Echinococcosis: Recent Advancements in OMIC Technologies

48

Rana Muhammad Athar Ali[1*], Rana Muhammad Mazhar Ali[2], Sadia Ahsan[3], Ghazanfar Hussain[4], Laiba Mateen[2], Ayesha Kabir[2], Hazrat Bilal[1], Jawad Younas[5] and Arooj[2]

1. INTRODUCTION

Cystic echinococcosis (CE) and alveolar echinococcosis (AE), two severe zoonotic tapeworm infections caused by *Echinococcus granulosus sensu lato* and *Echinococcus multilocularis*, respectively, are referred to as echinococcosis (McManus et al. 2012). The yearly occurrence of CE varies from 1 to 200 / 100,000 people in endemic regions, whereas the incidence of AE varies between 0.03 to 1.2 / 100,000 people (Schweiger et al. 2007). Ninety percent of AE patients who receive no treatment or insufficient treatment die within 10 to 15 years after their diagnosis (Budke et al. 2013). Although the CE fatality rate (2% to 4%) is less it might rise significantly in cases of insufficient care management. Echinococcosis is one of the 17 neglected illnesses that the World Health Organization (WHO) hopes to manage or eradicate by 2050. While CE is more prevalent and has a cosmopolitan distribution, several countries have claimed it to be eliminated (Craig and Larrieu 2006; Craig et al. 2007). Still, the disease is distributed all over the world with high prevalence in Asian, American, and African countries as given in Fig. 1 (Wen et al. 2019; Larrieu and Zanini 2012; Cucher et al. 2016; Alvi et al. 2021; Alvi et al. 2022; Alvi et al. 2023a; Alvi et al. 2023b; Alvi et al. 2023c). In fact, significant recent advancements are expected to bring revolution in control and management of CE and AE. However, due to less sensitivity and specificity of current diagnosis tools, side effects and less potency of available medications, the frequently inappropriate surgery, and the difficulties in preventive measures, the discovery of novel therapy and vaccine potential sites is highly needed.

CITATION

Ali RMA, Ali RMM, Ahsan S, Hussain G, Mateen L, Kabir A, Bilal H, Younas J and Arooj, 2023. Echinococcosis: recent advancements in OMIC technologies. In: Khan A, Abbas RZ, Hassan MF, Aguilar-Marcelino L, Saeed NM and Mohsin M (eds), Zoonosis, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. I: 659-669. https://doi.org/10.47278/book.zoon/2023.192

CHAPTER HISTORY Received: 26-March-2023 Revised: 14-April-2023 Accepted: 19-May-2023

[1]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan.
[2]Department of Zoology, Wildlife, and Fisheries, University of Agriculture, Faisalabad, Pakistan.
[3]Department of Zoology, Government College Women University, Sialkot, Pakistan.
[4]Institute of Feed Research, The Graduate School of Chinese Academy of Agricultural Sciences, Beijing, China.
[5]Department of Epidemiology and Public Health, University of Agriculture, Faisalabad, Pakistan.
***Corresponding author:** athar4545@gmail.com

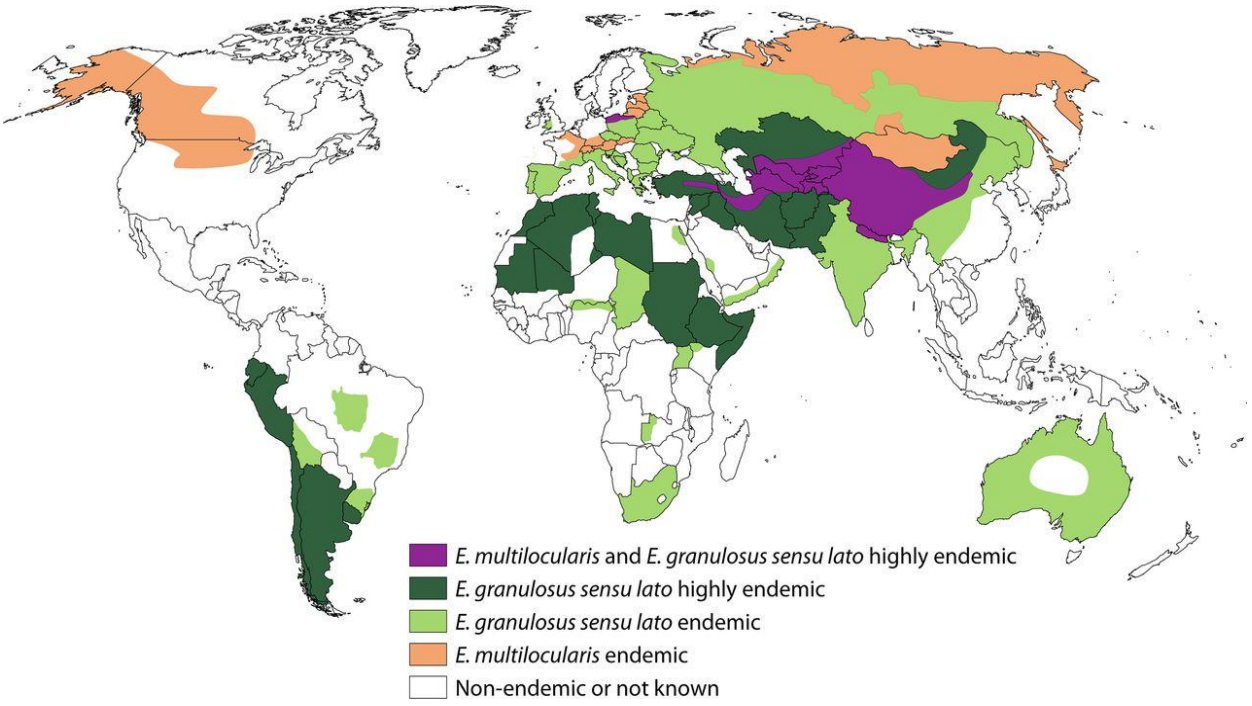

Fig. 1: Worldwide distribution of CE and AE with colors representing their endemicity (Wen et al. 2019)

