Chapter 4

Echinococcus shiquicus and *Echinococcus felidis*

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Abstract

Currently, ten genotypes (G1-G10) of *Echinococcus* Sensu Lato have been explicitly identified on the basis of taxonomic criteria. These include morphometric keys, host specificity, geographical distribution, phylogenetic analysis and genome mapping. However, a few emergent species of genus *Echinococcus* have been indigenously confirmed in some autonomous regions of Tibet plateau and Africa where there is little known about their biological aspects and potential pathogenicity in intermediate and definitive hosts. This chapter is focused on the parasite history, life cycle, phenotypic aspects, epidemiology, zoonotic potential and phylogeny relationship of two enigmatic parasites namely: *Echinococcus shiquicus* and *Echinococcus felidis*. This aims to provide a better understanding of their taxonomic status, public health problems and biological features in the mentioned regions.

Keywords: *Echinococcus shiquicus*, *Echinococcus felidis*, Biological Aspects, Phylogenetic traits

1. Introduction

Currently, ten genotypes (G1-G10) of *Echinococcus* Sensu Lato have been explicitly identified on the basis of taxonomic criteria. These include morphometric keys, host specificity, geographical distribution, phylogenetic analysis and genome mapping. However, a few emergent species of genus *Echinococcus* have been indigenously confirmed in some autonomous regions of Tibet plateau and Africa where there is little known about their biological aspects and potential pathogenicity in intermediate and definitive hosts. This chapter is focused on the parasite history, life cycle, phenotypic aspects, epidemiology, zoonotic potential and phylog-

eny relationship of two enigmatic parasites namely: *Echinococcus shiquicus* and *Echinococcus felidis*. This aims to provide a better understanding of their taxonomic status, public health problems and biological features in the mentioned regions.

2. *Echinococcus shiquicus*

2.1. History, morphology and biology

In ~2005, *Echinococcus shiquicus* was first described by Xiao, from the Tibetan fox in Shiqu county of the Qinghai–Tibet plateau, China called the ''Roof of the World'' [1]. Earlier than molecular analysis, the *E. shiquicus* isolated from Tibetan fox; *Vulpes ferrilata* was considered as a morphological strain of *E. multilocularis*, whilst it is metacestode from the plateau pika; *Ochotona curzoniae*, was then recognized as *E. granulosus* because of its unilocular cystic characteristics [2].

