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Trends in Parasitology

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Opinion

Fasciola Species Introgression: Just a Fluke or Something More?

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The threats posed by a range of viral and bacterial zoonotic diseases inevitably receive renewed attention in the wake of global pandemic events due to their overt and devastating impacts on human health and the economy. Parasitic zoonoses, however, many of which affect millions of people each day, are frequently ignored. In the case of fasciolosis, caused by infection with *Fasciola hepatica* or *Fasciola gigantica*, this oversight has allowed for the expansion of areas of parasite sympatry and thus increased the incidence of hybridization and possible introgression between the two species. Here we highlight how an increased demand for animal-derived protein, combined with a lack of appropriate tools for detection of these events, is changing the status quo of these zoonotic parasites.

Highlights

Increased demand for animal-derived protein from *Fasciola hepatica*-endemic countries has led to a growing number of reports of hybridization between *F. hepatica* and *Fasciola gigantica* in Southeast Asia.

Hybridization and eventual introgression have been reported in a range of protozoan, helminth, and arthropod parasites and act as important drivers of evolutionary change and adaptation.

Introgression between *Fasciola* spp. remains unproven but has potentially serious human and animal health consequences as seen in other parasites.

New tools for the characterization of hybridization and introgression events between *Fasciola* spp. are needed.

Parasitic Zoonoses of Livestock in Developing Countries

As seen during the current coronavirus disease 2019 pandemic, zoonotic diseases are one of the greatest threats to human health. The high death rates associated with the more overt viral and bacterial diseases demand global attention and a rapid and coordinated response in order to limit human health consequences and impacts to the economy. Chronic and subclinical diseases, however, such as those caused by infection with parasites, are largely overlooked despite affecting millions of people each day. Parasitic diseases of livestock are of particular concern due to the additional threat they pose to animal productivity and food security[i]. Despite these concerns, the propensity for these diseases to impact rural communities in developing countries means that they are often neglected by the wider scientific community (Figure 1, Key Figure).

Fasciolosis: Inequality in the Context of Parasitic Zoonoses

One example of a parasitic zoonosis that is largely overlooked in the developing world is fasciolosis, caused by infection with the digenean trematodes *F. hepatica* and *F. gigantica* (Box 1). The economic impacts of fasciolosis on livestock production outcomes are underestimated but are expected to exceed US$3 billion/year, with over 180 million people – primarily women and children – considered at risk of infection [1,2]. Livestock production impacts due to infection with either *Fasciola* species range from sudden death, in the case of acute infections, to chronic losses due to decreased milk yield and fertility, reduced body condition scores, liveweight gains, and wool growth [3–7]. Infection with *F. hepatica* has been shown to modulate the immune system of infected hosts by causing a shift towards a T helper 2 (Th2) cell response, leaving hosts more susceptible to infection with other bacterial pathogens such as *Mycobacterium bovis* and *Bordetella pertussis* [8,9]. This immunomodulation has also been shown to confound the outcomes of diagnostic tests for diseases, including bovine tuberculosis, due to the suppression of an effective Th1 response, increasing the rate of false-negative results [10].

In both human and animal infections the clinical signs of fasciolosis depend on the period of infection (either invasive/acute or chronic) and are related to the level of damage to the liver [11,12]. Ectopic or aberrant fasciolosis has been shown to occur when migrating immature flukes find their way to other

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Key Figure

The Distribution of *Fasciola hepatica, Fasciola gigantica*, and *Fasciola* Hybrids in Southeast Asia and the Diversity of PubMed Records since 2000

