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► **To cite this version:**

Bruno Polack, Eglantine Mathieu-Bégné, Isabelle Vallée, Anne Rognon, Jean-Jacques Fontaine, et al.. Experimental Infections Reveal Acquired Zoonotic Capacity of Human Schistosomiasis Trough Hybridization. *Journal of Infectious Diseases*, 2024, 10.1093/infdis/jiae152 . hal-04562390

**HAL Id: hal-04562390**

**<https://hal.science/hal-04562390>**

Submitted on 29 Apr 2024

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## **Experimental Infections Reveal Acquired Zoonotic Capacity of Human Schistosomiasis Through Hybridization**

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### **ABSTRACT**

We are currently witnessing the endemization of urogenital schistosomiasis in southern Europe. The incriminated parasite is a hybrid between a human parasite and a livestock parasite. Using an experimental evolutionary protocol, we created hybrid lines from pure strains of both parasite species. We showed that the host spectrum of the human parasite is enlarged to the livestock parasite after genomic introgression. We also evidenced that the tropism of the parasites within the host changes and that some hybrid lines are more virulent than the parental strains. These results engage a paradigm shift from human to zoonotic transmission of urogenital schistosomiasis

### **KEYWORDS**

emergence; hybrids; livestock; schistosomiasis; zoonoses

In summer 2013, urogenital schistosomiasis emerged in the south of Europe. Tourists from Germany and France have contracted this usually tropical disease in the Cavu River, Corsica Island, France [1]. During the first outbreak, 106 cases were referenced [2]. Since then, new cases keep being identified each year, and the disease recently jumped to a second river [3]. We are currently assisting to the endemization of this tropical disease in southern Europe. The etiologic agent responsible for this emergence is a hybrid between *Schistosoma haematobium* (*Sh*), a human parasite, and *Schistosoma bovis* (*Sb*), a parasite of livestock and rodents. This hybrid is largely present in several countries from West Africa [4]. Hybridization is of great concern because it raises the zoonotic potentiality of this typical human pathogen. This hybrid has not been found in cattle in Senegal [5] or Cameroon [6], but it has been evidenced in rodents in Senegal [7] and cattle and rodents in Benin [8, 9]. After a large campaign of serologic diagnostics, schistosomes were not found in cattle, goat, or sheep in Corsica Island, but some rodents were noted to host the hybrid parasite [10]. Inferring the zoonotic capability of a pathogen is paramount for controlling transmission in endemic areas, which concerns the 78 countries in which urogenital schistosomiasis is endemic, but it also makes it possible to predict the spread of epidemics in European countries where the snail vector is present (Spain, France, Portugal, and Italy). This zoonotic capability can be addressed by population genetic studies as previously done [8, 9] or by experimental infections on the natural animal host. In this article, we demonstrate for the first time the increase in the host spectrum of urogenital bilharziasis after crossbreeding with bovine bilharziasis using sheep infections.

## METHODS

### Parasite lines and vertebrate hosts

*Sh* and *Sb* were maintained in the Interactions Hotes-Pathogenes-Environnements Laboratory by using *Bulinus truncatus* snails as intermediate hosts and *Mesocricetus auratus* as definitive hosts. The parasite strains originated from Egypt and Spain for *Sh* and *Sb*, respectively. The *Sh* strain, isolated from the urine of an infected human, was provided by the Biomedical Research Institute (Rockville, Maryland). The *Sb* strain, isolated from the feces of an infected cow, was provided by Ana Oleaga from the Spanish laboratory of parasitology of the Institute of Natural Resources and Agrobiology in Salamanca. Eight- to 10-week-old female golden hamsters (*M auratus*) were provided by Janvier-Labs. Twelve- to 16-week-old female lambs of the Romane breed were provided by an authorized supplier.

### Experimental protocol

Parasite lines were constructed on experimental hamster hosts, and the cercariae were used to infect 3 series of sheep with pure species, F1 parasites, or backcrossed parasites (Figure 1). Methods for mollusk, hamster, and sheep infection were previously described [11, 12].

Hamsters and sheep were infected with a natural percutaneous immersion method. Miracidia from *Sb* or *Sh* were isolated from the liver of infected hamsters. Single miracidia were proposed to *B truncatus* infection. Fifty-five days postexposition, surviving positive snails were selected because they emitted cercariae. The sex of the clonal population hosted by each positive snail was determined by polymerase chain reaction [13]. The same number (150) of male and female cercariae of *Sb* and *Sh* was proposed to hamster infection to produce F1 hybrid crosses. We have constituted the 2 mirror hybrid lines: [ $\sigma Sb \times \text{♀} Sh$ ] and [ $\sigma Sh \times \text{♀} Sb$ ]. Ninety-days postinfection, the hybrid miracidia were recovered in the liver of the latter infected hamsters. Two batches of snails (A1 and A2) were exposed to 5 miracidia coming from the 2 F1 hybrid lines, [ $\sigma Sb \times \text{♀} Sh$ ] and [ $\sigma Sh \times \text{♀} Sb$ ], respectively. Two other batches of snails (B1 and B2) were exposed to single miracidia coming from the 2 F1 hybrid lines, [ $\sigma Sb \times \text{♀} Sh$ ] and [ $\sigma Sh \times$