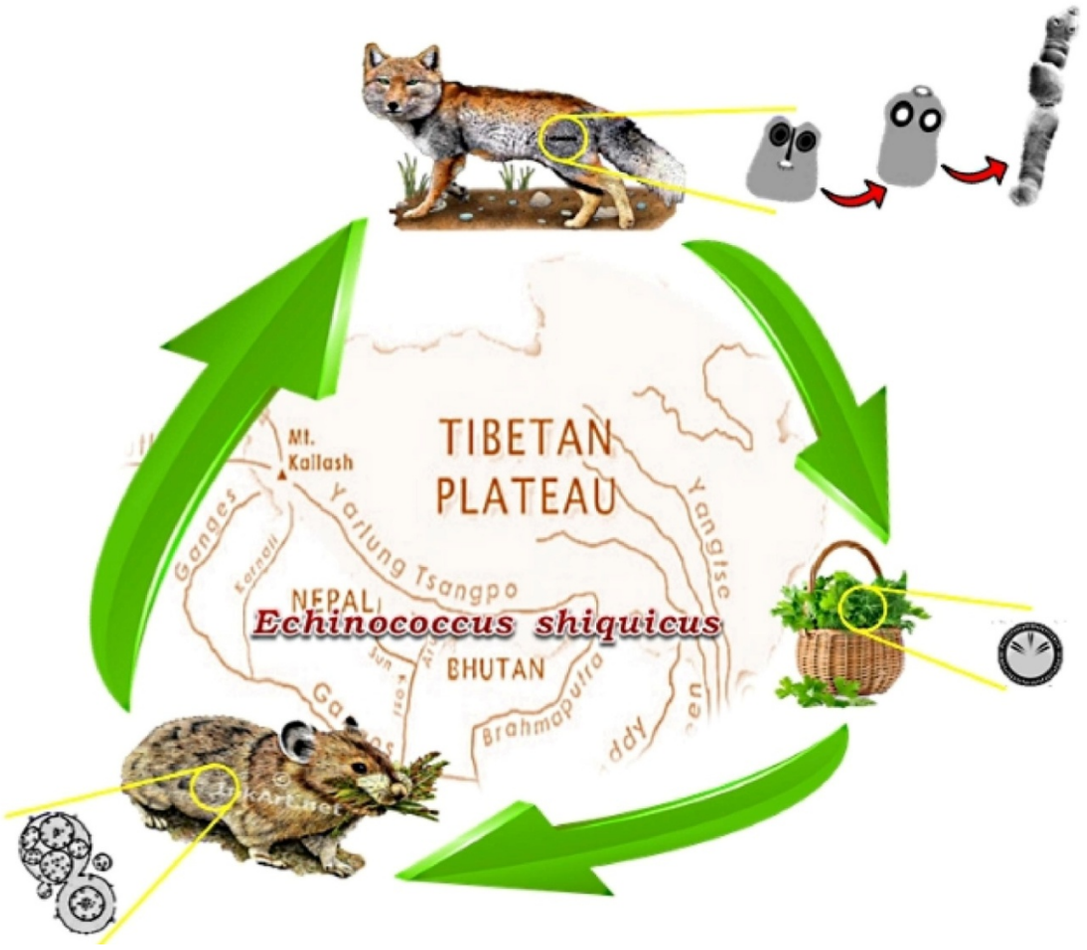

Figure 1. The life cycle of *Echinococcus shiquicus*. Credit: Image courtesy of Seyyed Ali Shariatzadeh.

Afterward, based on taxonomic criteria including morphology, host specificity, molecular characters and geographical distribution was considered as a new species of *Echinococcus* [1].

The sylvatic cycle of *E. shiquicus* is supported by the Tibetan fox and the plateau pika as the definitive and intermediate hosts, respectively, which are indigenous to the highlands of Tibet (Figure 1) [1]. However, *E. shiquicus* using copro-PCR and sequencing strategies has been naturally reported in dogs from eastern Qinghai–Tibet plateau region, China [3].

The adult worms of *E. shiquicus* are observed into two types. The majority of the samples included pre-mature and gravid proglottid which testes and ovary are placed in the pre-mature segment while the genital pore is closed (Figure 2A) [1]. The second type consisted of immature, mature and gravid proglottid (Figure 2B). The number of segments in an adult worm is limited to three. *E. shiquicus* is readily discriminated from *E. granulosus* by its shorter length, length of hooks on the rostellum, branchless gravid uterus and anterior position of genital pore in the gravid proglottid. Also, the upper position of genital pore in the mature segment, smaller rostellar hooks and the fewer eggs in gravid segment of *E. shiquicus* (less than 100) are useful for differentiation of *E. shiquicus* in compared to *E. multilocularis*.