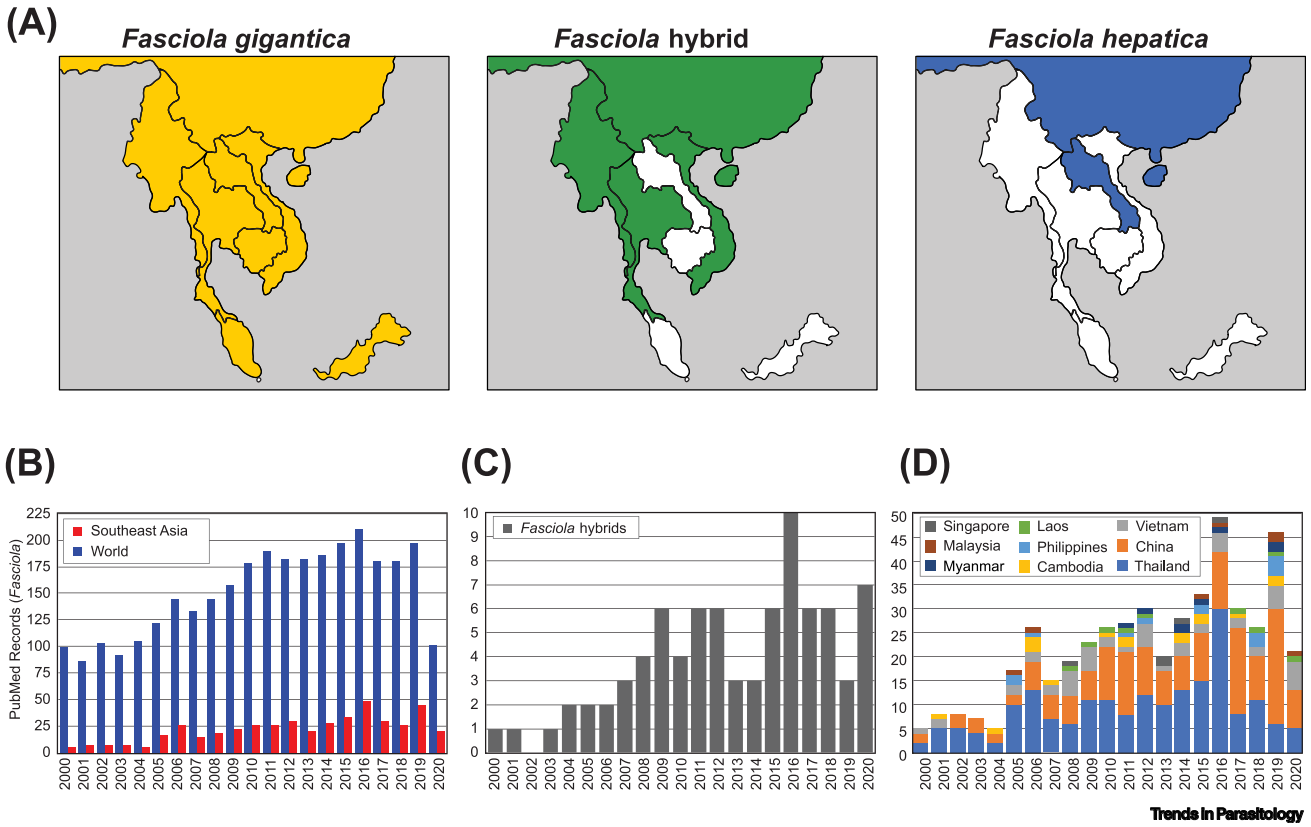

Figure 1. (A) The distribution of *F. hepatica, F. gigantica*, and *Fasciola* hybrids in Southeast Asia and China is shown in blue, yellow, and green, respectively, according to PubMed records where at least one instance of local infection was molecularly identified using a minimum of two markers, ideally one nuclear and one mitochondrial. (B) The number of PubMed records per year for Southeast Asia in comparison to global records demonstrates the neglect of these parasites in the developing world over the past two decades. (C) The number of PubMed records per year for *Fasciola* hybrids is increasing since 2000. (D) The distribution of PubMed records for *Fasciola* by country in Southeast Asia is slowly increasing, but is overwhelmingly dominated by records from China, Thailand, and Vietnam – the most developed countries in the region – with less than ten records per year for the remaining six countries combined since 2000.

organs, most commonly elsewhere in the gastrointestinal tract but also the abdominal wall, heart, lungs, and occasionally, the eyes or brain [13,14]. Reports of infection with *F. hepatica* are more common in human cases than infection with *F. gigantica*, leading to the assumption that *F. hepatica* has the greater zoonotic potential. The case may be, however, that human cases of fasciolosis caused by infection with *F. gigantica* are under-reported due to their occurrence in countries where limited access to medical care prevents diagnosis and disease reporting [15].

In countries where livestock production tends to be more industrialized, such as Australia and the UK, human infections are rare [11,16]. The considerable production impacts, however, make *F. hepatica* one of the primary foci of integrated parasite management programmes in these regions [4,17]. Alternatively, in countries where subsistence and smallholder farmers account for the majority of livestock owners, human and animal *Fasciola* spp. infections often go

unnoticed and remain largely untreated (Figure 1) [11,15,16]. This is despite the availability of a treatment donation programme as a result of collaboration between Novartis and the World Health Organization. Zoonotic outbreaks are primarily associated with the highlands of South America, particularly Bolivia and Peru, where fasciolosis is considered to be **hyperendemic** (see Glossary) [11]. More recently, however, there have been a growing number of reports from the Middle East and North Africa, in countries such as Egypt, Iran, and Ethiopia [15].

While fasciolosis is considered a neglected tropical disease in several countries, a reliance on aquatic rice production in conjunction with large ruminant husbandry presents an ideal scenario for disease re-emergence in Southeast Asia in particular. Limited access to healthcare services and a high incidence of outdoor defecation further contribute to the increased zoonotic disease risk in the region [11,15,18]. Studies on the prevalence of *Fasciola* spp. in Southeast Asia are primarily limited to Vietnam, one of the wealthier countries in the region (Figure 1) [19,20]. Here, 47 of the 63 provinces have recorded cases of human infection, with more than 10 000 cases reported between 2006 and 2009 [20]. Prevalence data on human and animal infections in the countries neighbouring Vietnam is limited. The similar agricultural activities and human sanitation practices in these countries, however, suggest that they are likely to be similarly affected by fasciolosis, yet the disease remains relatively unnoticed outside of Vietnam.

