

NARRATIVE REVIEW

Liver Fluke Infection Throughout Human Evolution

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The species of liver fluke that infect humans are zoonotic parasites that we share with other animals. The complex way in which humans have interacted with their environment, and the animals that live alongside them, has affected our ancestors' risk of infection by these helminths for millions of years. Here, we describe the range of flukes that can survive in the human liver and the health consequences that result from infection. Our focus is on *Clonorchis sinensis* (Chinese liver fluke), *Fasciola hepatica* (sheep liver fluke), *F. gigantica* (giant liver fluke), *Opisthorchis viverrini* (Southeast Asian liver fluke), *O. felinus* (cat liver fluke), and *Dicrocoelium dendriticum* (lancet liver fluke). We use our knowledge of where different kinds of liver flukes are endemic to estimate when and where human ancestors would have been exposed to infection over deep time. DNA evidence is used to investigate the evolutionary origins of the major species of liver flukes. The archaeological evidence for different species of fluke helps to show in which periods of human history they were most common. These flukes spread to humans from wild animals in early prehistory because of our ancestor's hunter-gatherer lifestyle and later from farm animals after the development of agriculture. We explore when and how some species have been able to spread across the world, whereas others are limited to particular geographic regions because of the endemic distribution of snail and vertebrate intermediate hosts. It is clear that human activity and migrations have played a key role in the expanding geographical range where we find liver flukes endemic today.

Keywords: *Clonorchis*; *Dicrocoelium*; *Fasciola*; *Opisthorchis*; Paleopathology

Introduction

Flukes are flat, leaf-like trematode worms of the phylum Platyhelminthes. Those that infect humans may reside in the lungs (eg, *Paragonimus* spp.), blood vessels (eg, *Schistosoma*), intestines (eg, *Echinostoma* and *Fasciolopsis*), or the liver. Although a wide range of species can occasionally infect us, the main parasitic liver flukes to cause disease in humans are the Chinese liver fluke (*Clonorchis sinensis*), sheep liver fluke (*Fasciola hepatica*), giant liver fluke (*F. gigantica*), Southeast Asian liver fluke (*Opisthorchis viverrini*), cat liver fluke (*O. felinus*), and the lancet liver fluke (*Dicrocoelium dendriticum*).¹ Although there has been past work investigating how humans have interacted

with various types of parasites throughout our evolution,^{2–5} there has been little specifically focusing on liver flukes. The aim of this article is to explore in detail how liver flukes have interacted with humans during our evolution. We consider the origins of each of the common flukes, when and how they infected our ancestors, how this interaction changed as humans moved from being hunter-gatherers to settled farmers, and how some species have been spread to new regions by human migrations.

The Biology of Liver Flukes That Infect Humans

The Chinese liver fluke infects humans and fish-eating animals in China, Korea, northern Vietnam, and far eastern Russia. It is thought that about 35 million people are infected by *C. sinensis* today. Humans may become infected if they eat raw or undercooked freshwater fish. Flukes are 1–2.5 cm in length and infect the bile duct and gall bladder.^{6,7} The sheep liver fluke is found worldwide, whereas the giant liver fluke is endemic in Africa and Asia. The life cycle requires a mammal primary host and intermediate hosts of freshwater snails. Human infection follows the consumption or larval forms encysted on edible aquatic plants and vegetables. As the most common definitive hosts are ruminant herbivores, *Fasciola* spp. often transmit to humans when consuming plants or water that is contaminated by the feces of sheep or cattle. It is thought that between 2.6 and 17 million people are infected by *Fasciola* flukes worldwide. *F. hepatica* flukes are about 4 cm in length, whereas *F. gigantica* are larger at 7–8 cm in length.^{8,9} The Southeast Asian liver fluke is found in Thailand, Laos, Cambodia, and Vietnam, where about 10 million people are infected.¹⁰ Its close relative the cat liver fluke is endemic in Russia, Belarus, Ukraine, Germany, and Italy, and about 1.2 million people are thought currently to be infected.¹¹ Both species of *Opisthorchis* measure about 1 cm in length and are contracted by eating raw or undercooked freshwater fish. The lancet liver fluke is found across the world and causes dicrocoeliasis. *D. dendriticum* has a

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Table 1. Information on the Species of Liver Fluke That Can Infect Humans