Our methods for examining biological systems have been significantly transformed by "OMICS," which is defined as exploring and analyzing a significant amount of information that represents the structure and function of the entire composition of a specific biological system at a particular level (Wen et al. 2019; Dai and Shen 2022).

In this chapter, we have discussed how current advancements in genome and transcriptome analysis, are exploring the interaction between a parasite and its host, giving access to the data that can help to prepare new drug interactions and treatments against CE and AE.

2. ENHANCING INFORMATION ON THE DIVERSE LIFECYCLE OF CE AND AE AS WELL AS REVEALING PHENOTYPIC VARIATIONS IN PARASITES

Transcriptomic analysis of various forms of CE has revealed different aspects of biological and parasitological processes (Zheng et al. 2013). Moreover, the current reports on the complete genome of *E. granulosus* as well as *E. multilocularis* (Tsai et al. 2013) have disclosed other important things, linked with parasitism, comprising of details of family domains, obtained during the evolution. Other genes discovered relate to signaling pathway, the neurological and endocrine systems, development and reproduction, as well as other processes that played their part in evasion mechanisms. For a deeper comprehension of cestode biological processes, differentiation, growth and development, evolution, disease, and other host-parasite relationships, genetic and transcriptomic data thus serve as a crucial foundation.

CE and AE life cycles give information about the significance of the parasite's secretory/ excretory products being translated from new genes. The phenotypic changes connected to the various phases of parasite life and the corresponding modifications of the immunity in each host are probably driven by up or down-regulation of gene expression. For the purpose of identifying the specific roles of these genes and searching

for important genes linked to these alterations, comprehensive transcriptome analysis is essential. The protoscoleces have unbelievable potential to convert into a cyst or adult which is a distinguishing characteristic of *E. granulosus* and *E. multilocularis*. A particular host stimulus, controls how a development will proceed (Constantine et al. 1998). 3,900 of the 11,325 genes anticipated to be in the genome of *E. granulosus* (s.s.) could not be assigned a function; of these, 361 genes did not transcribe in adult worms, and among these, 21 showed significant expression and might be related to adult worm growth (Zheng et al. 2013). The eggs from the gravid proglottid excreted into the surroundings to infect intermediate hosts including humans. 55 genes were preferentially expressed in adult *E. granulosus* compared to immature stages, and were among the 361 genes that mRNA transcriptome analysis revealed to be well-expressed in these species. The unlimited asexual development related to the metacestode stage contrasts with the limited sexual development associated with adult worms. Of the 8,361 genes expressed in the two phases, 498 genes were strongly expressed in the adult worm whereas 502 genes were involved in the metacestode (Zheng et al. 2013). Future research using gene deletion methods may reveal their functional properties.

The knowledge of morphology, anatomy, and clinical features between two cestodes will therefore depend heavily on a thorough relevance of genetic structure and transcribed proteins of CE and AE. The form and shape of the metacestode are one of the main differences between the two. The cysts of *E. granulosus* have a distinctive shell-like adventitia that distinguishes them from the hepatic and pulmonary and brain. In contrast, the *E. multilocularis* metacestodes are infiltrating lesion that continuously advances irregularly and harms the liver or other target organs. It is made up of compiled cells as a result of immunological reactions in the form of necrosed or fibrosed tissue.

Using RNA sequencing (RNA-Seq) or microarray technological advances, comparative investigation into differing or conjoining gene pairs and their course of expression can be utilized for recognizing patterns that are exhibited by multiple or specific species. Such gene pair study has shown that both species contain 5418/10,018 genes with strong sequence analogy, despite the fact that research on *Echinococcus* spp. is yet in its beginning. The identification and characterization of nonsimilar/unique genes will be the next step in elucidating the fundamental differences in biology or pathogenesis in two species.

3. ADVANCING ECHINOCOCCOSIS DIAGNOSIS AND TREATMENT

The comprehensive genomic and transcriptome data currently accessible might be helpful for creating new public health treatments against *E. granulosus sensu stricto*, such as enhanced diagnostic procedures and the discovery of fresh therapeutic targets. One-third (n3,903) of the genes in the *E. granulosus* genome have no gene similar in other taxonomic group, according to BLAST sequence analysis. This finding suggests the distinctive nature and biological features of *E. granulosus* genes. The byproducts of these genes might likewise be useful as fresh suspects in echinococcosis diagnostics and as novel medication targets. Certain proteins may be effective as chemotherapeutic targets along with enhanced immunodiagnosis or immunotherapy because they function as chemical mediators for networking between CE and its mammal host (Zheng et al. 2013; Tsai et al. 2013). For example, genes of a germinal layer of the parasite prepare polypeptides and proteins that can be used in the development of vaccines such as GPCRs, MAPK, neuro-peptides, and ion channel (Lu et al. 2016; Lin et al. 2011; Gelmedin et al. 2010; Gelmedin et al. 2008).