Fasciola spp. Distribution in Southeast Asia as a Result of International Livestock Movements

The global distribution of *F. hepatica* and *F. gigantica* was historically associated with postdomestication livestock movements [18]. Translocation events are limited by the indirect life cycle of *Fasciola* spp., which requires a variety of prerequisites to be met in order to facilitate their successful expansion into a region. These include the availability of permissive hosts (both snail and vertebrate) and environments (water bodies to enable transmission), most of which have already been extensively reviewed [2,21]. The role that the international trade of livestock plays in the more recent dispersal of these parasites, however, is often overlooked despite sustained growth in global live export markets [2][ii,iii,iv]. This is especially concerning in Southeast Asia where a rapidly growing middle class in countries such as China and Vietnam has driven an increase in the demand for animal-derived protein[iv]. As demand outstrips local production, and higher-quality meat is preferred, animals are sourced from the major live cattle exporters, including Australia, Argentina. and Brazil, where *F. hepatica* is endemic [22][iv]. In 2019 over a million cattle were imported into China, Vietnam, and Indonesia from Australia alone, an increase of 40% on the year before[ii,iii]. Many of these animals are then moved via international livestock corridors throughout the region for fattening, slaughter, or breeding purposes[ii,iii].

Leaking into Local Livestock Systems

The impacts of international livestock movements are well recognized in the case of the more overt and devastating viral and bacterial diseases, such as foot and mouth disease and African swine fever[iv]. In an attempt to mitigate these transboundary disease risks, livestock corridors throughout Southeast Asia have been well documented[iv]. Regardless, undocumented livestock movements across land borders occur commonly, providing ample opportunity for 'leakages' of both animals and the diseases they carry into local livestock systems as they are trafficked towards their final destination[iv]. Unlike many other production-limiting diseases, fasciolosis is often subclinical in large ruminants, allowing infected animals to move through these systems unnoticed. The subclinical nature of the disease further contributes to the neglect of this economically important parasite in the region, allowing translocation events to go undocumented. While the conditions supporting these parasite translocation events are most apparent in Southeast Asia, similar *Fasciola* spp. translocations are likely to be occurring elsewhere, such as in the Middle East where the

Glossary

Adaptive introgression: the permanent establishment of beneficial alleles from one species in the gene pool of another through repeated backcrossing.
Cercaria (*plural*: cercariae): a free-swimming larval stage of *Fasciola* spp. that emerges from the intermediate snail host prior to encystation.
Hybridization: the process of mating between two species resulting in a hybrid with theoretically equal genetic makeup from each parental species.
Hyperendemic: high prevalence in a population (>10%) with very high faecal egg loads (>1000 eggs per gram of faeces). Human sanitation practices (lack of adequate sewerage and a high rate of defecation in the environment) contribute to the maintenance of the life cycle.
Introgression: the transfer of genetic material from one species into the genome of another.
Metacercaria (*plural*: metacercariae): the most environmentally resistant encysted stage of *Fasciola* spp., infectious to mammalian hosts.
Miracidium (*plural*: miracidia): the short-lived free-swimming larval stage of *Fasciola* spp. that emerges from the eggs; it is passed in the faeces of mammalian hosts and infects the intermediate snail hosts.
Parthenogenesis: reproduction without the need for fertilization of the ovum.
Phenotype: any observable characteristic resulting from the combination of genotype and the environmental factors.
Polyploids: individuals with more than two pairs of homologous chromosomes (vs. diploid, in which one set of chromosomes is inherited from each parent).
Prepatent period: the period of time from infection of the mammalian host to the detection of *Fasciola* spp. eggs in faeces.
Redia (*plural*: rediae): the second stage of *Fasciola* spp. after infection of the intermediate snail hosts; it can produce daughter rediae by clonal expansion, and eventually cercariae.
Sporocysts: elongated sacs resulting from infection of snails by *Fasciola* spp. miracidia that are capable of producing daughter sporocysts or rediae, the first larval stage within the intermediate snail hosts.

importation of sheep and goats from *F. hepatica*-endemic countries is equally common[ii,iii]. Hence the sustained increase in the movement of animals from *F. hepatica*-endemic to non-endemic regions is likely to be quietly changing the status quo of these parasites around the world and expanding regions of parasite **sympatry** (Figure 1) [22].

Sympatry: the occurrence of two or more species within the same geographic locality at a given time.

The Proof Is in the Pâté

While it seems only natural that an increase in the trade of livestock from *F. hepatica*-endemic to non-endemic regions would facilitate parasite translocation events, there is a lack of data available to support this hypothesis. This is primarily because, until recently, all molecular *Fasciola* spp. identification was restricted to the analysis of DNA from adult flukes collected from the livers of infected animals during postmortem examination [23–25]. A reliance on access to adult parasites for species identification via molecular methods precludes widespread surveillance in a system that inherently requires the movement of live animals. However, the postmortem examination of sheep livers in Saudi Arabian abattoirs suggests that between 10% and 22% of imported animals are infected [25,26]. In one instance, the morphological and molecular characterization of adult flukes revealed the presence of not only *F. hepatica* and *F. gigantica*, but also an 'intermediate' *Fasciola* species in imported animals [25]. By carrying the genetic signatures of both species, this 'intermediate' form is assumed to be the outcome of interspecific **hybridization** between *F. hepatica* and *F. gigantica* [27]. Although limited to the Middle East, these results provide evidence for the capacity of the international livestock trade to facilitate the movement of these parasites via infected animals and suggest that similar translocation events may be occurring in Southeast Asia.