Liver fluke species	Modern geographic distribution	Intermediate hosts	Potential definitive hosts
Chinese liver fluke <i>Clonorchis sinensis</i>	China Korea Vietnam Eastern Russia	Freshwater snails Freshwater fish	Humans Dogs Cats Rats Pigs Buffaloes Weasles Foxes
Sheep liver fluke <i>Fasciola hepatica</i>	Asia, Africa, Europe, Oceania, Latin America	Freshwater snails	Sheep Cattle
Giant liver fluke <i>Fasciola gigantica</i>	Asia, Africa		Humans
Southeast Asian liver fluke <i>Opisthorchis viverrini</i>	Thailand, Laos, Cambodia, Vietnam	Freshwater snail Freshwater fish	Cats Dogs
Cat liver fluke <i>Opisthorchis felineus</i>	Italy, Germany, Russia, Belarus, Ukraine		Other fish-eating mammals Humans
Lancet liver fluke <i>Dicrocoelium dendriticum</i>	North Africa, Asia, Europe, Canada	Land snails Ants	Cattle Sheep Humans

complex lifecycle, requiring both land molluscs and ants as first and second intermediate hosts.^{12,13} The adult fluke measures 6–10 mm in length and is mostly found in the bile ducts of ruminants, pigs, and deer. In humans, the ingestion of ants containing infective stage metacercariae may cause infection, whereas consuming undercooked animal liver will result in parasite eggs in the feces but no active infection.¹⁴ Therefore, true infection follows if ants are deliberately eaten as a food source or if they are accidentally eaten when the ants are by chance present on other foods. A summary of the liver flukes that more commonly infect humans is found in Table 1.

Symptoms are most common in *Fasciola* infection but can occur with other types of liver fluke. When symptoms are present, they include weakness, loss of appetite, nausea, vomiting, flatulence, abdominal pain, diarrhea, and jaundice. Pathogenesis is mainly caused by immature flukes burrowing through the soft tissues, especially the liver parenchyma. The physical presence of the flukes in bile ducts, coupled with their feeding activity, can also lead to inflammation, obstruction, and cholangitis. Children with infection often develop anemia and impaired cognitive development.⁹ The range of defense strategies that enable the helminths to evade the human immune system can allow the flukes to survive for decades within their host. Over this time, Chinese liver fluke and Southeast Asian liver fluke can cause malignant change in the form of cholangiocarcinoma. This cancer of the bile duct is 100 times more common in those with Southeast Asian liver fluke and Chinese liver fluke infection compared with those not infected.^{1,15}

Genetic Evidence for the Evolutionary Origins of Liver Flukes

Fasciola liver flukes are characterized by high levels of genetic diversity, lack of population structure, high gene flow, and having one of the largest pathogen genomes. They also possess substantial levels of polymorphism in genes specific to parasitic digeneans, suggesting potential for rapid adaptation to changes in host availability, climate change, and drug treatment.^{16,17} The ability of *Fasciola* to adapt to a wide range of definitive mammals is pertinent to its evolutionary history. Between 65 and 50 million years ago, a switch of intermediate hosts and a shift from intestinal to hepatic habitats appears to have occurred in the *Fasciola* lineage. At that time, climate and ecologies changed rapidly, which might have shaped the adaptive range of *Fasciola* spp.¹⁸ It has been proposed that the basal fluke species probably originated in African proboscideans (elephants) that migrated into Eurasia, where the parasite was able to infect other herbivores. The split between *F. hepatica* and *F. gigantica* is thought to have occurred around 5 million years ago, coinciding with the reduced faunal exchange between Africa and Eurasia. Therefore, *F. hepatica* appears to have its origins in Eurasia.^{18,19}

Clonorchis is thought to have shared a common ancestor with *Opisthorchis*, with the split estimated to have occurred about 3 million years ago based on mitochondrial DNA sequence analysis.²⁰ Chinese liver fluke genetic diversity has been investigated in a number of studies.^{21–24} Some have found a low degree of genetic diversity between different isolates, whereas others have found significant variation, especially in the mitochondrial genes. Sun et al²² argue that the