♀Sb]. Fifty-five days postexposition, the cercariae emitted by the A1 and A2 batches were used to infect sheep with the 2 F1 hybrid lines (3 sheep by line). The sex of the clonal population hosted by each positive snail of the B1 and B2 batches was determined by polymerase chain reaction [13]. The same number (150) of male and female cercariae of F1 hybrid lines and pure strains was proposed to hamster infection to produce 4 backcrossed lines:

♀ [ $\sigma$ Sb × ♀Sh] ×  $\sigma$ Sb

♂ [ $\sigma$ Sb × ♀Sh] × ♀Sb

♀ [ $\sigma$ Sh × ♀Sb] ×  $\sigma$ Sh

♂ [ $\sigma$ Sb × ♀Sh] × ♀Sh

Ninety days postinfection, the backcrossed miracidia were recovered in the liver of the latter infected hamsters. Four batches of snail (C1-C4) were exposed to 5 miracidia from each of the 4 backcrossed lines. Fifty-five days postexposition, the cercariae emitted by batches C1 to C4 were used to infect 3 sheep by line. Three control sheep were exposed to the pure strains: Sb and Sh. The same number of cercariae (1500) was used to infect each sheep. Sheep infection was done under anesthesia (ketamine and diazepam) at the level of a forearm previously wet with warm water. In each line, a minimum of 12 snails exposed to 5 miracidia was used to produce the cercariae for sheep infection.

Euthanasia was performed by deep anesthesia of the sheep with a combination of xylazine and ketamine, followed by bleeding the animals to collect blood. Autopsies were performed under the supervision of an European College of Veterinary Pathologists board member. All viscera were examined, and the vessels of the small intestine, cecum, colon, rectum, and bladder were examined by candling with a strong light.

### **Ethic statement**

Experiments were carried out according to national ethical standards established in the writ of 1 February 2013 (NOR: AGRG1238753A), setting the conditions for approval, planning, and operation of establishments, as well as the breeders and suppliers of animals used for scientific purposes and the controls.

Hamster infection and euthanasia were performed at the UMR5244 laboratory (Perpignan, France). The animal facility possesses agreement A66040 delivered by the French Ministry of Higher Education, Research and Innovation. The investigator possesses the official certificate for animal experimentation (decret 87-848, 19 October 1987, authorization 007083).

Sheep infection and autopsy were performed at Ecole Nationale Vétérinaire d'Alfort (Maisons-Alfort, France). The animal facility possesses agreement E940462 delivered by the French Ministry of Higher Education, Research and Innovation. The sheep experiment was registered by the French Ministry of Higher Education, Research and Innovation and approved by an independent ethics committee (APAFIS 23786-2020012416159143 v2).

### **RESULTS**

Figure 2 shows the repartition of single males, females, and worm pairs for each parasite line and their localization in the sheep venous system. No parasite was found in the veins surrounding the bladder,

and as expected, the *Sh* parental strain was unable to infect sheep. *Sb* parasites strongly infected sheep, and the majority of parasites were paired and localized in the veins surrounding the small intestine. Even though they had half of the *Sh* human parasite genome, the F1 hybrids kept their capacities to infect sheep host. Interestingly, the tropism of the parasites shifted from the small intestine toward the colon except for single male parasites in the  $\sigma Sh \times \text{♀} Sb$  F1 line. When F1 was backcrossed with *Sh* (ie, approximately 75% *Sh* genome), parasite infectivity drastically decreased: mainly, males were able to infect sheep but some pairs were recovered. When F1 was backcrossed with *Sb* (ie, approximately 75% *Sb* genome), all crosses effectively infected sheep with no unique tropism and a great variance in parasite infectivity. The  $\text{♀} [\sigma Sb \times \text{♀} Sh] \times \sigma Sb$  and  $\sigma [\sigma Sb \times \text{♀} Sh] \times \text{♀} Sb$  crosses showed a mean  $\pm$  SD of  $381.7 \pm 92$  and  $704.7 \pm 63.7$  parasites in the 3 sheep, as compared with  $279.8 \pm 31.4$  parasites in pure *Sb* infection.

