomiasis. The presence of these hybrids seems to cause increased morbidity in infected individuals. However, it does not appear to result in differences in diagnostic tests or in clinical and analytical responses after treatment with standard doses of PZQ.

Parasite hybridization represents an emerging public health problem with the potential to have a significant impact on the evolution and epidemiology of parasites worldwide, with important challenges for the control of parasitic diseases [29]. Hybridization can originate from direct mating between species leading to viable hybrids (hybrids defined as offspring resulting from crossing between two species), but also from introgressions (the introduction of single genes or chromosomal regions from one species to another through repeated backcrossing) in parasites in both animal and human populations [29].

In the case of schistosomiasis, there is evidence that hybrid schistosomes may have a wider variety of intermediate (snails) and definitive hosts than their single pure parent species, which may also result in a wider geographical range for hybrid schistosome infections [12,29]. This could favour its establishment in geographical areas where it is currently absent, such as Europe. The increase in animal reservoirs (domestic and wild) may in turn complicate transmission prevention measures, even when human prevalence reaches levels suitable for the interruption [30]. From a human health perspective, "hybrid vigour" could lead to increased infectivity and virulence, and enhance the emergence of drug resistance [3,29].

The presence of hybrids outside endemic areas has been poorly studied thus far. In addition to reports of isolated cases in migrants [21,22], the Corsican outbreak revealed that 7/12 (58 %) of the people studied had *S. haematobium-S. bovis* hybrid eggs [13]. In our study, the prevalence of hybrids was almost 40 %, reaching 50 % in migrants from Senegal. Furthermore, a great variability was observed, affecting both

human and zoonotic species.

Few studies have examined the impact of schistosomiasis hybridization on morbidity in humans. Fall et al. [24], in a study conducted in Senegal mostly in children, compared urogenital morbidity in two areas, one with a high prevalence of *S. haematobium-S. bovis* hybrids and another with a predominance of pure forms of *S. haematobium*. The analysis including only children with genotyped miracidia showed no differences in urogenital morbidity between those with and without hybrids. However, an increased liver morbidity (hepatomegaly), and a decreased urogenital morbidity (ureteral lesions), were observed in hybrid as compared to non-hybrid infections, attributed to the effect of zoonotic intestinal species. In our study, which exclusively focused on the adult migrant population, we found higher morbidity in patients with hybrids, with a prevalence of hematuria in 100 % of patients (which could explain the lower haemoglobin levels), and a significantly greater number of bladder lesions. However, none of the patients had ultrasound findings of liver involvement. These differences may be determined by the different epidemiological characteristics of the patients, and the different *Schistosoma* hybrid populations, which may lead to differences in morbidity. If the presence of hybrids leads, as it seems, to a greater number of genitourinary lesions, an expected consequence is that severe complications of the disease, such as hydronephrosis or bladder cancer, could increase in the future.

In our study, serology was proven to be a very sensitive method, although it should be noted that all patients with hybrid schistosomes were also coinfected with "pure" *S. haematobium*. Regarding molecular techniques, PCR has shown an excellent sensitivity for the detection of hybrid species in clusters of patients with acute schistosomiasis [19,20]. Here, we used the LAMP technique for diagnosing the schistosomiasis. The sensitvity obtained was not very high, although results were similar in both groups of patients. This may have been due to the use of a single urine sample per patient and the very small sample volume used in the amplification (2 μL of DNA extracted from only 2 mL of total urine volume per patient), which most likely underestimated the molecular diagnosis.

The presence of a positive LAMP test at the final visit without any other clinical or analytical data of active disease was considered due to the persistence of the elimination of biological remains of schistosome adults or non-viable eggs. The length of time in which this technique can remain positive in urine once the infection is considered cured is yet to be determined.

Concerning the response to the PZQ treatment, the study by Fall et al. [24] showed that changes in morbidity were independent of the presence or absence of *S. haematobium-S. bovis* hybrids, except for anaemia, which tended to decrease more frequently in children with hybrid schistosomes, with bladder lesions decreasing less frequently in response to PZQ than in children without hybrids. Among the patients in our study, we found no differences between the two groups. On the other hand, standard doses of PZQ rapidly reduced bladder lesions in hybrid-infected patients, despite the significantly higher ultrasound score, and differences were not observed in treatment failure. Although the response to treatment does not seem to be a problem at present, it is possible that in the future new hybrids may develop resistance mechanisms to PZQ, a drug for which there are very few alternatives. Regarding patient number 8, with the appearance of new lesions in the ultrasound control at the 12-month visit, a possible explanation would be the failure of the echographer to detect the lesions during the first examination.

The limitations of our study are mainly related to the small sample size and failure to complete all scheduled patient visits. The unscheduled visits were mainly due to the characteristics of the population studied, which has a high geographic mobility for work-related reasons. Additionally, the sample size does not allow drawing conclusions about possible differences in morbidity between the different hybrid species. Nevertheless, the strengths of our study include the epidemiological characterization of patients, the follow-up within the predetermined times established in the protocol, and the avoidance of biases that could be due to reinfection during follow-up, as occurs in studies in endemic regions.

5. Conclusion

In conclusion, hybridization of schistosome species is a phenomenon that appears to be increasing, and may have an impact on disease control in endemic regions, allowing the establishment of schistosomiasis in other regions where it is currently absent. However, at present, hybridization does not appear to have an impact on the diagnosis and clinical management of the disease. Future studies are needed to assess possible differences in morbidity between the different types of hybrids, as well as whether increased morbidity translates into a greater number of severe disease complications.

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CRediT authorship contribution statement

Joaquín Salas-Coronas: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **M. Dolores Bargues:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation. **Pedro Fernández-Soto:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation. **Manuel J. Soriano-Pérez:** Writing – review & editing, Investigation, Data curation. **Patricio Artigas:** Writing – review & editing, Investigation, Data curation. **José Vázquez-Villegas:** Writing – review & editing, Investigation, Data curation. **Antonio Villarejo-Ordoñez:** Writing – review & editing, Investigation, Data curation. **José C. Sánchez-Sánchez:** Writing – review & editing, Investigation, Data curation. **María I. Cabeza-Barrera:** Writing – review & editing, Investigation, Data curation. **Begoña Febrer-Sendra:** Writing – review & editing, Investigation, Data curation. **Alejandra De Elías-Escribano:** Writing – review & editing, Investigation, Data curation. **Beatriz Crego-Vicente:** Writing – review & editing, Investigation, Data curation. **María C. Fantozzi:** Writing – review & editing, Investigation, Data curation. **Juan García-Bernalt Diego:** Writing – review & editing, Investigation, Data curation. **Nerea Castillo-Fernández:** Writing – review & editing, Investigation, Data curation. **Jaime Borrego-Jiménez:** Writing – review & editing, Investigation, Data curation. **Antonio Muro:** Writing – review & editing, Investigation, Data curation. **María P. Luzón-García:** Writing – review & editing, Supervision, Investigation, Data curation.

Declaration of competing interest

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.

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Appendix A. Supplementary data

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