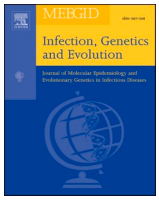
Infection, Genetics and Evolution 95 (2021) 105080

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid



Review

Infection, genetics, and evolution of *Trichinella*: Historical insights and applications to molecular epidemiology

Ewa Bilska-Zając [a,*], Peter Thompson [b], Benjamin Rosenthal [b], Mirosław Różycki [a], Tomasz Cencek [a]

[a] National Veterinary Research Institute in Puławy, Poland
[b] USDA-Agricultural Research Service, Animal Parasitic Diseases Lab, Beltsville, MD, USA

ARTICLE INFO

Keywords:
Trichinella spp.
Molecular epidemiology
Phylogeny
Evolution
Population genetics
Epidemiological investigations

ABSTRACT

Genetic variation in pathogen populations provides the means to answer questions in disease ecology and transmission, illuminating interactions between genetic traits, environmental exposures, and disease. Such studies elucidate the phylogeny, evolution, transmission and pathogenesis of viruses, bacteria and parasites. Here, we review how such studies have fostered understanding of the biology and epidemiology of zoonotic nematode parasites in the genus *Trichinella* spp., which impose considerable economic and health burdens by infecting wildlife, livestock, and people. To use such data to define ongoing chains of local transmission and source traceback, researchers first must understand the extent and distribution of genetic variation resident in regional parasite populations. Thus, genetic variability illuminates a population's past as well as its present. Here we review how such data have helped define population dynamics of *Trichinella* spp. in wild and domesticated hosts, creating opportunities to harness genetic variation in the quest to prevent, track, and contain future outbreaks.

1. Introduction

Parasitologists first began to interrogate genetic variation in the late 1980s, when molecular genetic tools became more widely available. An early review (Hide and Tait, 1991) delineated these opportunities: discriminating the etiological agents of disease, resolving strains and species of parasites, identifying vectors and intermediate and reservoir hosts, and defining those strains responsible for particular outbreaks and disease characteristics. Recent years have brought more sophisticated analytic techniques, and the means to amass far more genetic (and genomic) data. Nonetheless, the basic questions remain much the same (Traub et al., 2005; Conway, 2007; de Meeûs et al., 2007; Lymbery and Thompson, 2012; Bobes et al., 2014).

Molecular variation in populations (the primary data of molecular epidemiology) contains rich information about a parasite's history. That history constrains the extent and distribution of extant variation within and among parasite populations. Thus, the past creates (and constrains) opportunities for understanding and managing the present. Here, we review how molecular epidemiological investigations have benefitted our understanding of parasites in the genus *Trichinella*, a group of direct-developing nematode parasite species that infect a variety of vertebrate species (Pozio, 2005).

1.1. Why do we need molecular tools to diagnose and trace outbreaks of trichinellosis?

Nematode parasites in the genus *Trichinella* historically cause widespread zoonotic disease. Today they remain responsible for costly inspection regimes and barriers to animal trade. Prior to the advent of molecular means to discriminate among the various species of *Trichinella,* all such occurrences were incorrectly ascribed to a single species: *Trichinella spiralis* (Duckett et al., 1970; Smith, 1983). People acquire each infection by ingesting meat and/or meat products containing live *Trichinella* larvae. The average yearly incidence of the disease in humans worldwide is close to 10,000 cases, but with a low mortality rate (about 0.2%) (Pozio, 2007). Underreporting of cases is most likely in countries where the healthcare system is still developing (Bruschi, 2012). Human trichinellosis linked to consumption of infected pork has been reported from Latin America (Mexico, Argentina and Chile), Asia (China, Laos, Myanmar, Thailand, Vietnam) and Europe (Bulgaria, Romania, Russia,

* Corresponding author.
E-mail address: ewa.bilska@piwet.pulawy.pl (E. Bilska-Zając).

https://doi.org/10.1016/j.meegid.2021.105080
Received 21 April 2021; Received in revised form 4 September 2021; Accepted 7 September 2021
Available online 9 September 2021

Serbia, and the Ukraine) (Larrieu et al., 2004; Chavez-Larrea et al., 2005;Cui et al., 2006; Murrell and Pozio, 2011; Bukina, 2014; Dobrescu et al., 2014; Rainova et al., 2016; Ng-Nguyen et al., 2017; Pavic et al., 2020; Ribicich et al., 2020).

High historical burdens of disease engendered implementation of highly efficacious, but expensive preventative measures (Gottstein et al., 2009). Biosecurity measures can be very effective in limiting the spread of *Trichinella* in domestic swine herds, and testing can further limit human exposure but cannot stop circulation of the parasites in sylvatic cycles (although sylvatic transmission may be diminished where on-farm interventions diminish pathogen spillover (Hill et al., 2010)). Therefore, game animals remain an important source of exposure to *Trichinella* spp. and have, in some countries, become the primary source of human infections (Pozio, 2015). Because species of *Trichinella* are known to naturally infect more than 150 host species (mostly mammals, but also birds and reptiles) they provide an interesting model for studying molecular epidemiology (Pozio, 2005). Mining of genetic data now enables prompt source tracking. Below, we review recent advances in defining the taxonomy, systematics, and evolution of *Trichinella* spp., discuss what such tools tell us about *Trichinella* spp. population dynamics, explore the persistent limitations in fully realizing the potential of molecular epidemiology, and finally introduce the potential of genomic epidemiology to resolve unmet needs of outbreak tracing.

2. Achievements of molecular epidemiology of *Trichinella* spp. in taxonomy, systematics and evolution

The earliest records of trichinellosis date from biblical times and associate pork consumption with the spread of disease whose symptoms resembled trichinellosis. The Jewish and Muslim edicts against pork consumption may derive from illnesses that followed pork consumption (Gould et al., 1955). This zoonosis was probably an undiagnosed health concern in ancient times, as was indicated by autopsies of 3000 year old Egyptian mummies, wherein researchers described the presence of cysts in muscle fiber similar to capsules of *Trichinella* spp.(de Boni et al., 1977; Clarke et al., 2014).