Both *E. multilocularis* and *E. granulosus* protoscoleces contain hormone- and cytokine-activated pathways, therefore, it is of the utmost importance that host components activate or deactivate them (Yang et al. 2017; Koziol et al. 2016a; Hemer et al. 2014; Lu et al. 2016; Gelmedin et al. 2008; Gelmedin et al. 2010; Hemer et al. 2014; Zhang et al. 2014; Brehm and Spiliotis 2008; Konrad et al. 2003; Brehm 2010;

Spiliotis et al. 2006; Spiliotis et al. 2005; Zavala-Gongora et al. 2003; Spiliotis et al. 2003). If we compare the genetic structure of both parasites, it will become obvious that parasites have a high level of similarity, revealing that many compounds are produced by both parasites and can be aimed at creating new therapies. A lot of research is being done right now to see whether MAPK inhibitors can kill metacestodes or protoscoleces. ML3403 interacts with the P38-like MAPK in CE and inhibits Egp58 function and causes considerable protoscolex mortality within five days in vitro (Lu et al. 2016). Similar outcomes were bought with *E. multilocularis* specifically SB202190, and ML3403, a different pyridinyl imidazole, investigated on protoscoleces vesicles propagated in vitro resulting in the removal of phosphate group of the parasite's EmMPK2 and ultimately destroy the vesicles at the levels that did not influence grown cells of mammals (Gelmedin et al. 2008).

Various metabolic processes have been investigated as an outcome of the publication of the full genetic structure of CE and AE, along with different inhibitors are now being researched (Siles-Lucas et al. 2018; Joekel et al. 2018; Flo et al. 2017; Koziol et al. 2016b; Schubert et al. 2014; Hemer and Brehm 2012). Nilotinib altered protoscoleces structure of *E. multilocularis* in vitro; but, neither of these drugs prevented the development of the parasite in *E. multilocularis*-infected rats (Joekel et al. 2018). It was discovered that the Polo-like kinase inhibitor, BI2536, inhibited EmPlk1 activity and prevented the development of protoscoleces from cultured *E. multilocularis* inner germinal cells as shown in Fig. 2A and 2B. Additionally, it removed the inner cell growth in laboratory, producing worm tissue that was not able to undergo development (Schubert et al. 2014). Imatinib is an a different ABL inhibitor considered for treatment of cancer. It has been demonstrated to interact with kinases in AE and to be very efficient in eliminating stem cells of *Echinococcus*, vesicles of metacestode, and protoscoleces in vitro (Hemer and Brehm 2012). However, it is yet uncertain if such kinase inhibitors have the ability to cure AE in vivo.

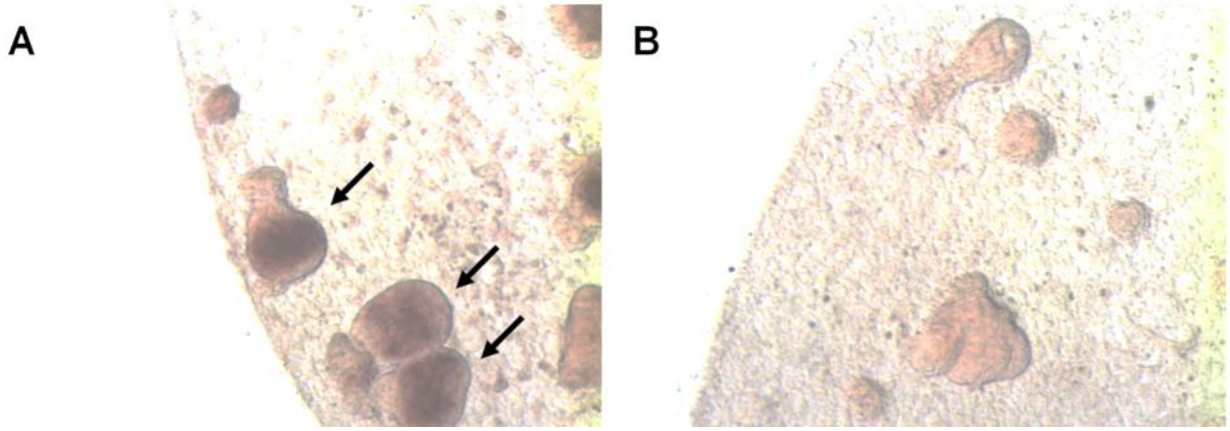

Fig. 2: Protoscoleces are enriched with EmPlk1 as indicated by arrows (A). Cells are stained with EmPlk1 antisense probe (A and B).

4. ENHANCING KNOWLEDGE OF IMMUNOLOGICAL PATHWAYS AT THE PARASITE-HOST INTERFACE TO PREPARE NOVEL THERAPY

Despite of high vulnerability of CE parasite, and particularly of AE cestode, to the defense system cells of the host was known from the last three decades (Vuitton 2003), the majority of a thorough understanding of immunological processes underlying the delicate equilibrium between protection of hosts and growth of parasites has been attained in the twenty-first century (Vuitton and Gottstein 2010; Gottstein et al. 2017). The advancement of genomics in this area has been beneficial by pointing to novel molecular

pathways and potential therapeutic targets. Investigations of the transcriptional patterns seen in the hepatic tissue of rats inoculated with AE parasite and the rat models used with specific gene losses have been essential in this regard (Wang and Gottstein 2016; Wang et al. 2014; Gottstein et al. 2010; Siracusano et al. 2012a). According to recently discovered evidence, immunotherapy may be able to cure echinococcosis in combination with anti-infective medication therapy. On the other hand, deeper comprehension of the defense system of hosts diseased with AE and CE parasites may result in the development of new therapeutic strategies for the treatment of ongoing inflammatory illnesses.