## DISCUSSION

This experiment reveals for the first time that the widening of the definitive host spectrum of the human *Sh* parasite can be acquired through genomic introgression from the *Sb* livestock infecting parasite. Interestingly, the tropism of the parasite changed following genetic introgression. This can be explained by the fact that *Sb* lives in the veins surrounding the intestine while *Sh* lives in the vein surrounding the bladder. Because we did not observe parasites in the sheep's bladders, we deduced that regardless of the type of hybrid cross-created, the hybrids lose their capacity to join the urogenital venous system. This last point could explain why eggs of hybrids have never been found in the urine but in feces of field-infected animals. This engages the future visual prospection of hybrids more in the cecum and colon as compared with the small intestine. The 75% *Sh*/25% *Sb* genomic ratio is comparable to the genomic introgression level in hybrid human parasites collected in the field that emerged in Corsica [14]. In our experiment, these hybrid lines were not very infective, but some pairs were detected in the animal, with an excess of single males.

The preponderance of males in these last lines and some other crossed lines could be explained by the Haldane rule, which proposes that in situations of interspecific crosses, the heterogametic sex (ZW female in schistosome) is absent, rare, or sterile [15]. Finally, some *Sb* backcrossed lines seemed to be particularly infective because of hybrid vigor by introgression.

The recent discovery of hybrids in humans and animals in Benin [8, 9], with these experimental infections on a natural, ecologically realistic host, engages a paradigm shift from human to zoonotic transmission of urogenital schistosomiasis with important consequences in terms of control measures.

### *Author contributions :*

Sheep infection: P. B., M.-B. E., V. I., R. A., F. J.-J., T. M., and B. J.

Hamster infection: R. A. and B. J.

Writing manuscript: P. B., M.-B. E., V. I., T. E., and B. J.

Conceptualization: P. B., V. I., F. J.-J., T. E., and B. J.

*Financial support :*

This work was supported by the program HySWARM, ANR (ANR-18-CE35-0001); and is set within the framework of the Laboratoire d'Excellence TULIP (ANR-10- LABX-41).

*Potential conflicts of interest :*

All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

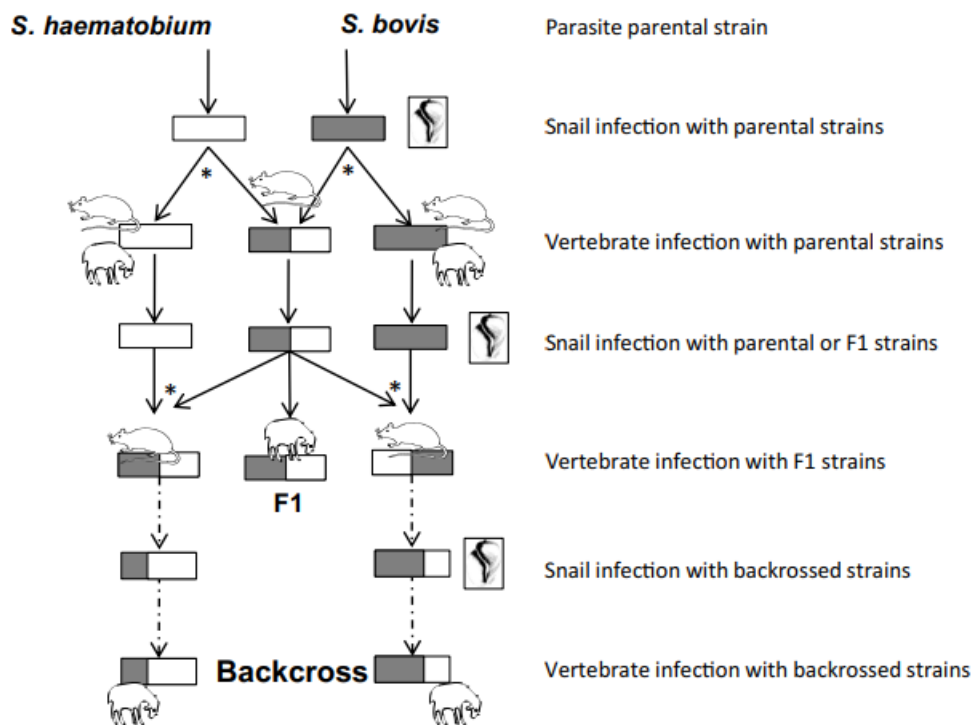
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**Figure 1.**

Protocol of infection. Snails are infected with a single miracidium (ie, only 1 sex of the parasite). The emitted cercariae are sexed to constitute the F1 lines. F1 parasites are used to infect mollusks with miracidia and sheep with cercariae. The F1 lines are then backcrossed with males or females of the pure lines to form the backcrossed lines. The backcrossed lines are used to infect mollusks with miracidia and sheep with cercariae.

\*Parasite sexing by polymerase chain reaction. *S. bovis*, *Schistosoma bovis*; *S. haematobium*, *Schistosoma haematobium*





**Figure 2.**

Mean  $\pm$  SD of single females, single males, or worm pairs recovered in sheep infected by *Schistosoma bovis* (Sb)  $\times$  *Schistosoma haematobium* (Sh) hybrid schistosomes. Worm locations are presented according to their localization in the mesenteric vein system ;