2.1. Genetic tools enable differential diagnosis of species in the genus

Multiplex polymerase chain reaction (PCR) assays targeting variable segments of ribosomal RNA, followed by restriction fragment length polymorphism (RFLP) assays enabled recognition of several species in a genus once only understood as including the type species, *Trichinella spiralis* (Murrell et al., 2000; Zarlenga et al., 2006; Pozio, 2007). The parasite was described and named soon after the advent of microscopy. In 1835, a small worm was found in the “sandy diaphragm” of a deceased man and described as *Trichina spiralis* by Owen and renamed *Trichinella spiralis* later when it was found that the genus name had previously been used for an insect (Railliet, 1895). For the next 140 years, all *Trichinella*-like parasites were ascribed to a monospecific genus with a single morphological phenotype.

In the 1970s, multiple researchers noted, independently, that differing biological characteristics of certain isolates warranted division of the genus into subgroups; three new species, named *Trichinella nativa* (T2), *Trichinella nelsoni* (T7) and *Trichinella pseudospiralis* (T4), were described (Garkavi, 1972; Britov and Boev, 1972; the presence or absence of a collagen capsule surrounding the larvae in the muscle was found to distinguish two clades: encapsulated (T1, T2, T7) and non-encapsulated (T4). A decade later, using allozymes and biological features, Pozio et al. (1992) proposed a revised genus with one additional new species *Trichinella britovi* (T3), and genotypes *Trichinella* T5, T6 and T8. *Trichinella* genotype T5 has since been elevated to species level and is now called *Trichinella murrelli* while T6 and T8 remain unnamed. Later, based on molecular analysis of the cytochrome oxidase subunit I gene, the large subunit ribosomal-DNA gene and the expansion segment five, two new non-encapsulated species were described: *T. papuae* (T10) (Pozio et al., 1999) and *T. zimbabwensis* (T11) (Pozio et al., 2002) and new genotype T9 (Nagano et al., 1999). Most recently, two encapsulated species have been described in the Americas, *T. patagoniensis* (T12) (Krivokapich et al., 2008) in South America and *T. chanchalensis* (T13) in subarctic regions of Canada (Sharma et al., 2020).

The multiplex PCR-RFLP assays described above enabled differentiation of genotypes in various hosts, and, understanding the habits and ranges of those hosts, laid the foundation for initial estimations of each *Trichinella* species’ range (Pozio, 2007; Pozio, 2013; Gottstein et al., 2009; Pozio, 2013). Molecular phylogenies were then reconstructed to understand the descent relationships among these lineages (Pozio and Zarlenga, 2005; Zarlenga et al., 2006; Korhonen et al., 2016). Molecular characteristics traditionally supported division of the phylum Nematoda into the Secernentea and the Adenophorea, to which the genus *Trichinella* belongs (Maggenti et al., 1983). Variation in the small subunit ribosomal DNA (Blaxter et al., 1998) subsequently divided nematodes into five clades, a proposal confirmed in the studies of Voronov et al. (1998). *Trichinella* branched off early in the history of the first of these clades which contains certain free-living soil and marine taxa as well as some parasites of plants, insects, and vertebrates. Subsequent genetic data refined this framework (Lavrov and Brown, 2001; De Ley and Blaxter, 2002; Holterman et al., 2006).

Trichinella species were found to share about half of its expressed-sequence tags (EST) with *Caenorhabditis elegans* (assigned to clade V) (Mitreva et al., 2004, 2005). Analysis of mtDNA (Lavrov and Brown, 2001) provided additional evidence for the placement of *Trichinella* spp. as an early branch in the history of the nematodes. Mitreva and Jasmer (2006) estimated that the divergence of lineages leading to *Caenorhabditis* and *Trichinella* may have commenced 600 million years ago (mya). Furthermore, *Trichinella* genus diverged from its closest nematode lineage, Trichuridae, approximately 250–300 mya (Zarlenga et al., 2006); a subsequent analysis based on comparative whole genome analysis (Korhonen et al., 2016) is broadly consistent with that estimate (281 mya, at the transition from the Oligocene to the Miocene). Known species of *Trichinella* began diverging from a common ancestor ~29 mya, when the encapsulated and non-encapsulated clades separated in the upper and uppermost Miocene and continuing into the Pliocene and Pleistocene (Zarlenga et al., 2006; Korhonen et al., 2016).

2.2. Genetic variation elucidated the early diversification and dissemination of Trichinella parasites

Phylogenetic and phylogenomic methods sustain broadly congruent hypotheses concerning the early diversification of species in the genus, setting the stage for contemporary epidemiological investigations (Zarlenga et al., 2006; Korhonen et al., 2016). Eurasia is the likely ancestral home for all species. Divergence into encapsulated and non-encapsulated species likely occurred in the mid-Miocene as parasites of Eutherian mammals (omnivores, scavengers etc). The spread of the parasites into Africa, Eurasia and North America likely occurred by interregional faunal exchange after an early Miocene glaciation. Divergence of non-encapsulated group into *T. pseudospiralis* and the common ancestor of *T. papuae* and *T. zimbabwensis* may have occurred during a period of climate change accompanying the Tibetan plateau uplift (10–8 mya); the split between *T. papuae* and *T. zimbabwensis* may have occurred 4.9–2.3 mya (Korhonen et al., 2016).

The scenario described by Korhonen et al. (2016) supposes that independent expansion events around 7.5 mya (Miocene), 4.5–4 and 3.5 mya (Pliocene) and 2.0 mya (Pleistocene) caused divergence of *T. nelsoni* (7.8–4.1 mya) and *T. britovi* + T8 (3.2–1.7 mya) on the African continent, while separation of *T. nelsoni* + *T. spiralis* near the Miocene–Pliocene boundary may be linked with hominins and humans and later domestication of primary suid hosts for *T.spiralis*. Anthropogenic dissemination of *T. spiralis* appears to explain its notably global reach (Rosenthal et al., 2008). Diversification of T8 and *T. britovi* may have occurred in Africa (Pozio et al., 2005) or in Eurasia (Pozio et al., 2009;

Zarlenga et al., 2006).

