Int. J. Morphol.,
40(1):115-121, 2022.

Exosomes and Cystic Echinococcosis. Systematic Review

Exosomas y Equinococosis Quística. Revisión Sistemática

Carlos Manterola[1,2] & Pamela Leal[1,3,4]

MANTEROLA, C. & LEAL, P. Exosomes and cystic Echinococcosis. Systematic review. *Int. J. Morphol., 40(1)*:115-121, 2022.

SUMMARY: Exosomes are small and single-membrane secreted organelles, up to 200 nm in diameter that have the same topology as the cell, but are enriched in proteins, nucleic acids, lipids, and glycoconjugates. It can be found in any type of body fluids such as plasma, urine, saliva, sperm, bile, etc.On the other hand, cystic Echinococcosis (CE) has been studied from different points of view, among others, from genomics and proteomics, and the presence of CE exosomes in humans and other intermediate hosts has been reported in very few articles.The aim of this review was to report the evidence available regarding exosomes and CE. Systematic review. Data sources: Trip Database, SciELO, WoS, MEDLINE, EMBASE and SCOPUS. Eligibility criteria: Studies related to CEin any type of host and state of the parasite, without language restriction, published between 1966-2021. Variables: Year of publication, geographical origin, species isolated, location of exosomes. Forty-two studies were initially identified. After scrutinizing titles and abstracts, checking duplications and in-depth analysis of the studies selected, 12 articles including human, bovine, sheep, dog samples were also included. All were case reports or case series. The highest proportion of articles was published between 2019 and 2021 (58.3 %). Publications were predominantly from China (58.3 %). Evidence about exosomes and CE is scarce and reduced range of articles and cases.

KEY WORDS: Exosomes; Extracellular Vesicles; Echinococcosis; Hydatid cyst; Hydatidosis; Cystic Echinococcosis.

INTRODUCTION

Echinococcosis, caused by different species of Echinococcus spp., has been defined as a "neglected disease" (WHO Informal Working Group, 2003; Booth, 2018); being cystic Echinococcosis (CE), one of the most frequent due to, among others, the health burden and the health and economic costs involved (Kern *et al*., 2017).

The development of molecular biology techniques and the knowledge of the E. granulosus genome (Zheng *et al*., 2013), have allowed the identification of new species and genotypes within the genus Echinococcus, which has increased the taxonomic understanding and geographic distribution and by hosts of this parasitosis (Manterola *et al*., 2020, 2021). The same has happened with proteomics and metabolomics (Zhang *et al*., 2014; Wen *et al*., 2019). In this sense, progress has been made in the description of extracellular vesicles in the larval stage of E. granulosus (Nicolao *et al*., 2019), as well as exosomes obtained from sheep CE and human plasma (Siles-Lucas *et al*., 2017; Fratini *et al*., 2020).

A series of proteins have been identified in CE samples, some associated with immunological processes, gluconeogenesis, glycogenolysis and glycogenesis. On the other hand, the release of exosomes to the hydatid fluid by the protoescolex and germ layer has been documented; structures in which virulence factors associated with cyst survival have been identified (Manterola *et al*., 2019).

Exosomes are small organelles secreted by cells, composed of a single membrane ~30 to ~200 nm in diameter, that have the same topology as the cell and are enriched in proteins, lipids, nucleic acids, and glycol conjugates (Pegtel & Gould, 2019; Kalluri & LeBleu, 2020). They contain several membrane-associated oligomeric protein complexes, have pronounced molecular heterogeneity, and are produced

[1] PhD program in Medical Sciences, Universidad de La Frontera, Chile.
[2] Center of Excellence in Morphological and Surgical Studies (CEMyQ), Universidad de La Frontera, Chile.
[3] Center of Excellence in Translational Medicine (CEMET) & Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Chile.
[4] Department of Agricultural Sciences and Natural Resources, Faculty of Agricultural and Forestry Science, Universidad de La Frontera, Chile.
Funding Source: This study was partially financed by project CONICYT MEC 80190026 and DIUFRO DI19-0030.

Received: 2021-10-04 Accepted: 2021-11-09

by budding in plasma and endosome membranes (Kalluri & LeBleu).

Exosome biogenesis is a protein quality control mechanism. Once released, they have activities as diverse as remodeling the extracellular matrix and transmitting signals and molecules to other cells. This intercellular vesicle trafficking pathway plays an important role in various aspects of human health and disease, including development, immunity, tissue homeostasis, cancer, and neurodegenerative diseases (Kalluri & LeBleu).

It has been widely reported that cells subjected to any type of stress generate greater release of exosomes (Marcilla *et al*., 2014). Furthermore, being able to use exosomes for cell-cell communication, under physiological or pathological conditions, has been described as a true biological advantage (Mathivanan *et al*., 2010).