At the later phase of infection in people, there is the majority of helper T cells, comprising of immunoglobulin-E mediated responses and elevated amounts of the cytokine IL-10 (Vuitton 2004). After adventitial fibrous barrier formation, a strong T helper-2 profile is quickly developed in CE (Siracusano et al. 2012a; Tuxun et al. 2018). In AE, the immune system's response advances in three stages, with the initial phases being characterized by a mixed Th1/Th2 profile, the middle stage being distinguished by a dominant Th2/Treg profile, including IL-10 and transforming growth factor (TGF) regulatory cytokines, and the final phase of infection being marked by a T-cell exhaustion status (Gottstein et al. 2017; Zhang et al. 2017). According to clinical investigations on CE, therapeutic sensitivity is linked to a Th1 profile, whereas therapy resistance is linked to a T helper 2 profile (Gottstein et al. 2017; Siracusano et al. 2012b). Certain proteins may be beneficial as targets for enhanced immuno-diagnosis or follow-up of patients because they function as code of communication between the parasites and their hosts as shown in Fig. 3 (Tsai et al. 2013; Zheng et al. 2013).

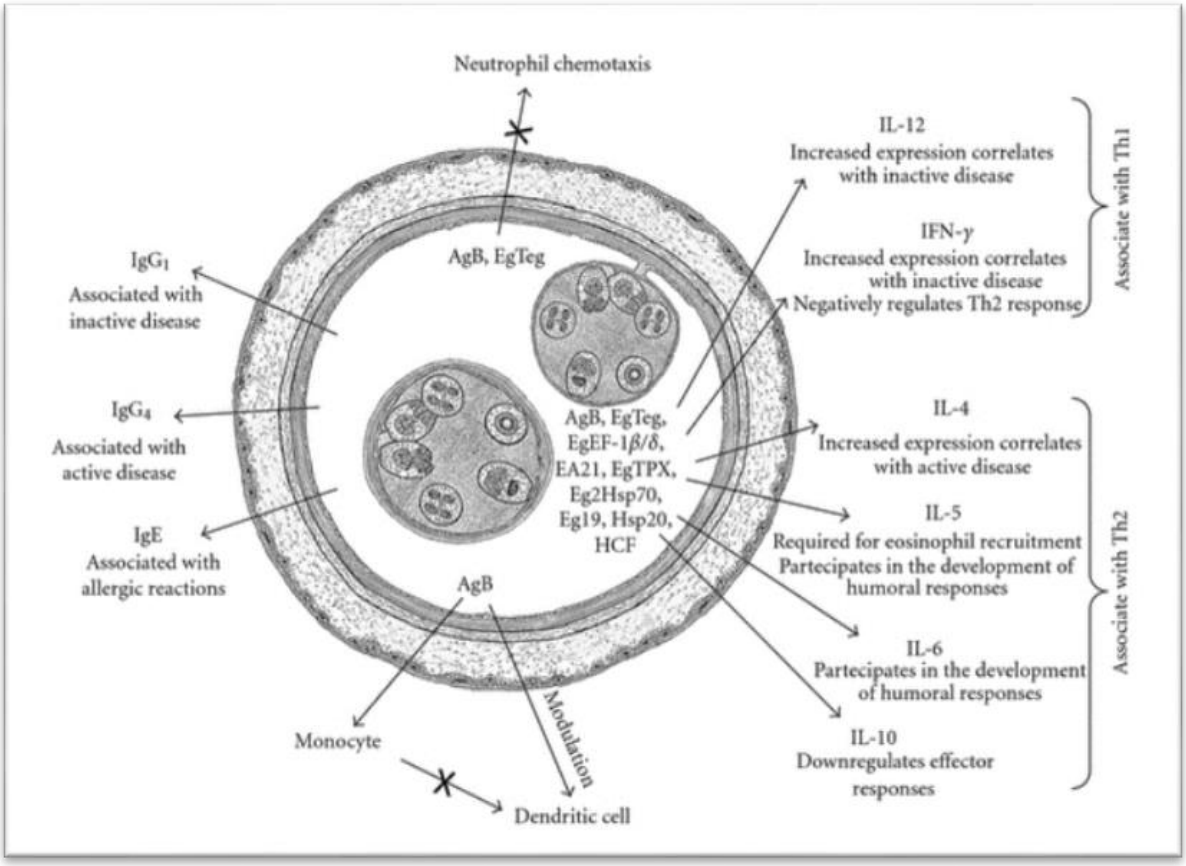

Fig. 3: Major products produced as a response to hydatid cyst components which can be used as drug targets

With effects varying from resistance (self-cure) to quickly increasing host mortality (high vulnerability), the content and kind of feedback produced by CE parasites causally affect the results and course of disease (Wang et al. 2014). In *E. multilocularis*, this feedback as well as the body cells, are strongly influenced by the parasite burden, which may be statistically measured in a laboratory setting including infection by intraportal inoculation of protoscoleces (Zhang et al. 2017). A major element in resistance, according to current investigations, is Th1/Th17 polarization, whereas FoxP3 Tregs are essential to immune regulatory mechanisms that support *E. multilocularis* metacestode survival (Wang et al. 2017). After being infected with *E. granulosus sensu stricto*, mice were treated in vivo with a single IV administration of 200 l recombinant IL-17A at the ideal dose of 125pg/ml. This dropped the infection rate by 2/3 and suppressed metacestode production by over 90 percent (Labsi et al. 2018).