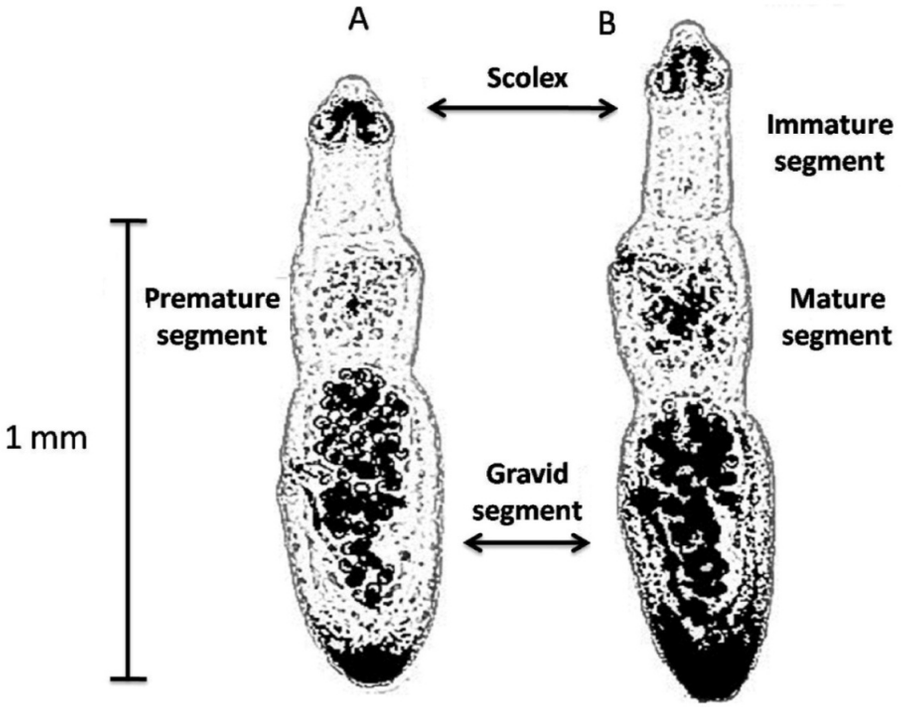

Figure 2. Different adult types (A and B) of *Echinococcus shiquicus* in a naturally infected Tibetan fox.

The metacestode stage of *E. shiquicus* is characterized by presence of unilocular minicyst containing developed brood capsules with no daughter cysts. The morphometric aspects of *E. shiquicus*, *E. multilocularis* and *E. granulosus* are shown in Table 1.

The concomitant (dual) infections of *E. shiquicus* with *E. multilocularis* have been recently identified in *O. curzoniae* and Tibetan foxes [4]. The zoonotic potential of *E. shiquicus* is still

Features	*E. shiquicus*	*E. granulosus*	*E. multilocularis*
Body Length (mm)	1.3 -1.7	2.0–11.0	1.2– 4.5
Number of segments	2–3	2– 7	2–6
Length of large hook on the rostellum (microns)	20.0– 23.0	25.0– 49.0	24.9– 34.0
Length of small hook on the rostellum (microns)	16.0–17.0	17.0– 31.0	20.4– 31.0
Number of testicles	12–20	25– 80	16– 35

Table 1. Comparison of the morphological aspects of *Echinococcus* spp.

unknown; although recently, some cases with unilocular and alveolar echinococcosis inclusive atypical ultrasound images have been characterized in Tibetans [5].

Although, no human infection of *E. shiquicus* has been reported yet, nevertheless the public health problems of *E. shiquicus* should not be neglected in control programs such as follow-up, monitoring and surveillance in an at risk population.

Therefore, additional studies are required to survey the possibility of human infections which can be employed in animal's models by serial passages using diagnostic antigens. The biological aspects of *E. shiquicus* including definitive host, intermediate hosts, genetic similarity and *etc*. are shown in Table 2.

Hydatid characters	Infectivity to humans	Genetic similarity	Distribution	Intermediate host	Definitive host	Species
Unilocular	Uncertain	*Echinococcus multilocularis*	Tibetan plateau	Pika	Tibetan fox	***Echinococcus shiquicus***
Unknown	Uncertain	*Echinococcus granulosus*	Africa	Warthog (possibly zebra, wildebeest, bush pig, buffalo, various antelope, giraffe, hippopotamus)	Lion	***Echinococcus felidis***

Table 2. A list of *Echinococcus felidis* and *Echinococcus shiquicus* features.

2.2. The evolutionary markers and molecular approaches in identifying *Echinococcus* spp.

The evolution in *Echinococcus* spp. can be considered as a combination of three major mechanisms that separately affect the composition of the genome of each species: mutation, selection and genetic drift [6].

Mutations occurred in the non-synonymous sites (called replacement sites because mutations at these sites lead to a change in the protein sequence) and the synonymous sites (called silent sites because mutations there do not lead to a change in the protein sequence) [6, 7].

It is worth noting that the nucleotide substitutions (Transition/Tranversion models) and/or indels (insertion or deletion) in the first and second positions of codons usually lead to create a new species/strain, which directly affect the frame shift of amino acids in case of any amino acid shifting or changing protein functionality while, changing in third position of codons (wobble site) is frequently leaded to creating novel haplotypes [8–11].