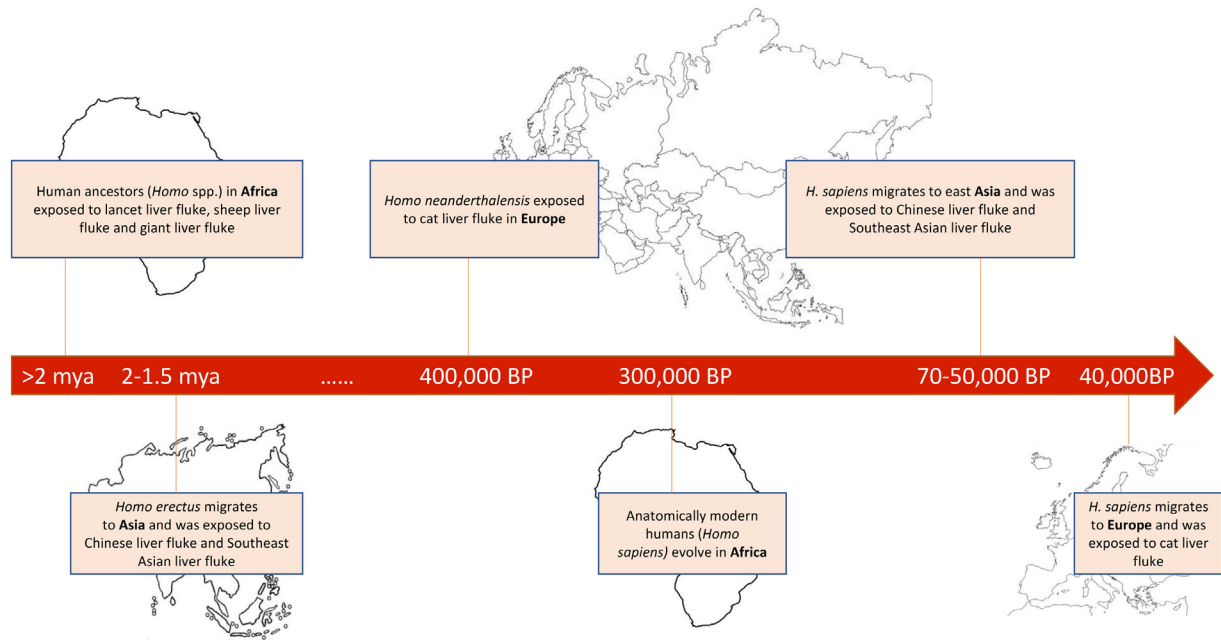


Figure 1. A timeline of key liver fluke-related events during human evolution.

different clusters of *C. sinensis* were experiencing divergent evolutionary trajectories, with the high diversity, low population differences, coupled with geographically defined structures, suggesting a model of slow population expansion. As the samples collected from central China were positioned throughout the network of their haplotype analysis, they suggest that the parasite originated in China.

Opisthorchis shares many characteristics with Chinese liver fluke, which suggests that they probably share a common ancestor.²⁵ Brusentsov's study of the genetic structure of *O. felinus* notes that the genetic diversity revealed from several mitochondrial DNA and nuclear recombinant DNA loci is low. They suggest that the primary cause of the lack of genetic diversity is the strong founder effect induced by the Pleistocene glacial events, followed by a sudden group growth and geographical expansion. The authors then suggest that the rapid expansion in the range of this parasite after a genetic bottleneck indicates its adaptability and high dispersal potential. With its split from Chinese liver fluke proposed to have taken place about 3 million years ago, its spread has been linked initially to the movement of fish-eating mammals and later by human migrations.²⁵ The *O. viverrini* genome has not undergone such detailed interrogation to assess its evolutionary origins.²⁶ However, as it is found in Southeast Asia, and its endemic area overlaps that of Chinese liver fluke, this would suggest the species originated there.

Likely Exposure to Liver Fluke Infection in Early Humans

The skeletal and genetic evidence suggests that humans and their ancestors evolved in Africa. The hominin family tree over the last 6 million years includes the *Ardipithecus* group of

species, the *Australopithecus* group, the *Paranthropus* group, and finally, the *Homo* group about 2 million years ago. The genus *Homo* includes *H. rudolfensis*, *H. habilis*, *H. erectus*, *H. heidelbergensis*, *H. neanderthalensis*, *H. floresiensis*, and our own species, *H. sapiens*.²⁷ Some early hominin species were only found in Africa and so would only have been exposed to those species of liver fluke endemic there, such as giant liver fluke, sheep liver fluke, and lancet liver fluke. Other species such as *H. erectus* were present in Africa, Asia, and Europe, as they migrated out of Africa 2.0–1.5 million years ago, well before our own species evolved.²⁸ In this way, they would have been exposed to those species present in Africa and also those present in the regions to which they migrated. Therefore, *H. erectus* in Asia would have likely been exposed to Chinese liver fluke and Southeast Asian liver fluke, whereas those in Europe would have been exposed to cat liver fluke. Further hominin species were only found outside Africa, such as Neanderthals in Europe and Asia and Denisovans in Asia, indicating that they probably evolved from earlier hominin species in those regions. In consequence, Neanderthals and Denisovans would again have been exposed to the species of liver fluke endemic in those regions. *Homo sapiens* is thought to have evolved in Africa around 300,000 years ago, and serial migrations over time led to populations moving into Asia at least by 60,000 years ago²⁹ and into Europe at least by 40,000 years ago.³⁰ Through this process, we can predict how early humans may have been exposed to different species of liver fluke (see Figure 1).