Newer molecular methods that enable *Fasciola* spp. differentiation from faecal samples have extended our ability to conduct surveillance of parasite translocation events beyond the abattoir (Box 2) [28]. The molecular detection of *F. hepatica* DNA in faecal samples from local cattle in Northern Laos, a major livestock thoroughfare into China and Vietnam, suggests that not only are these parasites being imported, but that their life cycles are likely to have already become established in the region [28]. The introduction of *F. hepatica* into Southeast Asia is of cause for concern in its own right due to the considerable human and animal health impacts and potential for the development of anthelmintic resistance in livestock compared to *F. gigantica*. Perhaps more alarming, however, are the possible outcomes of animals coinfected with both species.

Hybridization and Introgression between *F. hepatica* and *F. gigantica*

Hybridization between *F. hepatica* and *F. gigantica* has been increasingly reported to occur in areas of *Fasciola* spp. sympatry as the result of interspecific mating (Box 3) [24,27,29,30]. Experimentally,

Box 1. The Life Cycle of *F. hepatica* and *F. gigantica*

F. hepatica and *F. gigantica* have an indirect life cycle, requiring both a mammalian definitive host and a freshwater snail intermediate host. The definitive hosts become infected by ingestion of **metacercariae** that are either encysted on vegetation or free-floating in water (Figure I). Metacercariae are the most resistant stage of the life cycle and can survive in the environment for over a year when adequate moisture is maintained. Once metacercariae are consumed, excystation occurs in the small intestine within an hour of ingestion and the newly excysted juveniles burrow through the intestinal wall into the abdominal cavity. From here the newly excysted juveniles move towards the liver, eventually penetrating the liver capsule 4–6 days postinfection. The immature flukes migrate through the liver for 5–6 weeks in the case of *F. hepatica,* or for up to 11 weeks for *F. gigantica*. The flukes reach and mature in the bile ducts either 8–12 weeks postinfection (WPI) for *F. hepatica,* or 12–16 WPI for *F. gigantica*, after which they commence egg laying. Eggs are shed via the bile duct into the gastrointestinal tract where they exit the mammalian host into the environment. Embryonation and hatching occur when eggs are free from faeces and exposed to light, respectively, and must be in the presence of water to facilitate infection of the intermediate snail hosts. Newly hatched **miracidia** are highly mobile but short-lived and must infect an intermediate snail host within 24 h. Various aquatic lymnaeid snails act as intermediate hosts, most of which prefer shallow pools and ponds, and are capable of aestivation when these areas dry up. Miracidia infect snails via penetration of the foot, mantle, or tentacles, after which they migrate to the digestive gland (liver) of the snail as **sporocysts**. Here, sporocysts undergo clonal expansion, producing **rediae** from which **cercariae** are later produced. This process is complex and ongoing, with rediae able to produce both cercariae and daughter rediae. Cercariae emerge from snails 4–7 WPI. Their tadpole-like tails provide motility in water where they encyst on plants and nearby vegetation. After encystation they become metacercariae and are almost immediately infective. This summary is based on the description of the life cycle as elucidated by Thomas [54] and summarized in *Fasciolosis* [55].