To date, the status of *Echinococcus* spp. is identified evidently by sequencing of nuclear DNA [12] and mitochondrial genome (mitogenome) accompanied by employing phylogenetic analysis on the basis of cladistics methods (Maximum Likelihood and Maximum Parsimony or Bayesian statistics and uses allele frequencies to study the nature and extent of genetic variation within and between populations) [13]. Although recently, a diagnostic pattern has been reported based on RFLP technique in differentiation of *Echinococcus* spp. using non-coding conserved gene [14].

It is important that the employing extra nuclear (mitochondrial) markers with low copy numbers and high variation characteristics are able to identify the unknown species/strains even haplotypes in exceptional regions where several intermediate hosts are circulating unequivocally [15].

2.3. Phylogenetic findings

Sequencing, phylogenetic and bioinformatics' analyses of mtDNA [16] and nuclear DNA [12] revealed that *E. shiquicus* and *E. multilocularis* are sister species with identical branch lengths in a specific clade and bootstrapping value of 99%.

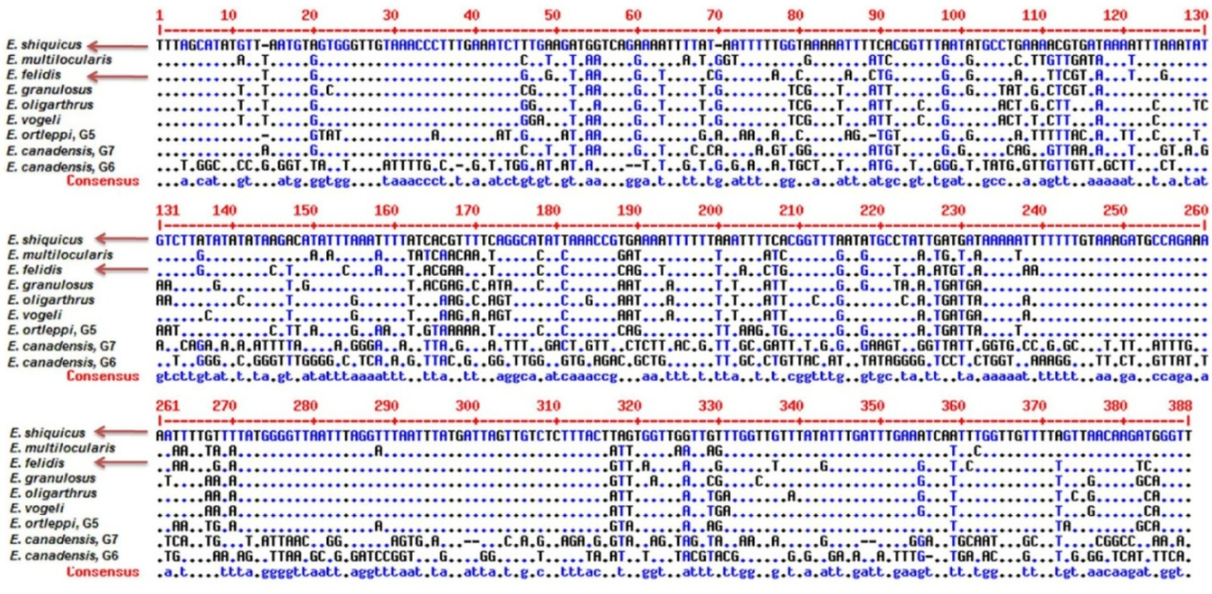

Figure 3. Nucleotide sequence alignments of Cox1 gene in *Echinococcus* spp.

The molecular characterization of *Echinococcus* spp. in the Tibetan plateau has demonstrated that the richness of genetic diversity indices of *E. shiquicus* is higher than those of *E. granulosus s.s* and *E. multilocularis* (Figure 3).

E. shiquicus, however, is placed with *E. multilocularis* in a common clade of ancestor with bootstrap value >70% (Figures 4 and 5).

This heterogeneity can be elucidated by description of three assumptions. First: presence of two turnover mechanisms, namely; unequal crossing over/transposition and slippage in the sequence length of parasite [17]. Second: lack of any bottleneck effects after its ancestor had been isolated on the Tibetan Plateau by colonizing alpine mammals (genetic drift or founder effect) [18]. And third: the long term geographic segregation into the plateau.