Encapsulated species likely spread to North America before Northern Hemisphere glaciations, when permanent land linked Eurasia and the Nearctic. Earlier, around 10 mya, *T.patagoniensis* diverged from the common ancestor of the encapsulated group (around when the Panamanian Isthmus formed, enabling its establishment in South America). Korhonen et al. (2016) indicate also that diversification of *T. nativa* + T6, *T. murrelli* + T9 and *T. pseudospiralis* in Northern America, reflects complex responses to climate variation and habitat change during the emergence of the Bering land bridge after 2.5 and 2.0 mya, facilitating independent host-switching events and expansion from Eurasia into North America. In their hypothesis, T9 diverged from a common ancestor with *T. murrelli* before geographic colonization of the New World, or following isolation across Bering Strait during a glacial maximum. The most recent study of Sharma et al. (2020) added one more piece to the puzzle of *Trichinella* biogeography, describing a newly discovered freeze-resistant encapsulated species, *T. chanchalensis,* in North Canada. Their molecular analysis indicated that *T. chanchalensis* diverged from other lineages even before *T. patagoniensis.* The geographical range of this ancient species likely resulted from episodic expansion, geographic and host colonization between Eurasia and North America through Beringia, consistent with the history of wolverines, its only known host.

2.3. Genetic tools elucidate the history of phenotypic adaptations in the genus

The phylogeny contextualizes important phenotypic adaptations that have occurred, for example the capacity to infect avian and crocodilian predators of mammals (in *T. pseudospiralis* or in *T. papuae* and *T. zimbabwensis,* respectively) and the acquisition of freeze resistance in species endemic to the Arctic (*T. nativa*, T6 and *T. chanchalensis*). These biological properties provide hypotheses regarding the evolution and epidemiology of these parasites. The freeze resistance investigated in several studies appeared different for various species and hosts, likely owing to different histories for the individual species. Survival at temperatures below 0 °C in carnivore muscle is best for *T. nativa* and T6; tolerance for cold climates seems also true for the newly- described *T. chanchalensis,* though further research is required to understand the limits to its freeze-resistance (Sharma et al., 2020). This characteristic may have evolved twice in the history of *Trichinella*, or the trait may have developed early and then been subsequently lost in lineages other than *T. nativa* and T6. Although freeze resistance of these three genotypes favor their survival in cold climates, their geographical distribution is not limited to only those areas. *T. nativa* has been found in temperate climates below the isotherm 4 °C, and not only in carnivores, which are the preferred host for this species, but also omnivores, which increases the risk to humans who eat wild-meat products (Pozio et al., 2009; Bilska-Zajac et al., 2017).

2.4. Genetic tools substantiate the occurrence of hybridization in the genus

Hybridization occurs among certain species of *Trichinella*. It occurs between T6 and *T. nativa* (La Rosa et al., 2003; Dunams-Morel et al., 2012), and between *T. spiralis* and *T. britovi* (Franssen et al., 2015). Attempted laboratory crosses vary in their success (La Rosa et al., 2003). When mixed infections were followed over five generations, genotypes indicated that hybridization had occurred in 15% of crosses, but over time a single species became dominant indicating fitness differences among pure-breeding and hybrid offspring (Hecht et al., 2016). Nevertheless, limited hybridization might facilitate introgression of important phenotypic traits, such as freeze resistance, from one species to another. This possibility merits further investigation.

3. Achievements of *Trichinella* molecular epidemiology in population genetics

3.1. The extent of genetic variation varies widely among parasite species and populations of Trichinella species

Molecular tools have also been used to describe intraspecific variability, beginning with the analysis of allozymes (La Rosa et al., 1992). Subsequently, other researchers utilizing different molecular tools and genetic datasets displayed some polymorphisms in *T. spiralis* (Wu et al., 1999; Wu et al., 2000; Gasser et al., 2005). Distinct levels of variability were thereby identified among the species (Odoevskaya and Spiridonov, 2016; Bilska-Zajac et al., 2019) suggesting important differences in the processes underlying the distribution of genetic variation within and among parasite populations (Lymbery and Thompson, 2012).

The structure of *Trichinella* spp. populations was first examined using isolates collected from Europe, the Americas, Africa and Asia using microsatellite markers, and demonstrated that *T. spiralis* isolates were nearly uniform throughout Europe and the Americas. The parasite may therefore have been brought to the Americas by Europeans, who were also responsible for introducing this parasite's major hosts (pigs and rats). Greater variability occurred in just 6 Asian isolates of *T. spiralis* isolates than in 50 western isolates (Rosenthal et al., 2008), underscoring the severity of a population bottleneck in Europe.

3.2. Uniformity of European and American populations of T. spiralis limit the ability to trace outbreaks there using available genetic tools

The notable uniformity of *T. spiralis* in Europe and the Americas poses practical difficulties in differentiating among even those subpopulations of this parasite separated by great geographical distance (Rosenthal et al., 2008). The uniformity of those populations, and evidence of significantly inbred larval cohorts in most animals, enabled such insights to be derived from larval pools. Subsequent analyses using microsatellites, applied to individual larvae, confirmed (especially for infected animals in the west) that larval pairs sampled from a given host tend to be related far more closely than full siblings would be if drawn from a randomly mating deme (La Rosa et al., 2012). Testing observed allele frequencies significantly different from equilibrium expectations. More than 60% of the time, only one allele was identified when a given locus was characterized for 48 larvae derived from a given host. In only 8% of isolates were heterozygous loci identified. The outcomes indicated that most 'non-Asian' isolates were comprised of full sibling cohorts. In one notable exception, two distinct homozygote genotypes were identified without the heterozygotes that would be expected if these larvae derived from a freely mating parental pool. Instead, these larvae appear to have derived from two successive infections. Again, Asian isolates were found to harbor far greater amounts of genetic variation, with two-thirds of clearly showing multiple parents contributing to the individuals isolated from a single host.

Greater variation still has been reported from certain Asian locations (Tiandong, Guangxi and Linzhi, Tibet) (Xi et al., 2019). In this study, the Bayesian model-based clustering analysis identified larval cohorts necessarily derived from more than two parents. The most recent study by Li et al. (2020) using 16 newly designed microsatellite markers, demonstrated abundant genetic variation in 12 Chinese isolates. The observed number of alleles per locus ranged from 7 to 19, the most polymorphism yet reported. (Four of the 16 loci deviated from Hardy-Weinberg Equilibrium, which may compromise their use for many purposes). It is not yet known whether these new markers hold the potential to identify genotypic differences among samples outside of Asia.