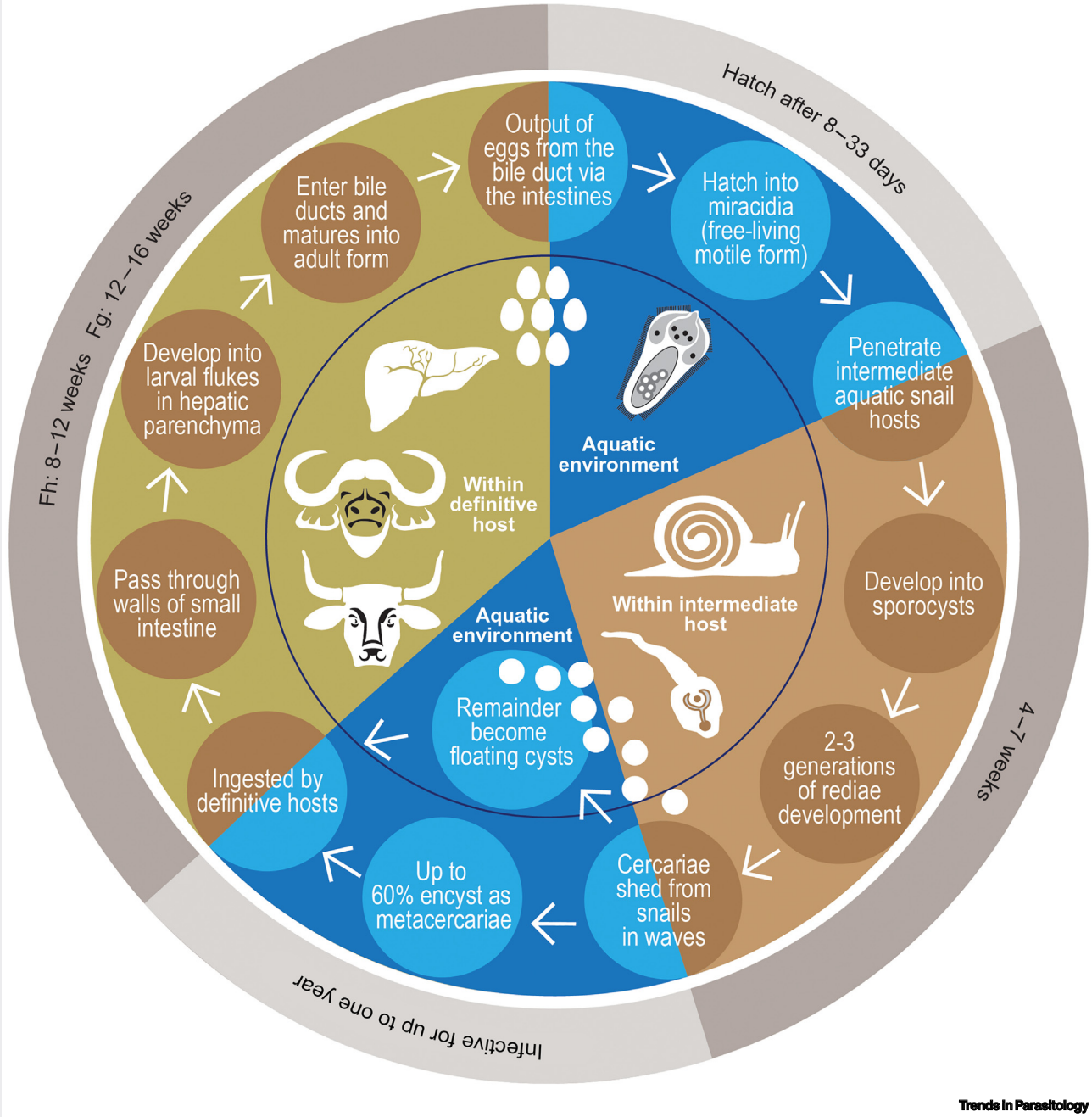

Figure I. The Indirect Life Cycle of Liver Fluke in Southeast Asia. The flukes (*Fasciola hepatica* and *Fasciola gigantica*) cycle between ruminant and snail hosts, exploiting the environment provided by the combination of aquatic rice and smallholder ruminant production. Snail populations are maintained in rice paddies through the wet season, while animals remain tethered at home to prevent the destruction of rice prior to harvest. In the dry season animals are allowed to free roam in the dry rice paddies, grazing on rice stubble harbouring the infective metacercariae.

successful hybridization between these parasites has been demonstrated via coinfection of Wistar rats and morphological and molecular characterization of the resulting F1 and F2 generations [31]. Experimental *Fasciola*-hybrid adults demonstrate an intermediate body-length to body-width ratio

Box 2. Antemortem Differentiation of *F. hepatica* and *F. gigantica* Infection

Fasciola species differentiation prior to completion of the **prepatent period** (**PPP**) using molecular methods is inherently prohibited by the localization of the immature stages within the liver of infected hosts. Once mature flukes commence egg-laying, the faeces of infected hosts become an abundant and noninvasive source of genetic material. When considered in the context of parasite sympatry, however, the limitation of molecular methods for species differentiation from faecal samples becomes apparent. Specifically, it is clear that the isolation of DNA from many eggs within an individual sample makes it impossible to differentiate between coinfected animals and those harbouring infections with *Fasciola* hybrids (Figure I). Newer molecular tools, such as TaqMan probe and next-generation sequencing assays, enable higher resolution of these ambiguous states but do not resolve them completely [28]. Instead, they provide the foundation for further investigation via highlighting areas of parasite sympatry and/or hybridization. New approaches with single *Fasciola* egg DNA isolation and SNP detection could overcome these limitations.

Traditionally, infection with either *Fasciola* species is diagnosed via sedimentation and counting of eggs in faeces [56]. While this method is low-cost, it is time-consuming and has limited sensitivity, particularly in large ruminants where the increased faecal volume dilutes the number of eggs on offer for detection. Immunologic techniques, including commercialized antibody and coprological antigen ELISAs, have been developed for earlier detection of *Fasciola* spp. infection before completion of the PPP [57,58]. Although more expensive than a traditional sedimentation, the antibody ELISA enables screening of large numbers of animals via the use of bulk milk tank samples [59]. This method is capable of detecting infection in naïve sheep as early as 4 WPI [58]. The maintenance of positive antibody titres post-treatment, however, limits the application of the antibody ELISA to the detection of *Fasciola* spp. exposure [60,61]. A coprological antigen ELISA (coproELISA) enables the diagnosis of current infections via the detection of *F. hepatica* and *F. gigantica* antigens as early as 6 and 9 WPI, respectively, with antigen levels declining rapidly post-treatment [62]. However, as with traditional sedimentation methods, it is limited in its ability to diagnose infection in large ruminants due to antigen dilution in their increased faecal volumes [63]. Regardless of their capacity to diagnose large numbers of animals earlier in infection than traditional methods, neither the antibody ELISA nor the coproELISA enable *Fasciola* species identification.