FoxpP3 has the potential to be a useful target in immunotherapy in humans (Wang et al. 2018a). Another strong contender is the PD-1/PD-L1 signaling process, which is important for the onset of Foxp3 CD25 CD4 Tregs, influences IL-10 secretion favorably, prevents growth of effector T cell, and blocks the generation of Th1 cytokines (Liu et al. 2013). In contrast to healthy counterparts, those with CE had higher levels of soluble PD-L1 (Li et al. 2016) and more helper T cells that expressed PD-1 (Zhang et al. 2015; La et al. 2015). A PD-1/PD-L1 engagement blocker has shown promise in early trials (Wang et al. 2018c). Clinicians have access to various PD-1/PD-L1 inhibitors that have already been employed to address cancer (Swaika et al. 2015) for pilot immunotherapeutic studies in AE. Clinicians may also benefit from antiinfective and immunological therapy when treating CE cases that are severe and involve many organs. Attention has been drawn to the unique profile of the chronic phase of CE and AE parasite infections because it is a known tolerance phase that may be employed to lessen the harmful consequences of inflammatory processes in a range of clinical circumstances. While concurrent CE infection decreases inflammation in rodents (Wang et al. 2014), concurrent AE inoculation in the mice postpones refusal of a hepatic tissue allograft (Li et al.2011). In both cases, the outcomes were linked to elevated IL-10 levels in the animals being studied. Though it was also suggested that *Echinococcus* sp. substances would be effective in treating rheumatoid arthritis, this has not yet been proven (Apaer et al. 2016). The observations in the experimental colitis study provide the best support for regulating the immune function of developed *Echinococcus* spp. infection in its intermediate host. Infections with E. *granulosus sensu stricto* (Khelifi et al. 2017) and *E. multilocularis* (Wang et al. 2018b) can both prevent mice from developing dextran sulfate sodium (DSS)-induced colitis (Wang et al. 2018c).

The potential use of non-infectious *Echinococcus* spp. extracts is confirmed by findings provided after treating rodents regularly with extracts from *E. granulosus sensu stricto* laminated layer beginning three days prior to colitis induction. The medication significantly lowered clinical signs and intestinal histological parameters while maintaining mucus production by goblet cells and inducing a sufficient drop in IFN. The change that the immune system of the host may experience as a consequence of the immune system regulation has been extensively researched (Gottstein and Hemphill 2008; Vuitton and Gottstein 2010). This shift may boost metacestode proliferation and subsequently impair host defense. Recent studies identified some immunoregulating products of *Echinococcus* species such as AgB, Eg2, Em2, EmAP, and EgTeg (Siracusano et al. 2012a; Wang and Gottstein 2016).

5. ENHANCING THE DEVELOPMENT OF VACCINE

5.1. INTERMEDIATE HOST VACCINATION

In pilot and field studies, the EG95 antigen vaccination of *E. granulosus* intermediate hosts illustrated a significant protective efficiency, and it is presently being employed in endemic regions of China and South America (Lightowlers and Heath 2004; Heath et al. 2012; Larrieu et al. 2013; Craig et al. 2017; Larrieu et

al. 2015). The infection phase for people, as well as intermediate hosts, is the oncosphere of *Echinococcus*. Antibodies produced by the eg95 (oncosphere-specific) gene provides an elevated defense against egg infection in small and large ruminants (Chow et al. 2004; Heath et al. 2012) and the end results of other genes that are differently produced at this phase likely represent possible additional vaccine candidates. According to gene transcription analysis, Eg95 has significant expression in oncospheres (Zheng et al. 2013), and recent research has found that Eg95 is really a family of 7 different genes. Other products are also encoded by oncosphere genes that are potential targets for vaccination as given in Table 1.

Table 1: Potential targets for vaccines against CE and AE in intermediate hosts expressed in different life forms (oncosphere, mature and protoscoleces).

Gene (ID)	Number in Sequence reading				Gene information
	Oncosphere	Mature	Cyst	Protoscoleces	
Eg_05614	806	2	0	0	Eg95
Eg_08805	481	2	0	0	Eg95
Eg_10541	266	4	5	1	Eg95
Eg_06928	185	1	0	0	Eg95
Eg_11122	30	0	0	0	Eg95
Eg_06751	27	2	0	0	Eg95
Eg_10281	24	79	12	12	Eg95
Eg_08721	108	6	0	0	Serine protease inhibitor
Eg_06806	209	1733	83	21	Antigen B3
Eg_05439	329	2	0	1	Hypothetical protein
Eg_09040	133	16	0	0	Hypothetical protein
Eg_07993	554	3	0	0	Diagnostic antigen gp50
Eg_00010	533	1	0	0	Host-protective antigen
Eg_04657	98	18	50	30	Reticulon-4
Eg_08098	222	1	0	0	Gli pathogenesis-related 1
Eg_05449	66	0	23	12	Hypothetical protein
Eg_04921	65	1	0	0	Hypothetical protein
Eg_04940	125	2	1	13	Novel hemicentin protein
Eg_05345	55	48	42	11	Proteasome (macropain) beta 1
Eg_00715	33	146	154	69	Tetraspanin 1-TSP6
Eg_07633	2700	2	8	1	Hypothetical protein
Eg_03592	61	64	36	24	Low-density lipoprotein receptor
Eg_00394	72	0	0	0	e74-like factor 2