The regional homogeneity of *T. spiralis* in Europe and the Americas constrains attempts to differentiate among outbreak strains in a locality or infer the multiplicity of infection common in naturally-infected animals. La Rosa et al. (2018) tested 41 isolates of *T. spiralis* extracted from wild boars from Extremadura, Spain, and found that two-thirds of them

consisted of identical individuals derived from inbred parents; 10% of the investigated isolates were fixed at all assayed loci. The study also showed evidence of mixed infections (larvae derived from more than one parental pair). These studies confirm that the life-cycle of *Trichinella* spp. promotes sibling inbreeding and results in reduced effective population size. When analyzing such highly inbred populations, genetically uniform isolates will often occur. The occurrence of mixed isolates however may slow the regional loss of allele richness (La Rosa et al., 2018).

3.3. Transmission dynamics favoring local homogeneity of parasite populations

What about the biology of this infection explains this genetic structure? The extent to which genetic variation is distributed among populations is determined by the interaction of various evolutionary forces, mainly genetic drift, selection and migration. These evolutionary forces are affected by several biological and ecological factors, such as reproduction, breeding system, effective population size and spreading capacity. The extent to which intraspecific diversity is structured between different hosts or geographical areas depends primarily on how the parasite reproduces and the fragmentation of the parasite population among host individuals.

Trichinella is transmitted when a carnivore or omnivore ingests the muscle tissue of an infected animal. No intermediate host or free-living stage exist, although larvae of certain species persist in host carcasses after death (Pozio, 2015; Rossi et al., 2019). Point sources lead to synchronous maturation of sibling larvae to sexually-mature adults, who then mate to produce the next generation. This favors local allelic loss and, eventually, genotypic fixation: genetic drift is stronger than gene flow in this situation. Genotypic dispersal may rely on vagile hosts, or transmission among distinct host species, but such forces do not seem to have been strong factors in the establishment of extant European populations. It seems that population size of founders was very small, which caused the development of a highly inbred population.

The deficit of genetic diversity in *T. spiralis* in Europe points to a past population bottleneck. Variation in a modest sample of whole mitochondrial genomes indicated that European lineages of *T. spiralis* probably did not descend from very recent migration of founders from China; rather Asian and European populations likely split much earlier (Thompson et al., 2021). European populations may then have suffered a uniquely marked bottleneck after the split. Recent reconstruction of population demographics from *T. spiralis* genomic sequences led to a similar conclusion: the population bottleneck limiting variation in European *T. spiralis* most likely occurred prior to domestication of pigs, perhaps during a decline of wild boar and other carnivores in Europe at the end of the last glacial maximum (Hecht et al., 2018).

In Europe, *T. britovi* is far more diverse than *T. spiralis* (Rosenthal et al., 2008). La Rosa et al. (2018) used 4 microsatellite markers to analyze isolates from continental Italy and France and the islands of Corsica and Sardinia; they found different levels of variability in each locale. The continental isolates were characterized by high levels of intra-isolate complexity and resembled isolates from other continental areas. Sardinian and Corsican isolates were more locally distinct and limited in variability. This was the first application variable genetic markers to trace distinct transmission chains, proving that Multi Locus Genotyping could be employed to trace back sources of *Trichinella* infection when sufficient genetic diversity is present. Given evidently abundant polymorphism in China, these approaches will likely suffice to trace outbreaks of *T. spiralis* there. Elsewhere in the world, limited genetic variability constrains the application of microsatellite markers to outbreak tracing of *T. spiralis*.

4. Practical application of population genetics in *Trichinella* outbreaks

Identifying sources of human infection can help stop its spread. Where alternative environmental sources occur, genetic data can be used to discern which gave rise to human infections. Such analyses have aided outbreak investigations and tracking transmission of other parasites (Ye et al., 2019; Tessema et al., 2019). New genomic sequencing technologies enable these types of analyses even for *T. spiralis* in Europe and the Americas, which have until very recently (Bilska-Zajac et al., 2021a) defied outbreak tracing using microsatellite markers.

In population studies of *Trichinella*, microsatellites have recently been the genetic marker of choice. They are co-dominant and tend to have high allelic diversity. However, they are also time-consuming to develop and impractical to genotype in large numbers, limiting studies to the use of low numbers of loci. At this time, only 6 papers examining the genetic structure of *T. spiralis* have been published, utilizing no more than 16 microsatellite loci. The results revealed the high homogeneity of this species with little indication that they can be used to track outbreaks in Europe or America.

Microsatellite studies of *T. spiralis* in Poland provide hope for tracing local outbreaks using genetic data. While *T. spiralis* from pig farms had remarkably uniform genotypes and displayed clonal character of isolates likely coming from common source, isolates from wild boar had higher genetic variability, indicating a more diverse local population than previously believed. The analysis was complicated by the generally very low genetic variability within the analyzed *T. spiralis* population; nonetheless, a degree of genetic differentiation between domestic and wildlife parasites was achieved. These results confirmed the capability of microsatellites to distinguish geographically distant isolates of *T. spiralis*. However, the study was arduous and time-consuming, and necessitated careful scoring of each microsatellite genotypes in ways that are difficult to apply from one laboratory to the next. Moreover, lack of heterozygosity complicated the process of estimating certain population parameters valuable in drawing conclusions about the structure of parasite populations (Bilska-Zajac et al., 2021a).

In general, outbreaks should entail a mere subset of a population's total genetic variability. But when the pool of total variability is limited, much more of the genome needs to be sampled (when seeking to discriminate what is unique about any given transmission chain). Careful attention is therefore required to choose sufficiently variable markers.

Given the limited resolution of existing marker systems when applied to European isolates, the addition of new microsatellite markers (for example the 16 markers recently reported by (Xi et al., 2019)) might facilitate progress. However, it bears emphasizing that interpreting microsatellite assays requires time and training, and the number of loci required to achieve needed resolution may be orders of magnitude more than what has been previously attempted.