In a study, three polymerase chain reaction (PCR) assays based on the amplification of a fragment within the NADH dehydrogenase subunit 1 (ND1) mitochondrial gene have been optimized for the detection of *E. shiquicus*, *E. granulosus* and *E. multilocularis* in co-endemic regions of Qinghai-Tibet plateau, China [19].

3. *Echinococcus felidis*

3.1. Introduction

In *Echinococcus* species position, *E. granulosus* Sensu Lato has been categorized into *E. granulosus* Sensu Stricto (G1–G3), *E. equinus* (horse strain; genotype G4), *E. ortleppi* (cattle strain; genotype G5) and *E. canadensis* (genotypes G6–G10).

However, the taxonomic position, human infection, intermediate hosts and DNA profile of enigmatic 'lion strain' from Africa [20] has been unknown due to unavailability of suitable isolates.

3.2. History and biology

In 1937, *Echinococcus felidis* (Cestoda: Taeniidae) was first described by Ortlepp, from the lion *Panthera leo*, in South Africa [21]. Then, Rausch and Nelson (1963) declared that the taxonomic status of *E. felidis* remained to be uncertain from other *Echinococcus* spp. owing to existence of the host specificity and its rostellar hooks [22]. Verster (1965) re-checked the morphological features of *E. felidis* proglottids and proposed as a sub-specific rank of *E. granulosus felidis* [23]. Afterward, Rausch (1967) invalidated the sub-specific/strain status regarding *E. felidis* as an *E. granulosus* synonym because of lack of a valid evidence for it being a geographically isolated sylvatic cycle [24].

Many records of this parasite remained unidentified from a large variety of African mammals, due to the lack of diagnostic criteria, mainly genetic characterization and the misdiagnosis with the sympatric *Echinococcus* spp. [25] (Figure 6).

Echinococcosis is a high public health priority in the endemic areas of the world especially Africa, where more than one species of intermediate host is present and there is the possibility

of interaction between cycles of transmission. Therefore the concomitant infections of *E. felidis* with other *Echinococcus* spp. may be sympatrically overlapped in under studied regions where it can be neglected.

The explicit status of *E. felidis* had not been determined until recent times. Huttner et al. (2008) presented its validity based on genetic classification and phylogenetic position [20].

The felids act as definitive hosts for enigmatic 'lion strain' however, It is still unclear which of the sympatric wild ungulates serve as intermediate hosts in life cycle of *E. felidis*. The different features of *E. felidis* are shown in Table 2. Like *E. shiquicus*, there is not enough data about human infection, hydatid feature and intermediate hosts. Therefore the zoonotic potential and public health concern among circulating isolates particularly those concomitant infections should be noticed absolutely.

3.3. Phylogenetic findings in *E. felidis*

Based on sequences of mitochondrial genes for cytochrome c oxidase subunit 1 (cox1), NADH dehydrogenase subunit 1 (nad1), cytochrome b (cob), rRNA (rrn), and nuclear genes for elongation factor 1 alpha (ef1a), ezrin-radixin-moesin (ERM)-like protein (elp) and

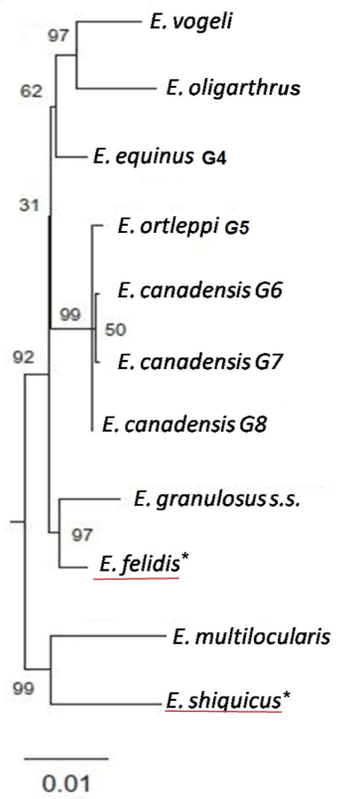

Figure 4. Phylogenetic tree of *Echinococcus* spp. inferred from nuclear protein-coding genes.

Internal transcribed spacer (ITS), *E. felidis* and *E. granulosus* Sensu Stricto are presumed to have a common ancestor from Asian Felidae [20, 26].