Archaeological Evidence for Liver Flukes in Humans

Evidence for liver fluke infection in past populations can be in the form of parasite eggs in feces or the flukes

Table 2. Examples of Archaeological Evidence of Liver Fluke in Human Contexts in Different Regions of the World

Liver fluke species	Period	Location	Reference
Chinese liver fluke <i>Clonorchis sinensis</i>	475–221 BCE	China	32
	700 CE	Japan	33
	Medieval	Korea	34
Sheep liver fluke <i>Fasciola hepatica</i>	8300–7000 BCE	Cyprus	35
	3000–2000 BCE	Germany	36
	400–350 BCE	Egypt	37
	1300–1700 CE	Belgium	38
Cat liver fluke <i>Opisthorchis felineus</i>	1100–1300 CE	Russia	39
Lancet liver fluke <i>Dicrocoelium dendriticum</i>	2400–150 BCE	Sudan	37
	78–233 CE	The Netherlands	40
	1620s CE	Canada	41

themselves in preserved liver. Ancient feces can be in the form of coprolites (a preserved piece of stool), sediment inside a latrine, the sediment from the pelvis of burials where the intestines were located during life, or feces remaining in the intestines of a mummy. Liver tissue may be preserved in mummies or in calcified structures formed by other parasites in the liver, such as hydatid cysts. Although most studies focus on microscopy, some also analyze ancient DNA. The earliest archaeological example of liver fluke so far discovered are of dicrocoelid eggs in hyena or bear feces found in cave in France dating to about 550,000 years ago.³¹ When considering the archaeological evidence from human contexts below, we should note that the presence of liver fluke eggs in human feces does not necessarily indicate active infection. As an example, *F. hepatica* eggs may get into the feces from genuine bile duct infection or by that individual eating the liver of an infected animal leading to fluke eggs passing through the human intestine without developing and causing disease. In the rare examples where the liver does survive in archaeological contexts, the presence of flukes or eggs in liver tissue must indicate true infection. However, even when it is not clear whether the eggs originated in a human or other animal, it does demonstrate the presence of the parasite in the ecosystem so that the human population would have been at risk of true infection. Some key examples of human infection by different liver flukes in past populations are given in Table 2.

Africa

In ancient Egypt and Nubia (now Sudan), the eggs of *Fasciola* sp. were recovered from Kerma (2400–150 BCE), Sai Island (700–300 BCE), and Saqqara (400–300 BCE) in embalming jars containing human internal organs, such as liver, and in feces recovered from naturally preserved mummies. *Dicrocoelium* sp. eggs have been identified at Kerma (2400–1750 BCE).³⁷ In South Africa, lancet liver fluke eggs have also been recovered from human coprolites at the Mapungubwe farming complex, dating from 1000 to 1300 CE, during the Iron Age.⁴²

Asia

Evidence for liver flukes in East Asian archaeological samples comes from China, Korea, and Japan. The oldest evidence is from 2 female mummies from Hubei province in China.^{32,43,44} During the Imperial period between 221 BCE and 1911 CE, multiple mummies from a range of provinces have been studied over the years.^{45–47} Apart from mummified evidence, one of the most interesting cases of Chinese liver fluke was found in the latrines of a Han Dynasty relay station named Xuanquanzhi (111 BCE to 109 CE) along the Silk Road.⁴⁸ The site was located near Dunhuang, an arid region of northwest China next to the Taklamakan desert where the fluke could not be endemic because there are no wetlands in the region. This relay station is at least 1500 km away from any place where the parasite is currently endemic. This suggests that the people who were infected by Chinese liver fluke must have traveled from somewhere in eastern or southern China to this relay station, making this the earliest evidence for travel with infectious diseases along the Silk Road (Figure 2).