Similar structures are extracellular vesicles: small membrane bounded vesicles formed by invagination of endocytic compartments generating multivesicular bodies and releasing to the extracellular space after fusion of it with plasmatic membrane (Riaz & Cheng, 2017).

On the other hand, viruses co-opt the biogenesis pathways of exosomes both to assemble infectious particles and to establish host permissiveness. Based on these and other properties, exosomes are being developed as therapeutic agents in multiple disease models (Pegtel & Gould).

The aim of this study was to inform the available evidence regarding exosomes and CE.

MATERIAL AND METHOD

This systematic review was carried out following the guidelines of MOOSE declaration (Meta-analysis of Observational Studies in Epidemiology) (Stroup *et al*., 2000).

Design: Systematic Review (SR).

Eligibility criteria: Studies related to CE, published between 1966 and 2021, without restriction of language, species or parasite stage from which study samples were obtained, were included. Articles not related to the objective, narrative reviews, consensus documents and discussion articles were excluded. The closing of the search for articles was on July 31, 2021.

Information sources: The following metasearch engines, libraries and databases were reviewed: Trip Database, SciELO, Web of Science, MEDLINE, EMBASE and SCOPUS. The deadline for the search and recruitment of articles was June 30, 2021.

Search criteria: This was carried out using the PECO components (population study [P], exposure [E], comparator [C] and result [O]).Studies related to CE OR hydatidosis OR hydatid cyst(P), in which exosomes or extracellular vesicles was analyzed (E), without comparators (C), in which proteomic analysis was realized (O), were searched. Sensitive searches were carried out using MeSH terms, free words, and Boolean connectors (AND and OR), with strategies adapted to each database (Table I). A manual and cross-reference search was also carried out.

Study selection: Identified documents in each information source were filtered by duplication between databases. They were subsequently examined by title and abstract, applying eligibility criteria. The articles were then extensively analyzed by the reviewers (CM, PL, TO), all of them experienced in searching and analyzing biomedical studies. Discrepancy situations were resolved by consensus.

Data collection: Critical review of each selected article, as well as the data extraction and its subsequent verification, was carried out by the researchers (CM and PL). Then, data was collected in an Excel spreadsheet (Mac Excel, version 15.24; 2016 Microsoft Corporation®).A list of excluded references including justification are summarize in Table II.

Table I. Search strategies and results Obtained for each source of information used.

Meta search engines, libraries, and databases	Search strategies
Trip Database (n=0)	(echinococcosis OR hydatidosis OR hydatid cyst) AND (exosome OR exosomes)
SciELO (n=1)	(Exosome) AND (Echinococcosis OR Hydatidosis OR Hydatid cyst)
WoS (n=11)	Exosomes (Topic) AND echinococcosis (Topic)
MEDLINE (n=11)	(("Exosomes"[Mesh]) OR "Extracellular Vesicles"[Mesh]) AND ("Echinococcosis"[Mesh] OR hydatid cyst OR hydatidosis" OR cystic echinococcosis))
EMBASE (n=2)	exosome:ab, ti AND echinococcosis: ab, ti
SCOPUS (n=17)	(TITLE-ABS-KEY (exosome) AND TITLE-ABS-KEY (echinococcosis) OR TITLE-ABS-KEY (cystic AND echinococcosis))

Table II. Characteristics of excluded studies. (N = 10).

Author	Publication year	Origin of the study	Samples used	Exclusion criteria
Ancarola	2020	Argentina	Metacestodes	*E. multilocularis* *
Ding	2019	China	Sera	*E. multilocularis* *
Macchiaroli	2021	Argentina	Metacestodes	*E. multilocularis* *
Zheng	2013	China	Metacestodes	*E. multilocularis* *

Outcomes: Year of publication, geographical origin of the studies, number of samples, host, and parasitic stage from which the study samples were taken were considered.

Statistics: Descriptive statistics was applied with calculation of percentages and averages.

Additional analyzes: No meta-analysis was performed as it was a qualitative review.

Risk of bias in individual studies: Assessment of confounding, quality and heterogeneity was not done.

Ethics: To reduce possible biases in the selection and analysis, masking of authors and study centers was implemented, coding and masking the primary articles and deleting the names of authors and centers.

RESULTS

Study selection: A total of 42 studies were retrieved from the search conducted. Of these, 19 were eliminated due to duplication of information sources. Therefore, 23 articles were examined by title and abstract, 9 of which were discarded because they were considered "not related" to the research; leaving 14 articles to evaluate eligibility by reading the full text. An in-depth analysis of the selected studies was then performed, leading to the exclusion of 2 studies based on the review criteria. Finally, 12 studies correspond to the study sample of this review (Virginio *et al*., 2012; Cui *et al*., 2013; Wang *et al*., 2015; Santos *et al*., 2016; Fratini *et al*.; Nicolao *et al*.; Siles-Lucas *et al*.; Wang *et al*., 2019; Zhou *et al*., 2019; Zhang *et al*., 2020; Wu *et al*., 2021; Yang *et al*., 2021) (Fig. 1).