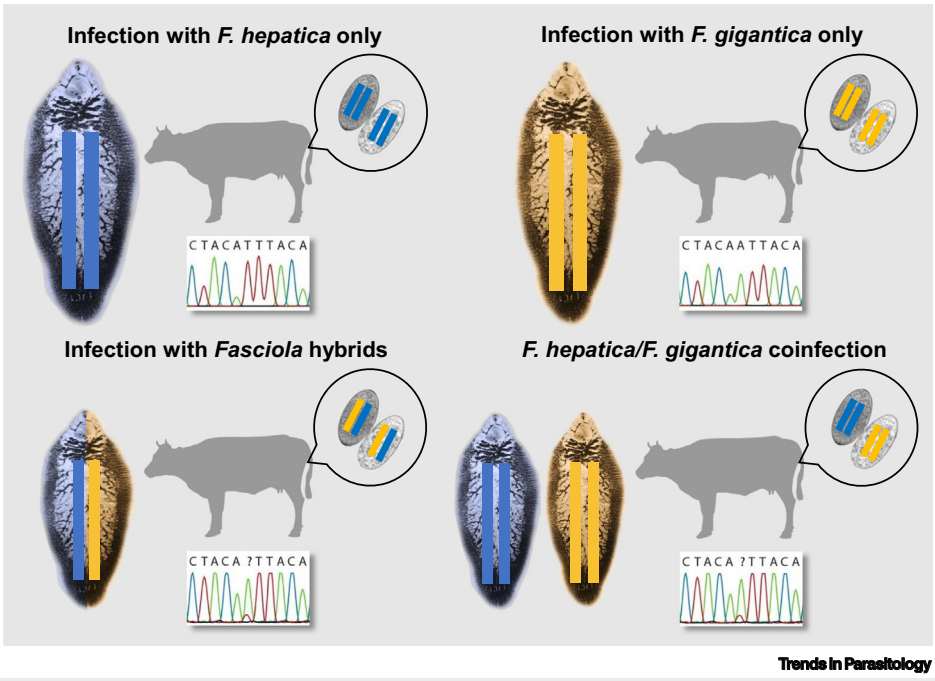

Figure I. *Fasciola hepatica*/*Fasciola gigantica* Coinfection and Infection with *Fasciola* Hybrids Are Indistinguishable Using Sanger Sequencing. Isolation of DNA from eggs in faecal samples does not allow for antemortem differentiation of *Fasciola* hybrid and coinfected animals. Eggs are represented by the bubbles emerging from the individual animals. The thick lines in the adult flukes and their eggs are representative of the genomes of each species, with blue indicating *F. hepatica* and yellow indicating *F. gigantica*. The two colours in the *Fasciola* hybrid adult and eggs is a theoretical representation of F1 generation as a result of hybridization between both parental species. The question mark in the chromatograms indicates ambiguous nucleotides from both parental species present when DNA is isolated and Sanger sequenced from multiple eggs. The chromatograms from *Fasciola* hybrid and *F. hepatica*/*F. gigantica* coinfected animals are identical, preventing the differentiation of these two states.

Box 3. What Do We Know about Hybridization and Introgression?

Hybridization has been experimentally demonstrated as the result of interspecific mating between *F. hepatica* and *F. gigantica*, with the F1 generations exhibiting an intermediate body-width to body-length ratio between the two species as shown (Figure I, left) [31]. Introgression is the transfer of genetic material from one species into the genome of another, usually as a result of backcrossing of hybrids with either of the original parental species [32,64].

The occurrence of introgression between *Fasciola* spp. remains unproven but is theoretically represented here as a result of the repeated backcrossing of *Fasciola* hybrids with *F. gigantica*. Adaptive introgression (Figure I, right) is the establishment of beneficial alleles within a population and may occur as a result of various forms of selection pressure. Initially, many alleles introgress, although incompatible alleles are eventually selected against and disappear. Over many generations, the advantageous allele is moving towards fixation, while other variants persist at a lower rate of recombination [45]. The source of selection pressure is population-dependent but may involve adaptations for drug resistance, increased virulence, adaptation to new definitive or intermediate hosts, higher metacercarial output from intermediate hosts, increased temperature tolerance of eggs and/or metacercariae, etc.

In both of the pure species, and in the *Fasciola* hybrid, accurate identification can theoretically be conducted regardless of the locus targeted. However, in introgressed specimens, these results may misrepresent the true status of the specimen (Figure I).