Considering the assumption, ancestral lineage of *Panthera leo* in Asia is refereed to late Pliocene [10], which had been invaded to Africa in the early Pleistocene [27]. Phylogenetic trees of *Echinococcus* spp. inferred from mitogenome and nuclear protein-coding genes shown that both *E. felidis* and *E. granulosus* have placed in *E. granulosus* Sensu Stricto complex (genotypes G1, G2 and G3; Figures 4 and 5). In Figure 4 the *E. felidis* and *E. granulosus* are characterized with different branch lengths in a common clade and bootstrapping value of 97%.

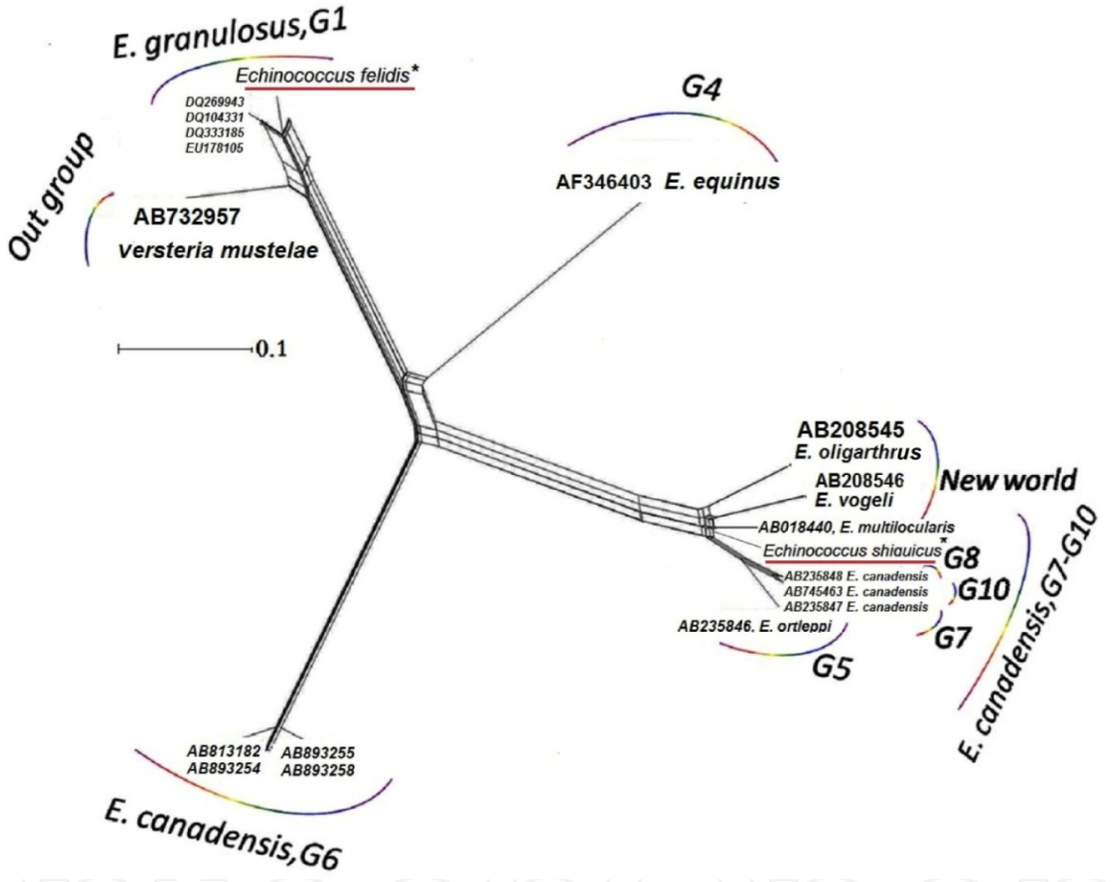

Figure 5. NeighborNet graph according to the Kimura-2 parameter model and concatenated sequences of *Cox1* gene (mitogenome) of *Echinococcus* spp. *Echinococcus felidis* and *Echinococcus shiquicus* have characterized by asterisk (*) and red underline in *E. granulosus* and *E. canadensis* complexes respectively

The tree was reconstructed by the maximum likelihood method and Kimura-2 parameter model. *Echinococcus felidis* and *Echinococcus shiquicus* have characterized by asterisk (*) and red underline.