5. High-resolution genomic approaches offer greater potential to trace outbreaks

Rapid techniques offering more resolution would aid outbreak investigation. The technological advances in next-generation sequencing (NGS) and their increasing availability provide a robust platform for the next generation of molecular epidemiology and population genetics. Whole genomes have been used to trace viral outbreaks, including the ongoing SARS-CoV2 pandemic (Zhang and Holmes, 2020). Whole genomes provide complete information, but may require significant resources depending on the size of the genome sequenced.

Reduced genome representation techniques provide an alternative to sequencing complete genomes. These generate large numbers of genetic loci from across the genome, facilitating investigations of population genetics, selection, linkage, and phenotypic association. Early methods like random amplified polymorphic DNA (RAPDs) and amplified

fragment length polymorphisms (AFLPs) proved useful in non-model organisms and have given rise to new sequence-based techniques such as restriction site associated DNA sequencing (RADseq) (Baird et al., 2008). RADseq produces DNA libraries for high-throughput sequencing using restriction enzymes that cut at specific motifs throughout the genome. Sequences adjacent to the restriction sites are collected and assembled into thousands of loci distributed across the whole genome. The results can be interrogated for single nucleotide polymorphisms (SNPs) which are essential for understanding the evolutionary history of the organism in question.

The technique has provided enhanced resolution in determining the genetic structure of populations (Morgan et al., 2017; Sunde et al., 2020) and phylogeographic history (Bohling et al., 2019) compared to microsatellite analysis. SNPs mutate more slowly than microsatellites gain or lose repeats, making RADseq data less informative about recent population divergence. However, RADseq compensates for this weakness by generating many more variable markers (Sunde et al., 2020). It has also been argued that the slower mutation rate of SNPs makes them better suited for revealing ancestral patterns of genetic structuring (Andrews et al., 2016). The power of both methods (microsatellite analysis and RADseq) has been compared in several empirical studies. Rašić et al. (2014) investigated the global population structure in the mosquito *A. aegypti* using 8 microsatellites and a large panel of SNPs and found that population structure could be more clearly resolved with SNPs. In a study genotyping great scallops (*Pecten maximus*), RADseq generated 10,539 SNPs, yielding greater power to detect genetic differences among the populations and resolving population structure over a much smaller geographical scale than 13 microsatellite loci (Vendrami et al., 2017).

As RADseq has the same potential as microsatellites to inform about the role of different evolutionary processes and has better resolution through increased sites, it is a technique that should be developed for these low-diversity organisms. We recently introduced Trich-Tracker, a sequence analysis pipeline that succeeded in rapidly extracting informative genomic variation from *T. spiralis* isolates derived from four Polish farms and from several wild boar (Bilska-Zajac et al., 2021b). The approach succeeded in distinguishing chains of transmission, linking parasites in one farm's pigs to parasites in its rats, and affirmed a suspected epidemiological connection between parasites infesting two proximate farms. This process thus yielded powerful and actionable insights by harnessing variation encoded throughout the parasite genome, and did so much more quickly than was previously possible using microsatellite markers. This successful proof of principle, achieved where historical inbreeding has frustrated attempts at traceback, heralds newfound opportunities to understand and mitigate outbreaks as they occur.

Declaration of Competing Interest

The authors declare that this article does not have any financial or non-financial conflict of interest.

Acknowledgements

This research was supported in part by an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA). ORISE is managed by ORAU under DOE contract number DE-SC0014664. All opinions expressed in this paper are the author's and do not necessarily reflect the policies and views of USDA, DOE, or ORAU/ORISE.

References

Andrews, K.R., Good, J.M., Miller, M.R., Luikart, G., Hohenlohe, P.A., 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nat. Rev. Genet. 17, 81–92.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3 (10), e3376.

Bilska-Zajac, E., Rozycki, M., Chmurzynska, E., Antolak, E., Prochniak, M., Gradziel-Krukowska, K., Karamon, J., Sroka, J., Zdybel, J., Cencek, T., 2017. First case of *Trichinella nativa* infection in wild boar in Central Europe-molecular characterization of the parasite. Parasitol. Res. 116, 1705–1711.

Bilska-Zajac, E., Franssen, F., Rozycki, M., Swart, A., Karamon, J., Sroka, J., Zdybel, J., Zietek-Barszcz, A., Cencek, T., 2019. Intraspecific genetic variation in *Trichinella spiralis* and *Trichinella britovi* populations circulating in different geographical regions of Poland. Int. J. Parasitol. Parasites Wildl. 10, 101–112.

Bilska-Zajac, E., Tonanzi, D., Pozio, E., Rozycki, M., Cencek, T., Thompson, P.C., Rosenthal, B.M., La Rosa, G., 2021a. Genetic evidence substantiates transmission of *Trichinella spiralis* from one swine farm to another. Parasit. Vectors 14, 359.

Bilska-Zając, E., Rosenthal, B., Thompson, P., 2021b. Trich-tracker – a practical tool to trace Trichinella spiralis transmission based on rapid, cost-effective sampling of genome-wide genetic variation. Int. J. Parasitol. https://doi.org/10.1016/j.ijpara.2021.08.002.

Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., Vida, J.T., Thomas, W.K., 1998. A molecular evolutionary framework for the phylum Nematoda. Nature 392, 71–75.

Bobes, R.J., Fragoso, G., Fleury, A., García-Varela, M., Sciutto, E., Larralde, C., Laclette, J.P., 2014. Evolution, molecular epidemiology and perspectives on the research of taeniid parasites with special emphasis on *Taenia solium*. Infect. Genet. Evol. 23, 150–160.

Bohling, J., Small, M., Von Bargen, J., Louden, A., DeHaan, P., 2019. Comparing inferences derived from microsatellite and RADseq datasets: a case study involving threatened bull trout. Conserv. Genet. 20, 329–342.

Britov, V.A., Boev, S.N., 1972. Taxonomic rank of various strains of *Trichinella* and their circulation in nature. Vestin. Akad. Nauk. SSSR 28, 27–32 (in Russian).