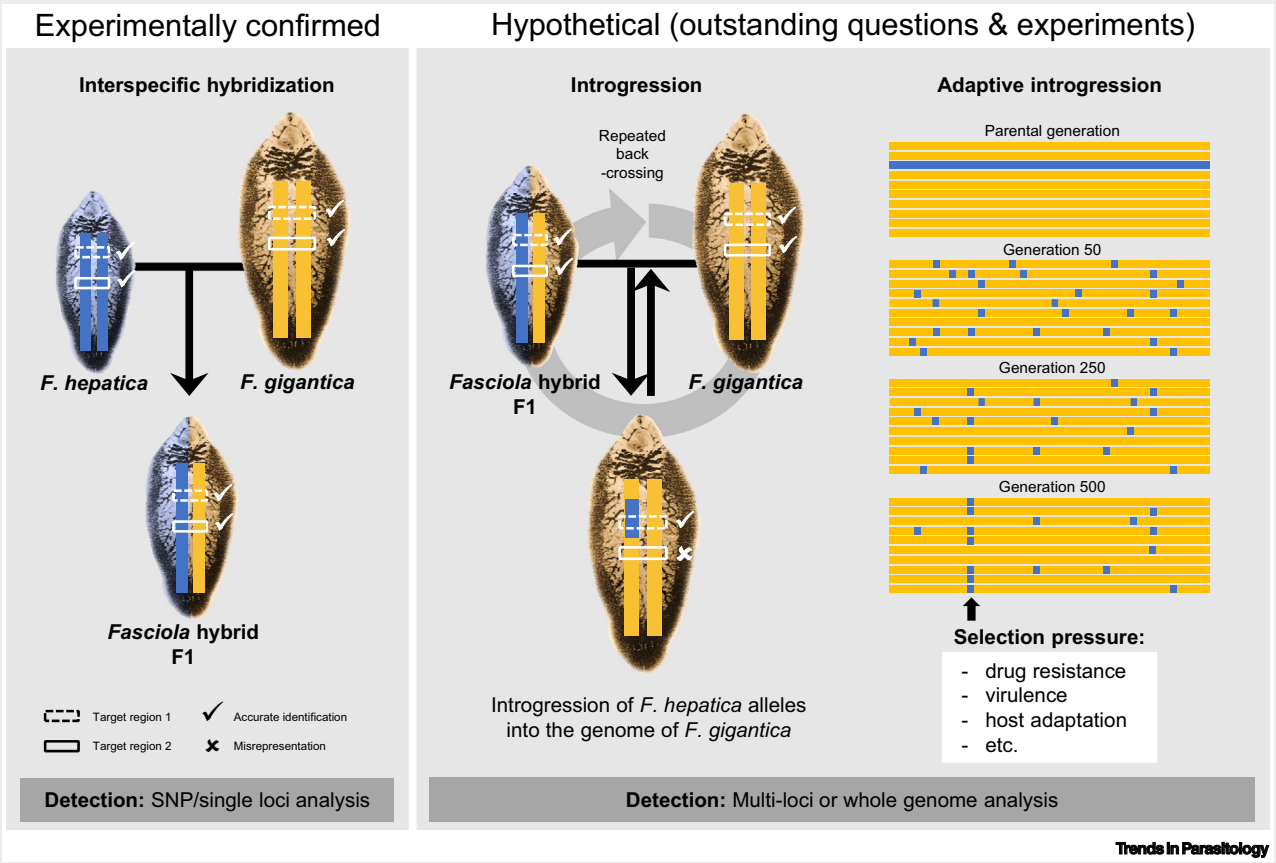

Figure I. Hybridization and Introgression between *Fasciola hepatica* and *Fasciola gigantica* and the Influence of Possible Selection Pressures. The thick lines in the adult flukes are representative of the nuclear genomes of each species, with blue indicating *F. hepatica* and yellow indicating *F. gigantica*. The two colours in the *Fasciola* hybrid adult is a theoretical representation of F1 generations as a result of hybridization between both parental species. A simplified diagram demonstrating the potential random drift of introgressed alleles from *F. hepatica* (blue) into the genome of *F. gigantica* (yellow), and the fixation of new alleles at a single locus as one outcome of selection pressure, is shown (right). The limitations of single-locus methods for differentiation between *F. hepatica* and *F. gigantica* adults, their hybrid and introgressed forms using rDNA compared to multilocus and/or whole-genome methods is illustrated using two hypothetical 'target' regions (1 and 2). The identification of introgressed individuals requires pre-existing knowledge of markers susceptible to introgression. Ticks indicate regions that have been accurately identified, while crosses indicate misidentification due to the selection of inappropriate markers that have not undergone introgression.

between that of their parent species and appear to be more infectious than *F. gigantica* alone based on higher than previously reported recovery rates [31]. Hybridization does not necessarily generate permanent genetic change, however, and the intermediate **phenotypes** observed in

the experimental *Fasciola* hybrids may be inconsequential in the long term due to the apparent limited viability of successive generations under laboratory conditions [31,32]. While *Fasciola* hybrids may exist only transiently, a more permanent and thus concerning consequence of these interspecific mating events is the outcome of backcrosses between hybrids and either of the parental species. That is, the potential for **introgression** of advantageous traits from one species into the other.