In Korea, the Later Silla Kingdom lasted from 668 to 935 CE. Five cesspits from this period at Chilgok-gun in Daegu City were found to contain parasite eggs, including Chinese liver fluke.⁴⁹ Twenty-four Korean mummies from the Joseon Dynasty (1392–1910 CE) have also undergone examination of their intestinal contents. Twenty-five percent (6/24) were found to contain the eggs of Chinese liver fluke.⁵⁰

In Japan, the eggs of Chinese liver fluke were found in the latrines of Fujiwara Palace in the city of Kashihara, which was the capital of Japan from 694 to 710 CE.³³ During the 8th and 9th centuries, CE Akita Castle was used as government offices for the Tohoku region. Sediment from the flushing toilets was found to contain Chinese liver fluke eggs.³³

Study of the trends in the rates at which different parasites have changed over time in East Asia has highlighted some interesting results. In both China and Korea, improvements in sanitation led to a marked drop in the prevalence of parasites spread by the fecal contamination of food and drink, such as roundworm and whipworm,



Figure 2. *Clonorchis sinensis* egg from the Xuanquanzhi relay station on the Silk Road in China, dating from 111 BCE to CE 109. Egg dimensions $29 \times 16 \mu\text{m}$. Black bar indicates $20 \mu\text{m}$. Image credit: Hui-Yuan Yeh. This figure was published in *Journal of Archaeological Science: Reports*, Vol. 9, Yeh, H., Mao, R., Wang, H., Qi, W., & Mitchell, P., Early evidence for travel with infectious diseases along the Silk Road, 758-764, Copyright Elsevier (2016).

between the 1970s and 1990s. However, the prevalence of Chinese liver fluke in both these countries has been noted to have fallen significantly prior to these improvements in sanitation. Investigation for China has implicated the draining of wetlands during the 1940s and 1950s as part of the drive to deal with schistosomiasis, a trend in culinary preferences to eat cooked fish rather than raw fish, and health education in the 20th century where toilets were moved away from fish ponds when they were previously placed there so fish could feed off human waste.⁵¹ In Korea, the change in prevalence of Chinese liver fluke seems to have been because of a combination of 20th-century industrial water pollution killing aquatic snails and a move away from eating raw fish to cooked fish.⁵¹

In Russia, cat liver fluke infection has been identified at the site of Zeleni Yar in western Siberia during the medieval period. *O. felinus* eggs were found in the pelvic sediment of a 1-year-old child who died in the 12th–13th century CE.³⁹ This indicates that raw fish was being fed to children in the region a thousand years ago, as is the case in the modern populations who live there. Eggs of flukes from the Opisthorchiidae family were also recovered from Stadukhinsky Fort in eastern Siberia dating from the 17th to 18th century. They may represent infection by cat liver fluke in Russians originating in western Siberia who were based in the fort. However, evidence of trade with China in the form of Chinese pottery, pipes, and other objects at the fort raises the possibility that these eggs may represent Chinese liver fluke from traders who traveled south to China.⁵² The final evidence for Asia comes from the Middle East, where 2 eggs of

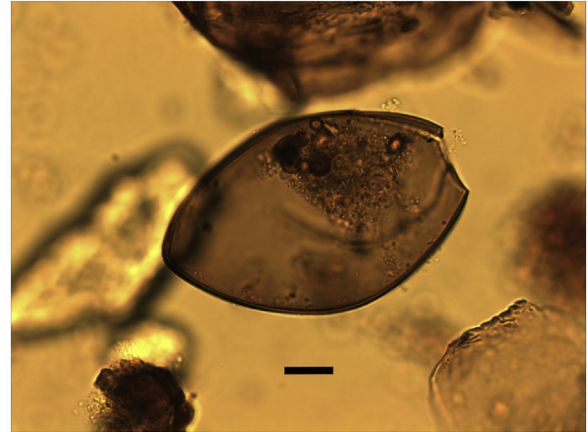


Figure 3. *Fasciola hepatica* egg from Brussels, Belgium, dating from 14th to 15th CE. Egg dimensions $130 \times 85 \mu\text{m}$. Black bar indicates $20 \mu\text{m}$. Image credit: Anna Graff.

lancet liver fluke were recovered from the pelvic sediment of a Bronze Age individual in western Iran, dating from 3600 to 2200 BCE.⁵³