Bruschi, F., 2012. Trichinellosis in developing countries: is it neglected? J. Infect. Dev. Ctries 6 (3), 216–222.

Bukina, L.A., 2014. Possible sources of *Trichinella* infection in the indigenous population of Chukotka. Med. Parazitol. (Mosk) 17–19.

Chavez-Larrea, M.A., Dorny, P., Moeller, L., Benitez-Ortiz, W., Barrionuevo-Samaniego, M., Rodriguez-Hidalgo, R., Ron-Roman, J., Proano-Perez, F., Victor, B., Brandt, J., Kapel, C., de Borchgrave, J., 2005. Survey on porcine trichinellosis in Ecuador. Vet. Parasitol. 132, 151–154.

Clarke, E.M., Thompson, R.C., Allam, A.H., Wann, L.S., Lombardi, G.P., Sutherland, M.L., Sutherland, J.D., Cox, S.L., Soliman, M.A., Abd El-Maksoud, G., Badr, I., Miyamoto, M.I., Frohlich, B., Nur El-din, A.H., Stewart, A.F., Narula, J., Zink, A.R., Finch, C.E., Michalik, D.E., Thomas, G.S., 2014. Is atherosclerosis fundamental to human aging? Lessons from ancient mummies. J. Cardiol. 63, 329–334.

Conway, D.J., 2007. Molecular epidemiology of malaria. Clin. Microbiol. Rev. 20, 188–204.

Cui, J., Wang, Z.Q., Hu, D.S., 2006. The epidemiology of swine trichinellosis in China during 1999-2004. Helminthologia 43, 21–26.

de Boni, U., Lenczner, M.M., Scott, J.W., 1977. Autopsy of an Egyptian mummy. 6. *Trichinella* spiralic cyst. Can. Med. Assoc. J. 117, 472.

De Ley, P., Blaxter, M.L., 2002. Systematic Position and Phylogeny. The Biology of Nematodes, Taylor & Francis, London, pp. 1–30.

de Meeûs, T., McCoy, K.D., Prugnolle, F., Chevillon, C., Durand, P., Hurtrez-Boussès, S., Renaud, F., 2007. Population genetics and molecular epidemiology or how to "débusquer la bête". Infect. Genet. Evol. 7, 308–332.

Dobrescu, C., Hriscu, H., Emandi, M., Zamfir, C., Nemet, C., 2014. Consumption of untested pork contributed to over two-thousand clinical cases of human trichinellosis in Romania. Folia Parasitol. 61, 558–560.

Duckett, M.G., Denham, D.A., Nelson, G.S., 1970. A difference in the longevity and distribution of adult *Trichinella spiralis* in two strains of laboratory mouse. Trans. R. Soc. Trop. Med. Hyg. 64, 25.

Dunams-Morel, D.B., Reichard, M.V., Torretti, L., Zarlenga, D.S., Rosenthal, B.M., 2012. Discernible but limited introgression has occurred where *Trichinella nativa* and the T6 genotype occur in sympatry. Infect. Genet. Evol. 12, 530–538.

Franssen, F., Bilska-Zajac, E., Deksne, G., Sprong, H., Pozio, E., Rosenthal, B., Rozycki, M., van der Giessen, J., 2015. Genetic evidence of interspecies introgression of mitochondrial genomes between *Trichinella spiralis* and *Trichinella britovi* under natural conditions. Infect. Genet. Evol. 36, 323–332.

Garkavi, B.L., 1972. The species of *Trichinella* isolated from wild carnivores. Veterinariia 10, 90–91.

Gasser, R.B., Hu, M., El-Osta, Y.A., Zarlenga, D.S., Pozio, E., 2005. Genetic analysis of *Trichinella* populations by 'cold' single-strand conformation polymorphism analysis. Vet. Parasitol. 132, 23–26.

Gottstein, B., Pozio, E., Nockler, K., 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin. Microbiol. Rev. 22, 127.

Gould, S.E., Gomberg, H.J., Bethell, F.H., Villella, J.B., Hertz, C.S., 1955. Studies on *Trichinella spiralis*. 1. Concerning the Time and Site of Insemination of Females of *Trichinella spiralis*. 2. Time of Initial Recovery of Larvae of *Trichinella spiralis* from Blood of Experimental Animals. 3. Effect on the Intestinal Phase of Trichinosis of Feeding Massive Numbers of Irradiated Trichina Larvae. 4. Effect of Feeding