Additionally, gene transcription analysis revealed that in contrast to the mature and cystic phases of *E. granulosus*, 340 (out of 3,811) genes had been significantly increased in oncospheres (Zheng et al. 2013) and 2% (74/3,811) of the genes transcribed in the oncosphere are secreted proteins that probably play a crucial role in the hatching oncosphere's passing through the mammalian intestine and in the growth of the oncosphere itself.

5.2. DEFINITIVE HOST VACCINATION

A step in integrated echinococcosis control that protects dogs from mature *Echinococcus* spp. infection would be extremely desired. There isn't presently a vaccination for this condition. In the canine gut, the protoscolex stage gives rise to a mature worm. In the protoscoleces or in the mature, proteins of genes that are significantly transcribed may offer promising vaccination candidates against adult parasites in the

target host. When dogs were vaccinated with egM gene and a necropsy was performed forty-five days after inoculation, they were found to have good immunity against parasites (Zhang et al. 2006; Zhang et al. 2018). These substances might be linked to adult worm growth and/or egg development. Mature *Echinococcus* parasites are found in the center of their definitive hosts' small intestines, which contain high concentrations of trypsin and trypsin-related enzymes as well as an extensive amount of nutrients, including amino acids. It is probable that the worms serve a crucial protective function in avoiding proteolytic enzyme assault and preserving the survival of *E. granulosus* inside its definitive hosts by secreting specific inhibitors that neutralize the potentially harmful outcomes of host GIT enzymes. These presumably indicate additional vaccination options that require further investigation, along with molecular receptors for neurotransmitters and transporters, as well as other inhibitions of protease that are specifically translated in adult worms (Zheng et al. 2013; Ranasinghe et al. 2015; Behrendt et al.2016; Cuesta-Astroz et al. 2017; Morais et al. 2018).

6. CONCLUSION

Due to novel proteomics data, the entire sequencing of the CE ad AE genomes, and improved knowledge of interactions between hosts and parasites for AE and CE, novel pharmacological or immunological treatment sites have been discovered. While a precise understanding of the pathogenic species/genotypes can assist healthcare organizations better concentrate and maximize the efficacy of control initiatives, limiting the spread of *Echinococcus* spp. remains a significant challenge. However, significant advancements in molecular assays to identify *Echinococcus* spp. in specific hosts and the environment has made CE ad AE control program easier to understand.

REFERENCES

Alvi et al., 2021. Veterinary Pathobiology & Public Health: Introduction to echinococcosis and a review of treatment panels. 1st ed.; Unique Scientific Publishers: Faisalabad, Pakistan, 128-143.

Alvi MA et al., 2022. Herbal medicines against hydatid disease: A systematic review (2000–2021). Life 12: 676.

Alvi MA et al., 2023a. Revealing novel *cytb* and *nad5* genes-based population diversity and benzimidazole resistance in *Echinococcus granulosus* of bovine origin. Frontiers in Veterinary Science 10: 1191271.

Alvi MA et al., 2023b. Phylogeny and population structure of *Echinococcus granulosus* (*sensu stricto*) based on full-length cytb-nad2-atp6 mitochondrial genes–First report from Sialkot District of Pakistan. Molecular and Biochemical Parasitology 253: 111542.

Alvi MA et al., 2023c. Past and Present of Diagnosis of Echinococcosis: A Review (1999-2021). Acta Tropica 106925.

Apaer S et al., 2016. Parasitic infection as a potential therapeutic tool against rheumatoid arthritis. Experimental and Therapeutic Medicine 12:2359–2366.

Behrendt P et al., 2016. A helminth protease inhibitor modulates the lipopolysaccharide-induced proinflammatory phenotype of microglia in vitro. Neuroimmunomodulation 23:109–121.

Brehm K and Spiliotis M, 2008. The influence of host hormones and cytokines on *Echinococcus multilocularis* signalling and development. Parasite 15:286–290.

Brehm K, 2010. The role of evolutionarily conserved signalling systems in *Echinococcus multilocularis* development and host-parasite interaction. Medical Microbiology and Immunology 199:247–259

Budke CM et al., 2013. A systematic review of the literature on cystic echinococcosis frequency worldwide and its associated clinical manifestations. American Journal of Tropical Medicine and Hygiene 88:1011–1027.

Chow C et al., 2004. *Echinococcus granulosus*: oncosphere-specific transcription of genes encoding a host-protective antigen. Experimental Parasitology 106:183–186

Constantine CC et al., 1998. Factors influencing the development and carbohydrate metabolism of *Echinococcus granulosus* in dogs. Journal of Parasitology 84:873–881.

Craig PS et al., 2017. Echinococcosis: control and prevention. Advances in Parasitology 96:55–158.

Craig PS and Larrieu E, 2006. Control of cystic echinococcosis/hydatidosis: 1863-2002. Advances in Parasitology 61:443–508.