Europe

Fasciola liver fluke eggs have been recovered from the pelvic sediment of a human burial at Shillourokambos in Cyprus. This Neolithic settlement dates from 7800 to 7300 BCE, where the inhabitants farmed sheep, goats, and cattle.³⁵ Although it is not always clear if the parasites originated in animals or humans, other evidence for *Fasciola* and *Dicrocoelium* liver flukes dated to the Neolithic period have been identified at a range of lakeside villages built on wooden stilts in France, Germany, Spain, Sweden, and Switzerland.^{36,54–56} Bronze age sites where *Dicrocoelium* or *Fasciola* was identified include Halstatt in Austria⁵⁷ and Gresine in France.⁵⁸ Liver flukes remained widespread during the Roman period, with the eggs of either *Dicrocoelium* or *Fasciola* being found at the Hallein salt mines in Austria,⁵⁹ Arlon in Belgium,⁶⁰ and multiple sites in Britain, France, and Italy.^{61,62} One particularly interesting example is that of a naturally mummified female bog body from the Netherlands dating from 78 to 233 AD, where microscopy of a liver biopsy identified the eggs of *D. dendriticum*.⁴⁰ In the medieval period evidence for *F. hepatica* and/or *D. dendriticum* liver flukes have so far been found in Belgium (see Figure 3), Britain, the Czech Republic, Denmark, and France.^{38,58,63–67}

Human and Animal Migrations Causing the Spread of Liver Flukes

We have discussed how some liver flukes appear to have had a very broad geographic spread in past populations, whereas others have been limited to specific regions of the world because of the range of their intermediate hosts. Chinese liver fluke and Southeast Asian liver fluke have not been

spread by migrations in the way some of the other flukes have. Here, we will consider why this has been the case.

In the late 1800s and early 1900s, migrants from China traveled to the west coast of North America to work as manual laborers. This led to the potential for those infected by parasites to spread them to the Americas. Wong Nim was a Chinese businessman who provided accommodation for some of these migrants in San Bernardino, CA. Latrines from his property were in use from the 1880s to 1930s. Analysis of sediment from the latrine showed they contained the eggs of Chinese liver fluke as well as other classes of parasite.⁶⁸ However, *C. sinensis* has never become endemic in North America because of the absence of the correct species of water snails required to complete its life cycle.

Lancet liver fluke does not appear to have been present in the early human settlers of the Americas who migrated from Asia 20,000 to 10,000 years ago. Despite analysis of coprolites and mummies from many sites across the Americas, no examples for *Dicrocoelium* have been identified before the arrival of Europeans.⁵⁸ The first archaeological evidence we have for lancet liver fluke in the Americas dates from a 17th-century cesspit at Ferryland in Newfoundland, Canada.⁴¹ The cesspit is thought to date from the 1620s and was used for the disposal of both human and animal feces. Therefore, it seems likely that the lancet liver fluke reached the Americas when farm animals from Europe were transported via sea with transatlantic shipping. Since then, the parasite has become established in the local wildlife. In Alberta, Canada, *Dicrocoelium* has switched to intermediate hosts in the form of snails and ants that are not present in Europe, enabling it to infect wild herbivores.⁶⁹

F. hepatica eggs have been identified in deer coprolites dating from 2300 years ago in Patagonia, Argentina.⁷⁰ Therefore, we know that *Fasciola* was present in the Americas before the transatlantic sea voyages that brought the Europeans. However, hybridization between different *Fasciola* species, as well as host-switching behaviors of this opportunistic parasite, continue to this day. Genetic analysis of *F. hepatica* in Brazil shows the strains originated in several different continents. The observed molecular variance, which is relatively high within populations (57.4% and 77.5%), but low between populations (9% and 36.8%), are in accordance with the regional cattle production system.⁷¹ This fits with the migration of people and farm animals from Europe and Africa in the last 500 years, adding their strains to the local South American ones.

Conclusion

Here, we have shown that liver flukes have had a close biological relationship with humans and their ancestors for millions of years. Individuals following a hunter-gatherer lifestyle would have originally been exposed to these parasites by their catching and eating uncooked wild animals, such as fish and ants. Once the practice of farming was sufficiently established (10,000–12,000 years BP), their

interaction with the herbivores they herded placed them at increased risk of infection by *Fasciola* if they ate aquatic plants growing in ponds where they brought their herds to drink. When Europeans migrated to the Americas with their farm animals, the evidence suggests they were responsible for the spread of lancet liver fluke to a new continent. They also introduced new strains of *Fasciola* to those already present in South America, so increasing genetic diversity. While *Dicrocoelium* and *Fasciola* do appear to have shifted to alternative intermediate hosts available in the Americas, the Chinese liver fluke and Southeast Asian liver fluke seem closely bound to the species of water snail endemic to their home range and that has prevented the spread of these flukes to other continents.

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