Irradiated *Trichinella* Larvae on Production of Immunity to Re-Infection. 5. Tests for a Strain of Trichina Larvae Resistant to Radiation. Am. J. Pathol. 31, 933–963.
Hecht, L., Thompson, P.C., Rosenthal, B.M., 2018. Comparative demography elucidates the longevity of parasitic and symbiotic relationships. Proc. Biol. Sci. 285 (1888), 20181032.
Hecht, L.B., Thompson, P., Lavin, E.S., Zarlenga, D.S., Rosenthal, B.M., 2016. Hybridization is limited between two lineages of freeze-resistant *Trichinella* during coinfection in a mouse model. Infect. Genet. Evol. 38, 146–151.
Hide, G., Tait, A., 1991. The molecular epidemiology of parasites. Experientia 47, 128–142.
Hill, D.E., Pierce, V., Darwin Murrell, K., Ratliffe, N., Rupp, B., Fournet, V.M., Zarlenga, D.S., Rosenthal, B.M., Ray Gamble, H., Kelly, K., Dulin, M., 2010. Cessation of *Trichinella spiralis* transmission among scavenging mammals after the removal of infected pigs from a poorly managed farm: implications for trichinae transmission in the US. Zoonoses Public Health 57, e116–e123.
Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., Helder, J., 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Mol. Biol. Evol. 23, 1792–1800.
Korhonen, P.K., Pozio, E., La Rosa, G., Chang, B.C.H., Koehler, A.V., Hoberg, E.P., Boag, P.R., Tan, P., Jex, A.R., Hofmann, A., Sternberg, P.W., Young, N.D., Gasser, R. B., 2016. Phylogenomic and biogeographic reconstruction of the *Trichinella* complex. Nat. Commun. 7.
Krivokapich, S.J., Prous, C.L.G., Gatti, G.M., Confalonieri, V., Molina, V., Matarasso, H., Guarnera, E., 2008. Molecular evidence for a novel encapsulated genotype of *Trichinella* from Patagonia, Argentina. Vet. Parasitol. 156, 234–240.
La Rosa, G., Marucci, G., Zarlenga, D.S., Casulli, A., Zarnke, R.L., Pozio, E., 2003. Molecular identification of natural hybrids between *Trichinella nativa* and *Trichinella* T6 provides evidence of gene flow and ongoing genetic divergence. Int. J. Parasitol. 33, 209–216.
La Rosa, G., Marucci, G., Rosenthal, B.M., Pozio, E., 2012. Development of a single larva microsatellite analysis to investigate the population structure of *Trichinella spiralis*. Infect. Genet. Evol. 12, 369–376.
La Rosa, G., Calero-Bernal, R., Pérez-Martín, J.E., Tonanzi, D., Galati, F., Serrano-Aguilera, F.J., Rosenthal, B.M., Pozio, E., 2018. Rare but evolutionarily consequential outcrossing in a highly inbred zoonotic parasite. Int. J. Parasitol. 48 (7), 543–553.
Larrieu, E., Molina, V., Albarracin, S., Mancini, S., Bigatti, R., Ledesma, L., Chiosso, C., Krivokapich, S., Herrero, E., Guarnera, E., 2004. Porcine and rodent infection with *Trichinella*, in the Sierra Grande area of Rio Negro province, Argentina. Ann. Trop. Med. Parasitol. 98, 725–731.
Lavrov, D.V., Brown, W.M., 2001. *Trichinella spiralis* mtDNA: a nematode mitochondrial genome that encodes a putative ATP8 and normally structures tRNAs and has a gene arrangement relatable to those of coelomate metazoansc. Genetics 157, 621–637.
Li, T., Tang, B., Bai, X., Wang, X.L., Luo, X., Yan, H., Zhu, H., Jia, H., Liu, X., Liu, M., 2020. Development of genome-wide polymorphic microsatellite markers for *Trichinella spiralis*. Parasit. Vectors 13, 58.
Lymbery, A.J., Thompson, R.C.A., 2012. The molecular epidemiology of parasite infections: tools and applications. Mol. Biochem. Parasitol. 181 (2), 102–116.
Nematode higher classification as influenced by species and family concepts. In: Maggenti, A.R., Stone, A.R., Platt, H.M., Khalil, L.F. (Eds.), 1983. Concepts in Nematode Systematics. Academic Press, London, pp. 25–40.
Mitreva, M., Jasmer, D.P., 2006. Biology and genome of *Trichinella spiralis*. WormBook 1–21.
Mitreva, M., Jasmer, D.P., Appleton, J., Martin, J., Dante, M., Wylie, T., Clifton, S.W., Waterston, R.H., McCarter, J.P., 2004. Gene discovery in the adenophorean nematode *Trichinella spiralis*: an analysis of transcription from three life cycle stages. Mol. Biochem. Parasitol. 137, 277–291.
Mitreva, M., Appleton, J., McCarter, J.R., Jasmer, D.P., 2005. Expressed sequence tags from life cycle stages of *Trichinella spiralis*: application to biology and parasite control. Vet. Parasitol. 132, 13–17.
Morgan, T.D., Graham, C.F., McArthur, A.G., Raphenya, A.R., Boreham, D.R., Manzon, R. G., et al., 2017. Genetic population structure of the round whitefish (*Prosopium cylindraceum*) in North America: multiple markers reveal glacial refugia and regional subdivision. Can. J. Fish. Aquat. Sci. 75, 836–849.
Murrell, K.D., Pozio, E., 2011. Worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerg. Infect. Dis. 17, 2194–2202.
Murrell, K.D., Lichtenfels, R.J., Zarlenga, D.S., Pozio, E., 2000. The systematics of the genus *Trichinella* with a key to species. Vet. Parasitol. 93, 293–307.
Nagano, I., Wu, Z., Matsuo, A., Pozio, E., Takahashi, Y., 1999. Identification of *Trichinella* genotypes by polymerase chain reaction-restriction fragment length polymorphism of mitochondrial cytochrome c oxidase subunit I gene. Int. J. Parasitol. 29, 1113–1120.
Ng-Nguyen, D., Stevenson, M.A., Traub, R.J., 2017. A systematic review of taeniasis, cysticercosis and trichinellosis in Vietnam. Parasit. Vectors 10, 150.
Odoevskaya, I.M., Spiridonov, S.E., 2016. *Trichinella* nativa haplotypes in Russia show diversity in cytochrome oxidase mtDNAgene. Vet. Parasitol. 231, 39–42.
Pavic, S., Andric, A., Sofronic-Milosavljevic, L.J., Gnjatovic, M., Mitic, I., Vasilev, S., Sparic, R., Pavic, A., 2020. *Trichinella britovi* outbreak: Epidemiological, clinical, and biological features. Med. Mal. Infect. 50, 520–524.
Pozio, E., 2005. The broad spectrum of *Trichinella* hosts: from cold- to warm-blooded animals. Vet. Parasitol. 132, 3–11.