At this time, there are no valid data on the pathogenicity of *E. felidis* to humans and livestock although its close relationship with *E. granulosus* s.s proposed a zoonotic potential [28].

On the other hand, information about intermediate hosts of this parasite is still unknown, even though a hydatid cyst was identified as *E. felidis* from warthog in Queen Elizabeth National Park, Uganda [29].

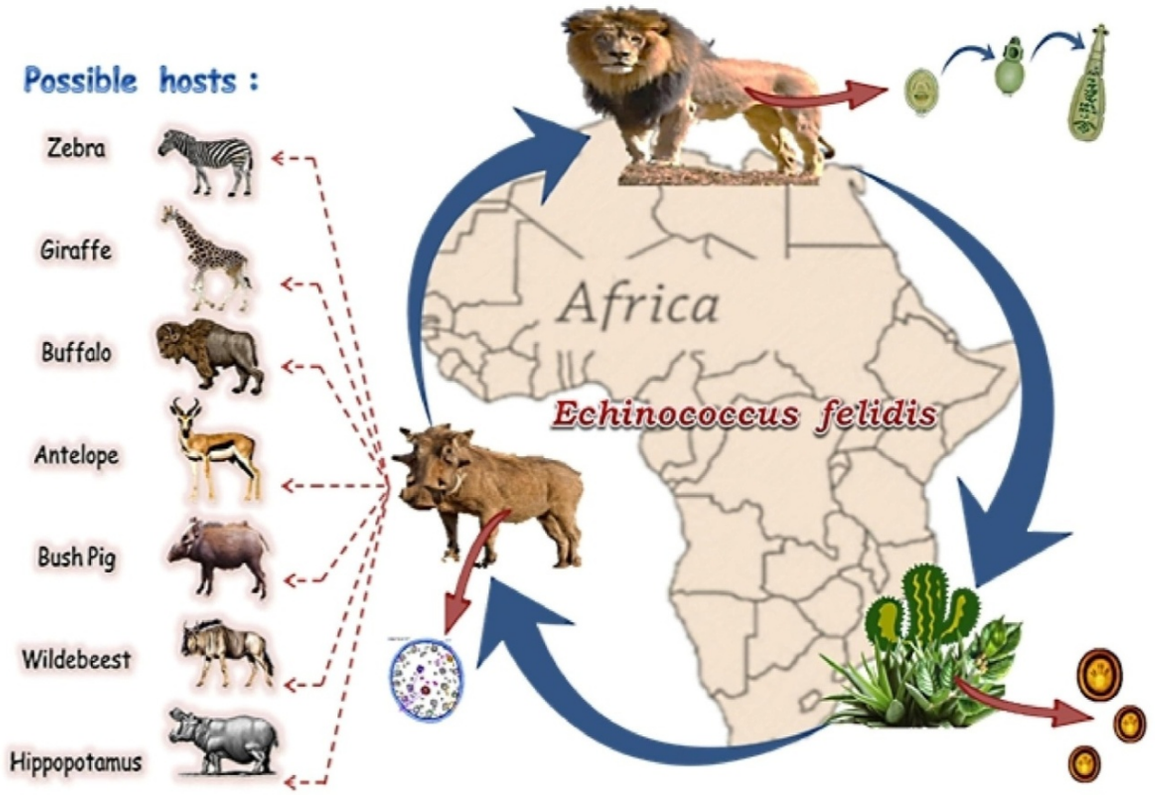

Figure 6. The life cycle of *Echinococcus felidis*. Credit: Image courtesy of Seyyed Ali Shariatzadeh.

4. Conclusion

In the past decade, *E. shiquicus* and *E. felidis* have been indigenously confirmed in some sympatric regions of Tibet plateau and Africa, however, there is no more known about their zoonotic potential, human infection and public health problems. The contents of this chapter can be useful in the parasite history, biology, morphometric aspects, epidemiology, zoonotic potential and phylogeny relationship of mentioned *Echinococcus* which is provided a better understanding of their taxonomic position, public health priority and biological aspects in the regions.

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