Changing face of paragonimiasis

1. As *Paragonimus* is one of your specialized areas of research, please shed some light on its current trends.

Paragonimus (lung fluke) species cause paragonimiasis, one of the neglected tropical diseases that many people might not be familiar with. Around 50 species have been named, some of which will prove not to be valid. Different species are known from Africa, the Americas, and Asia (especially Eastern and Southern Asia). As with many other trematodes, the life cycle is complex: cercariae develop in a freshwater snail, metacercariae develop in freshwater (sometimes brackish water) crabs or crayfish and adults develop in the lungs of mammals that eat crabs or crayfish. Typically, two adult worms live together in a cyst in the lungs and pass eggs out into bronchioles. Paragonimiasis is a zoonosis. Natural mammal hosts include members of the cat family, dog family, rodents, monkeys, marsupials (in the Americas), and more. Only a few species of *Paragonimus* infect humans. Some of these occupy cysts in human lungs, the usual site in their natural animal hosts, but other species end up in atypical sites such as the brain, where they can cause serious disease.[1]

Estimates from 2005 suggested that about 292 million people were at risk of paragonimiasis, with around 23 million infected, mostly in China. Overall numbers of cases have decreased slightly since then, but there remains a high degree of uncertainty about these estimates.[1]

Trends vary according to country and local culture. In much of eastern Asia, including China, paragonimiasis has largely been eliminated through a combination of public-health interventions, changing life-styles (with increasing affluence, fewer people eat undercooked crabs or crayfish) and loss of crab/snail habitats. In South Korea, for example, human paragonimiasis has almost disappeared and circulates only at low levels in natural hosts. In Japan, where it used to be common especially in schoolchildren, the disease has become rare and usually seen in middle-aged men. This is due to the opportunistic inclusion of paratenic hosts in the life cycle of *Paragonimus westermani*. If a crab containing metacercariae of this species is eaten by a mammal host other than the definitive host, the metacercariae can survive as juvenile worms for long periods. Such paratenic hosts include wild boar, which are hunted for sport in parts of Japan and their meat eaten raw. Thus hunters become infected. Occasional outbreaks of paragonimiasis are reported from China, where the disease is otherwise increasingly rare. Such outbreaks are often unfortunate sequels to celebrations that have included a feast of freshwater crabs.[2] In North America, locally acquired human paragonimiasis is the reward for bravado by young men eating freshwater crayfish raw as a "dare."

David Blair retired a few years ago and is currently an adjunct professor at James Cook University in Townsville, Australia. His career started in Glasgow, UK, where he gained a PhD with a study of life cycles of fish parasites in Scotland. In Australia, he worked at James Cook University in the late 1970s and again from 1989 until retirement. For several years in the 1980s, he worked in the Department of Zoology, University of Canterbury, Christchurch, New Zealand. His early work was on the trematode parasites of local wildlife, including marine mammals, sea turtles and crocodiles. He was an early adopter of molecular techniques in taxonomy and phylogeny, and applied these to a range of parasites, including schistosomes, liver flukes and lung flukes. This led to participation in projects in China and elsewhere to identify and characterise the large range of lung fluke species reported there. Molecular taxonomy led on to an interest in genomics, and especially the evolution of mitochondrial genomes in parasitic flatworms. Prof. Blair is a Fellow of the Australian Society for Parasitology and a Visiting Professor at Khon Kaen University, Thailand.

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Paragonimiasis, although often asymptomatic, can present a suite of symptoms (pulmonary symptoms including hemoptysis) that broadly overlap those of tuberculosis (TB). Misdiagnosis is therefore common and paragonimiasis is often discovered during investigations of what were initially thought to be cases of drug-resistant TB.[3] This happened, for example, in NE India and led to the development of combined surveys for TB and paragonimiasis.[4] It is difficult to say much about trends of paragonimiasis in West and Equatorial Africa, and parts of South America: data are limited. Researchers in Ecuador have been the most active in South America and have reported many cases there.[5]

2. What are your other areas of interest in parasitology research?

Much of my early work was on the taxonomy of trematode parasites of wildlife, for example, marine mammals, sea turtles and crocodiles. Later, I developed an interest in phylogeny and molecular taxonomy of trematodes and in mitogenomics of trematodes. This led also to work on population genetics of parasites, especially schistosomes. I have also worked on the temnocephalan symbionts of freshwater crayfish in Australia.

3. Please share your work experience in the morphological and molecular characterization of metacercaria of *Paragonimus caliensis* which separates it from *P. mexicanus*.

I can't claim much credit for this work. It was mostly that of Roderico Hernández-Chea, working at the time in Costa Rica.[6] His study helps illustrate the difficulties of identifying *Paragonimus* species and the challenges in their taxonomy. The name *P. caliensis* was first applied to adult lung flukes from marsupial hosts in Colombia, but it was often regarded as not distinct from *P. mexicanus*. Hernández-Chea found two morphological types of metacercariae in Costa Rica and obtained DNA sequences from these. The results showed without doubt that the different morphological types were distinct species: *P. mexicanus* and *P. caliensis*. In molecular phylogenies, these two species were not each other's closest relatives, thus emphasising that they are distinct.

4. Can you tell us regarding new intermediate hosts which are found for various Paragonimus species with special reference to snails?