Pozio, E., 2007. World distribution of *Trichinella* spp. infections in animals and humans. Vet. Parasitol. 149, 3–21.
Pozio, E., 2013. The opportunistic nature of *Trichinella*-exploitation of new geographies and habitats. Vet. Parasitol. 194, 128–132.
Pozio, E., 2015. *Trichinella* spp. imported with live animals and meat. Vet. Parasitol. 213, 46–55.
Pozio, E., Zarlenga, D.S., 2005. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. Int. J. Parasitol. 35, 1191–1204.
Pozio, E., La Rosa, G., Murrell, K.D., Lichtenfels, J.R., 1992. Taxonomic revision of the genus *Trichinella*. J. Parasitol. 78, 654–659.
Pozio, E., Owen, I.L., La Rosa, G., Sacchi, L., Rossi, P., Corona, S., 1999. *Trichinella papuae* n.sp. (Nematoda), a new non-encapsulated species from domestic and sylvatic swine of Papua New Guinea. Int. J. Parasitol. 29, 1825–1839.
Pozio, E., Foggin, C.M., Marucci, G., La Rosa, G., Sacchi, L., Corona, S., Rossi, P., Mukaratirwa, S., 2002. *Trichinella zimbabwensis* n.sp. (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. Int. J. Parasitol. 32, 1787–1799.
Pozio, E., Pagani, P., Marucci, G., Zarlenga, D.S., Hoberg, E.P., De Meneghi, D., La Rosa, G., Rossi, L., 2005. *Trichinella britovi* etiological agent of sylvatic trichinellosis in the Republic of Guinea (West Africa) and a re-evaluation of geographical distribution for encapsulated species in Africa. Int. J. Parasitol. 35, 955–960.
Pozio, E., Rinaldi, L., Marucci, G., Musella, V., Galati, F., Cringoli, G., Boireau, P., La Rosa, G., 2009. Hosts and habitats of *Trichinella spiralis* and *Trichinella britovi* in Europe. Int. J. Parasitol. 39, 71–79.
Rainova, I., Kaftandjiev, I., Harizanov, R., Tsvetkova, N., Jordanova, D., Marinova, I., Kurdova, R., Kantardjiev, T., Lalkovski, N., 2016. Outbreaks of human trichinellosis, still a challenge for the public health authorities in Bulgaria. J. Public Health-Heid 24, 291–297.
Rašić, G., Filipović, I., Weeks, A.R., Hoffmann, A.A., 2014. Genome-wide SNPs lead to strong signals of geographic structure and relatedness patterns in the major arbovirus vector, *Aedes aegypti*. BMC Genomics 15, 275.
Ribicich, M.M., Farina, F.A., Aronowicz, T., Ercole, M.E., Bessi, C., Winter, M., Pasqualetti, M.I., 2020. A review on *Trichinella* infection in South America. Vet. Parasitol. 285, 109234.
Rosenthal, B.M., LaRosa, G., Zarlenga, D., Dunams, D., Chunyu, Y., Mingyuan, L., Pozio, E., 2008. Human dispersal of *Trichinella spiralis* in domesticated pigs. Infect. Genet. Evol. 8, 799–805.
Rossi, L., Interisano, M., Deksne, G., Pozio, E., 2019. The subnivium, a haven for Trichinella larvae in host carcasses. Int. J. Parasitol. Parasites Wildl. 8, 229–233.
Sharma, R., Thompson, P.C., Hoberg, E.P., Scandrett, W.B., Konecsni, K., Harms, N.J., Kukka, P.M., Jung, T.S., Elkin, B., Mulders, R., Larter, N.C., Branigan, M., Pongracz, J., Wagner, B., Kafle, P., Lobanov, V.A., Rosenthal, B.M., Jenkins, E.J., 2020. Hiding in plain sight: discovery and phylogeography of a cryptic species of *Trichinella* (Nematoda: Trichinellidae) in wolverine (*Gulo gulo*). Int. J. Parasitol. 50 (4), 277–287.
Smith, H.J., 1983. Differentiation of *Trichinella spiralis spiralis* and *Trichinella spiralis nativa* based on resistance to low temperature refrigeration. Can. J. Comp. Med. 47, 501–502.
Sunde, J., Yıldırım, Y., Tibblin, P., Forsman, A., 2020. Comparing the performance of microsatellites and RADseq in population genetic studies: analysis of data for pike (*Esox lucius*) and a synthesis of previous studies. Front. Genet. 11, 218.
Tessema, S.K., Raman, J., Duffy, C.W., et al., 2019. Applying next-generation sequencing to track falciparum malaria in sub-Saharan Africa. Malar. J. 18, 268.
Thompson, P.C., Bilska-Zajac, E., Zarlenga, D.S., Liu, M., Cencek, T., Rozycki, M., Rosenthal, B.M., 2021. Divergence at mitochondrial and ribosomal loci indicates the split between Asian and European populations of *Trichinella spiralis* occurred prior to swine domestication. Infect. Genet. Evol. 88, 104705.
Traub, R.J., Monis, P.T., Robertson, I.D., 2005. Molecular epidemiology: a multidisciplinary approach to understanding parasitic zoonoses. Int. J. Parasitol. 35, 1295–1307.
Vendrami, D.L.J., Telesca, L., Weigand, H., Weiss, M., Fawcett, K., Lehman, K., et al., 2017. RAD sequencing resolves fine-scale population structure in a benthic invertebrate: implications for understanding phenotypic plasticity. R. Soc. Open Sci. 4, 160548. https://doi.org/10.1098/rsos.160548.
Voronov, D.A., Panchin, Y.V., Spiridonov, S.E., 1998. Nematode phylogeny and embryology. Nature 395, 28.
Wu, Z., Nagano, I., Pozio, E., Takahashi, Y., 1999. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for the identification of *Trichinella* isolates. Parasitology 118 (Pt 2), 211–218.
Wu, Z., Nakada, T., Nagano, I., Takahashi, Y., 2000. DNA fingerprints of *Trichinella* as revealed by restriction fragment length polymorphism and single-strand conformational polymorphism (RFLP-SSCP). Mol. Cell. Probes 14, 291–297.
Xi, Z., Lu, H.L., Xiu, H., Peng, J., Fei, N.Y., Quan, W.Z., Jing, C., 2019. Genotyping and phylogenetic position of *Trichinella spiralis* isolates from different geographical locations in China. Front. Genet. 10, 1093.
Ye, R., Tian, Y., Huang, Y., et al., 2019. Genome-wide analysis of genetic diversity in *Plasmodium falciparum* isolates from China-Myanmar border. Front. Genet. 10, 1065.
Zarlenga, D.S., Rosenthal, B.M., La Rosa, G., Pozio, E., Hoberg, E.P., 2006. Post-Miocene expansion, colonization, and host switching drove speciation among extant nematodes of the archaic genus *Trichinella*. Proc. Natl. Acad. Sci. U. S. A. 103, 7354–7359.
Zhang, Y.-Z., Holmes, 2020. A genomic perspective on the origin and emergence of SARS-CoV-2. E. C. Cell 181, 223–227.