Craig PS et al., 2007. Prevention and control of cystic echinococcosis. The Lancet Infectious Diseases 7:385–394.

Cucher MA et al., 2016. Cystic echinococcosis in South America: systematic review of species and genotypes of *Echinococcus granulosus sensu lato* in humans and natural domestic hosts. Tropical Medicine and International Health 21:166–175.

Cuesta-Astroz Y et al., 2017. Helminth secretomes reflect different lifestyles and parasitized hosts. International Journal of Parasitology 47:529–544.

Dai X and Shen L, 2022. Advances and trends in omics technology development. Frontiers in Medicine 9: 911861.

Flo M et al., 2017. Functional diversity of secreted cestode Kunitz proteins: inhibition of serine peptidases and blockade of cation channels. PLoS Pathogens 13:e1006169.

Gelmedin V et al., 2008. Characterization and inhibition of a p38-like mitogen-activated protein kinase (MAPK) from *Echinococcus multilocularis*: antiparasitic activities of p38 MAPK inhibitors. Biochemical Pharmacology 76:1068–1081

Gelmedin V et al., 2010. Molecular characterisation of MEK1/2- and MKK3/6-like mitogen-activated protein kinase kinases (MAPKK) from the fox tapeworm *Echinococcus multilocularis*. International Journal of Parasitology 40:555–567

Gottstein B and Hemphill A, 2008. *Echinococcus multilocularis*: the parasite host interplay. Experimental Parasitology 119:447–452

Gottstein B et al., 2017. Immunology of alveolar and cystic echinococcosis (AE and CE). Advances in Parasitology 96:1–54

Gottstein B et al., 2010. Hepatic gene expression profile in mice perorally infected with *Echinococcus multilocularis* eggs. PLoS One 5:e9779

Heath DD et al., 2012. Vaccination of bovines against *Echinococcus granulosus* (cystic echinococcosis). Vaccine 30:3076–3081.

Hemer S and Brehm K, 2012. In vitro efficacy of the anticancer drug imatinib on *Echinococcus multilocularis* larvae. International Journal of Antimicrobial Agents 40:458–462.

Hemer S et al., 2014. Host insulin stimulates *Echinococcus multilocularis* insulin signalling pathways and larval development. BMC Biology 12:5

Joekel DE et al., 2018. Evaluation of kinase-inhibitors nilotinib and everolimus against alveolar echinococcosis in vitro and in a mouse model. Experimental Parasitology 188:65–72

Khelifi L et al., 2017. Immune-protective effect of echinococcosis on colitis experimental model is dependent of down regulation of TNF-alpha and NO production. Acta Tropica 166:7–15.

Konrad C et al., 2003. Identification and molecular characterisation of a gene encoding a member of the insulin receptor family in *Echinococcus multilocularis*. International Journal of Parasitology 33:301–312

Koziol U et al., 2016a. Comparative analysis of Wnt expression identifies a highly conserved developmental transition in flatworms. BMC Biology 14:10.

Koziol U et al., 2016b. De novo discovery of neuropeptides in the genomes of parasitic flatworms using a novel comparative approach. International Journal Parasitology 46:709–721.

La X et al., 2015. Upregulation of PD-1 on CD4 CD25 T cells is associated with immunosuppression in liver of mice infected with *Echinococcus multilocularis*. International Immunopharmacology 26:357–366

Labsi M et al., 2018. In vivo treatment with IL-17A attenuates hydatid cyst growth and liver fibrogenesis in an experimental model of echinococcosis. Acta Tropica 181: 6–10

Larrieu E and Zanini F, 2012. Critical analysis of cystic echinococcosis control programs and praziquantel use in South America, 1974-2010. Revista Panamericana de Salud Publica 31:81–87.

Larrieu E et al., 2013. Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: early impact and preliminary data. Acta Tropica 127: 143–151

Larrieu E et al., 2015. Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: second study of impact. PLoS Neglected Tropical Diseases 9: e0004134.

Li T et al., 2011. Suppression of acute rejective response following orthotopic liver transplantation in experimental rats infected with *Echinococcus multilocularis*. Chinese Medical Journal (Engl) 124:2818–2823.

Li Y et al., 2016. Role of soluble programmed death-1 (sPD-1) and sPD-ligand 1 in patients with cystic echinococcosis. Experimental and Therapeutic Medicine 11:251–256.

Lightowlers MW and Heath DD, 2004. Immunity and vaccine control of *Echinococcus granulosus* infection in animal intermediate hosts. Parassitologia 46:27–31.

Liu H et al., 2013. PD-L1 signal on liver dendritic cells is critical for Foxp3() CD4 CD25 Treg and liver tolerance induction in mice. Transplantation Proceedings 45:1853–1855

Lu G et al., 2016. Molecular cloning and characterization of a P38-like mitogen activated protein kinase from *Echinococcus granulosus*. Korean Journal of Parasitology 54:759–768

McManus DP et al., 2012. Diagnosis, treatment, and management of echinococcosis. British Medical Journal 344:e3866.

Morais SB et al., 2018. *Schistosoma mansoni* SmKI-1 serine protease inhibitor binds to elastase and impairs neutrophil function and inflammation. PLoS Pathogens 14:e1006870

Ranasinghe SL et al., 2015. Cloning and characterization of two potent Kunitz type protease inhibitors from *Echinococcus granulosus*. PLoS Neglected Tropical Diseases 9:e0004268.