Snail intermediate hosts are not known for many *Paragonimus* species, especially in Africa[7] and South America (*P. mexicanus* being the exception). Prevalence in snails is often very low and the microcercous cercariae, if found, are difficult to identify to species without the use of molecular methods.[8] The confused taxonomy of possible snail hosts adds to the difficulties. In Vietnam, DNA sequences were used to identify infections of three different *Paragonimus* species, each in a different snail host.[9] A general rule in parasitology is that any given trematode species can only infect one, or a few related, species of snail. But the situation is complicated in *Paragonimus*: some "species" are really complexes of closely related forms, often with different biological properties. The *Paragonimus westermani* complex illustrates this well. Members of this complex occur across a broad range in eastern Asia, from Japan and the Philippines westwards to India and Sri Lanka. Three different members occur in Northeast India alone.[10] Adult worms are mostly morphologically similar, but biological properties differ substantially. For example, human infections are rarely found outside countries in the eastern part of the range of the complex. Different snail hosts are used by different geographical forms of *P. westermani*, but information is lacking for many regions.

5. What are the various factors which lead to the diversity and specific species distribution of *Paragonimus* spp. in different parts of the word? Is it just due to the availability of its intermediate host only?

Different *Paragonimus* species are found in different parts of the world and use different intermediate hosts (where known). Unlike, for example, *Fasciola hepatica*, *Paragonimus* species and some of their snail hosts have not spread around the world in recent times. The genus *Paragonimus* seems ancient with a distinct evolutionary history on each continent. Different, unrelated species on each continent have independently acquired the ability to infect humans.

6. What is the current understanding of genetic diversity in lung flukes?

The initial molecular work on genetic diversity in lung flukes focused on taxonomic and phylogenetic questions. This demonstrated the phylogenetic depth and complexity of the genus *Paragonimus* and the existence of several species complexes.[11] Molecular characterization of lung flukes using standard DNA sequence markers will continue into the future and will be essential to support descriptions of any new species.

The genomic era has not bypassed lung flukes, with two recent papers reporting genomes of *Paragonimus* species. One of these included draft genome data from four

species.[12] It will take some time for this flood of data to be digested and to be mined, for example, new diagnostic or therapeutic targets. Gene families that have expanded in lung flukes are largely the same as those found in liver flukes and blood flukes (schistosomes). Perhaps this is to be expected: all these groups of flukes are partially or completely tissue-dwelling. Comparisons have yet to be made with genomes of flukes living in the gut, by far the most common habitat for trematodes. Nevertheless, 256 orthologous groups of genes were identified that were specific to lung flukes and consistently transcribed in adults of all species. Included were genes involved with, for example, iron acquisition, immune modulation, and more.

7. Can you shed some light regarding any antigen or protein targets in *Paragonimus* spp which can help in its diagnostic aspect?

The diagnostic "gold standard" for paragonimiasis is the finding of eggs in sputum. However, shedding of eggs may be intermittent, and eggs are generally not found in cases of ectopic paragonimiasis. Immunodiagnostic approaches have therefore become widely preferred. Many different protein targets have been used and there has been a tendency for each laboratory group to develop their own. Some antigens used are poorly characterized: The Centers for Disease Control in the USA use a crude antigen extract of *P. westermani*. The use of purified or recombinant antigens has also been reported. Recombinant antigens have some clear advantages because they can be produced *in vitro* in quantity and with consistent quality. Full-length genes encoding many previously well-characterized diagnostic antigens were reported in the most recent genome paper.[12] This genomic resource will therefore facilitate future optimisation of diagnostic antigens.

8. You have been working all over the world, what is your opinion on research approach in developing countries when compared to developed nations?

There is no absolute cut off between the approaches and kinds of work done in developing versus developed countries. Having said that, workers in developing countries tend to focus on the immediate problem that confronts them, usually related to the presence of disease, diagnosis, treatment, and epidemiology. Many of these diseases are absent from developed countries, so priorities and outlook are different. In developed countries, workers can focus more on basic research that underpins understanding at all levels.

9. In your opinion, what newer perspectives can be addressed in these national programs for eliminating parasites with multiple hosts?

I am a great believer in the power of public-health education. What people don't know can hurt them. Once they understand the causes of disease, many people will take steps to avoid infection.[13] Another approach to eliminating parasites with multiple hosts in their complex life cycles is to attack the weakest link in the cycle: all of the host species are necessary or the parasite cannot be transmitted. Economic development itself can have this effect: natural populations of intermediate hosts (and of mammal final hosts) decline as human populations, urbanization, and pollution increase.

10. Please share few words of advice for budding scientists in the field of parasitology.

Parasitologists study all aspects of parasite biology, including taxonomy, life cycles, immune interactions with the host, ecology, epidemiology and public health, physiology and development of therapeutic drugs, genetics, genomics, and more. Hence, parasitology as a discipline is itself parasitic, feeding from expertise across a broad range of other fields. There is a tendency for budding scientists, fresh from MSc or PhD studies, to wish to continue research on their thesis topic for the rest of their career. This is understandable, but you can never know where opportunity might take you. I urge budding parasitologists to look beyond the often rather narrow disciplinary bounds of their higher degree work. Instead, they should develop expertise in more fields as their career develops. Cross-linking of disciplines makes for synergistic understanding. Don't be afraid to launch into new fields if they can provide the means to answer those questions that are buzzing around in your mind.

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