Although successful hybridization between *F. hepatica* and *F. gigantica* has been demonstrated experimentally, there is no empirical evidence supporting introgression between these two parasites. Regardless, the terms 'hybridization' and 'introgression' are frequently used without differentiation throughout the *Fasciola* spp. literature and often without any consideration of the functional and epidemiological implications of either state [27,29,33]. The current lack of differentiation between hybridization and introgression in *Fasciola* spp. is alarming given the phenotypic and epidemiological outcomes of introgression observed in other species. **Adaptive introgression** has seen the transfer of resistance genes against organophosphates and pyrethrum between *Anopheles* mosquito species, as well as the experimental induction of ivermectin resistance in laboratory-maintained *Haemonchus contortus* populations [34–37]. Thus, it is not inconceivable that adaptive introgression may result in the transfer of anthelmintic resistance in *Fasciola* spp. due to the importation of triclabendazole-resistant *F. hepatica* into *F. gigantica*-endemic regions. Introgression between these two species, if it exists, may have other important human and animal health impacts, including increased infectivity, virulence, and pathophysiology, not to mention any potential implications surrounding the infection of intermediate hosts and the survival of eggs and metacercariae in the environment.

Differentiating Hybridization and Introgression in *Fasciola* spp.

The differentiation between hybridization and introgression is far from trivial in *Fasciola* spp., where their hermaphroditic nature, assumed potential for **parthenogenesis**, and the occurrence of **polyploids** complicate our understanding of these events [38–41]. However, the major ambiguity surrounding the distinction of hybridization from introgression in *Fasciola* spp. is due to a tendency to investigate single loci when a multi-loci or whole-genome approach is required (Box 3) [33,41–45]. Most studies have relied on repetitive rDNA units to demonstrate hybridization, and only recently has a nuclear-encoded single-copy gene, PEPCK, been investigated [46,47]. Furthermore, the use of mtDNA alone, as evidence for hybridization in *Fasciola* spp., may be misleading due to a total lack of information concerning mitochondrial inheritance in these parasites and the limited recombination of mitochondrial genomes [41,48–50]. It quickly becomes clear that, without knowing more about the genetic makeup of these forms, over-interpretation of naturally occurring hybridization and introgression events should be avoided. The availability of the *F. hepatica* and *F. gigantica* draft genomes, and next-generation sequencing technologies that allow rapid sequencing and assembly of the 1.2 Gb large genome, will enable documentation of introgression between *Fasciola* spp. [51,52]. Similar applications of these technologies have allowed the mapping of introgression events of Neanderthal- and Denisovan-derived DNA into the genomes of modern humans [53].

Despite the apparent limited viability of hybrid offspring under laboratory conditions, experimental back-crossing of *Fasciola* hybrids with both parental species should be pursued to aid in the unambiguous confirmation of the occurrence of introgression between these parasites and to provide insights into appropriate markers going forward. Until then, the characterization of these forms in hybrid zones should involve the comparison of many markers from a pool of individuals to external populations where it is known that only a single species is present [27].

Concluding Remarks

The continued neglect of these parasites and their human and animal health impacts in the developing world means that few studies have examined the role of the international trade of livestock in the expansion of existing hybrid zones. The limited evidence available is damning, however, and when considered in relation to what we know about the impacts of introgression between other parasite species, begs the question: is introgression between *F. hepatica* and *F. gigantica* just a fluke, or something more?

New and emerging molecular methods are extending our capacity to monitor *Fasciola* translocation events beyond the abattoir for the first time, allowing increased surveillance of coinfection and hybridization – the precursors to introgression [28]. Both have been proven in *Fasciola* spp. and are likely to be occurring with increased frequency due to the movement of *F. hepatica*-infected animals into *F. gigantica* endemic regions [22,25,31]. So, while there is no evidence in support of adaptive introgression between these two species just yet, it is simply a matter of when, not if. Thus, it is time we started considering the longevity and functional and epidemiological implications of these events in order to determine the impacts on the region's food security and public health (see Outstanding Questions).

Outstanding Questions

Can experimental backcrosses of *Fasciola* hybrids with both parental species be used to prove or disprove the occurrence of introgression between the two species?

What is the mechanism of mitochondrial inheritance during hybridization events between *Fasciola* species?

What are the phenotypic and functional implications of experimental and naturally occurring *Fasciola* hybrids?

Are there differences in the immune responses of definitive hosts to infection with *F. hepatica*, *F. gigantica*, and their hybrids, and can this information be used to answer questions regarding their pathogenicity and control?

What is the scale and source of *Fasciola* spp. coinfections, and should surveillance be extended to include the diagnosis of human infections in known hybridization hotspots?

What methods (pre-export testing and treatment, commercialization of vaccines) are available to minimize coinfection of definitive hosts?

To what extent have *Fasciola* hybrids/introgressed forms been misidentified in the literature, and what impact does this have on known areas of parasite sympatry?

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Resources

[i]www.who.int/neglected_diseases/diseases/summary/en/

[ii]www.livecorp.com.au/about-us/livecorp-information/annual-reports

[iii]www.mla.com.au/prices-markets/overseas-markets/#

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