Schubert A et al., 2014. Targeting *Echinococcus multilocularis* stem cells by inhibition of the Polo-like kinase EmPlk1. PLoS Neglected Tropical Diseases 8:e2870

Schweiger A et al., 2007. Human alveolar echinococcosis after fox population increase, Switzerland. Emerging Infectious Diseases 13:878–882.

Siles-Lucas M et al., 2018. Progress in the pharmacological treatment of human cystic and alveolar echinococcosis: compounds and therapeutic targets. PLoS Neglected Tropical Diseases 12:e0006422

Siracusano A et al., 2012a. Cystic echinococcosis: aspects of immune response, immunopathogenesis and immune evasion from the human host. Endocrine, Metabolic and Immune Disorders Drug Targets 12:16–23

Siracusano A et al., 2012b. Host-parasite relationship in cystic echinococcosis: an evolving story. Clinical and Developmental Immunology 2012:639362

Spiliotis M et al., 2006. Characterisation of EmMPK1, an ERK-like MAP kinase from *Echinococcus multilocularis* which is activated in response to human epidermal growth factor. International Journal of Parasitology 36:1097–1112

Spiliotis M et al., 2003. Identification, molecular characterization and expression of the gene encoding the epidermal growth factor receptor orthologue from the fox-tapeworm *Echinococcus multilocularis*. Gene 323:57–65

Spiliotis M et al., 2005. Molecular cloning and characterization of Ras- and Raf-homologues from the fox-tapeworm *Echinococcus multilocularis*. Molecular and Biochemical Parasitology 139: 225–237

Swaika A et al., 2015. Current state of anti-PD-L1 and anti-PD-1 agents in cancer therapy. Molecular Immunology 67:4–17.

Tsai IJ et al., 2013. The genomes of four tapeworm species reveal adaptations to parasitism. Nature 496:57–63.

Tuxun T et al., 2018. Plasma IL-23 and IL-5 as surrogate markers of lesion metabolic activity in patients with hepatic alveolar echinococcosis. Scientific Reports 8:4417.

Vuitton DA and Gottstein B, 2010. *Echinococcus multilocularis* and its intermediate host: a model of parasite-host interplay. Journal of Biomedicine and Biotechnology 2010:923193

Vuitton DA, 2003. The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? Acta Tropica 85:119–132

Vuitton DA, 2004. Echinococcosis and allergy. Clinical Reviews in Allergy and Immunology 26:93–104

Wang H et al., 2014. *Echinococcus granulosus* infection reduces airway inflammation of mice likely through enhancing IL-10 and down-regulation of IL-5 and IL-17A. Parasite and Vectors 7:522

Wang J et al., 2014. Transcriptional profiles of cytokine/ chemokine factors of immune cell-homing to the parasitic lesions: a comprehensive one-year course study in the liver of *E. multilocularis* infected mice. PLoS One 9:e91638

Wang J and Gottstein B, 2016. Immunoregulation in larval *Echinococcus multilocularis* infection. Parasite Immunology 38:182–192.

Wang J et al., 2018a. Foxp3 Tregs as a potential target for immunotherapy against primary infection with *Echinococcus multilocularis* eggs. Infection and Immunity 86:e00542-18

Wang J et al., 2018b. Larval *Echinococcus multilocularis* infection reduces dextran sulphate sodium-induced colitis in mice by attenuating T helper type 1/type 17-mediated immune reactions. Immunology 154:76–88

Wang J et al., 2018c. Immunotherapy of alveolar echinococcosis via PD-1/PD-L1 immune checkpoint blockade in mice. Parasite Immunology 40:e12596

Wang J et al., 2017. Depletion of FoxP3() Tregs improves control of larval *Echinococcus multilocularis* infection by promoting co-stimulation and Th1/17 immunity. Immunity Inflammation and Disease 5:435–447

Wen H et al., 2019. Echinococcosis: advances in the 21st century. Clinical Microbiology Reviews, 32: 10-1128.

Yang M et al., 2017. Cloning and characterization of an *Echinococcus granulosus* ecdysteroid hormone nuclear receptor HR3-like gene. Parasite 24:36

Zavala-Gongora R et al., 2003. Identification and characterisation of two distinct Smad proteins from the fox-tapeworm *Echinococcus multilocularis*. International Journal of Parasitology 33:1665–1677.

Zhang W et al., 2006. Vaccination of dogs against *Echinococcus granulosus*, the cause of cystic hydatid disease in humans. Journal of Infectious Diseases 194:966–974.

Zhang C et al., 2014. Identification and characterization of functional Smad8 and Smad4 homologues from *Echinococcus granulosus*. Parasitology Research 113:3745–3757

Zhang F et al., 2015. CCR7(lo) PD-1(hi) CXCR5 CD4 T cells are positively correlated with levels of IL-21 in active and transitional cystic echinococcosis patients. BMC Infectious Diseases 15:45.

Zhang C et al., 2017. T-cell tolerance and exhaustion in the clearance of *Echinococcus multilocularis*: role of inoculum size in a quantitative hepatic experimental model. Scientific Reports 7:11153.

Zhang ZZ et al., 2018. Dog vaccination with EgM proteins against *Echinococcus granulosus*. Infectious Diseases of Poverty 7:61.

Zheng H et al., 2013. The genome of the hydatid tapeworm *Echinococcus granulosus*. Nature Genetics 45:1168–1175.