Study characteristics: All the studies analyzed were reports or case series representing more than 200 samplesfrom human and animal samples.

Synthesis of results: The highest proportion of articles was published during the period 2019-2021 (58.3 %). The country with the highest number of publications was China (58.3 %). The samples used (mostly hydatid fluid and protoescolex) came mainly from infected humans and sheep (33.3 % and 25.0 %, respectively) (Table III).

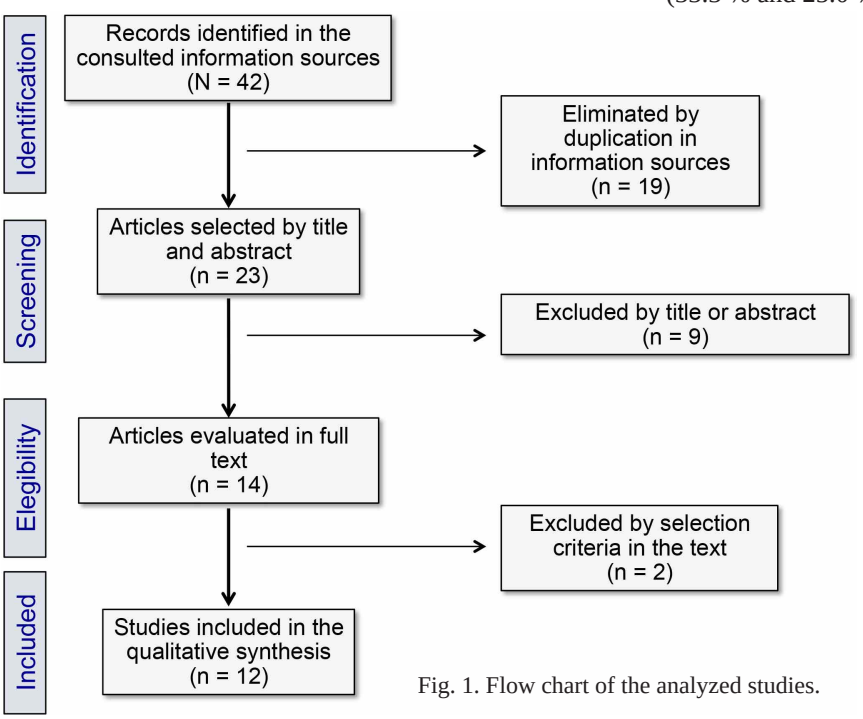

Fig. 1. Flow chart of the analyzed studies.

The studies could be summarized in the following lines of work: proteomics of plasma exosomes in humans with CE with active and inactive infection (Cui *et al*.; Wang *et al*., 2019; Fratini *et al*.); isolation and characterization of exosomes of hydatid cysts from intermediate hosts (Santos *et al*.; Siles-Luca *et al*.); noncoding RNA Profiles of Protoscolex Exosome-Like Vesicles and CE Fluid (Zhang *et al*., 2020).

Proteomics analysis was conducted with different techniques, highlighting mass spectrometry (58.3 %) and immunoblot (41.7 %).

Different proteins were detected in the primary studies (Table III).

Table III. Descriptive information for each study included. (N = 12)

Author	Publication year	Study origin	Hosts	Samples used	No. samples	No. proteins detected
Cui	2013	China	Human/dog	Larval / adults	?	?
Fratini	2020	Italy	Human	Plasma	25	202
Nicolao	2019	Argentina	In vitro	PS / metacestodes	5	298
Santos	2016	Brazil	Bovine	LH	85	498
Siles-Lucas	2017	Spain	Sheep	Cysts	?	?
Virginio	2012	Brazil	In vitro	PS	6	32
Wang	2015	China	Dog	Adult worms	40	9
Wang	2019	China	Human	Serum	3	?
Wu	2021	China	In vitro	PS	10	901
Yang	2021	China	Sheep	HF	15	581
Zhang	2020	China	Sheep	HF / PS	10	?
Zhou	2019	China	Human	HF	3	1026

HF: Hydatid fluid. PS: Protoescolex. ?: Unknown.

Possible biases in the review process: Additional information was requested from the authors to expand or verify some study data, unfortunately, with no response. Therefore, missing data may introduce bias in this review.

Risk of bias between studies: There may be publication bias, given the concentration of studies from China, and the paucity of studies from other geographic areas, places, and countries.

Publication bias was minimized by using different information sources (6 databases, libraries, and engine search) and rigorous search strategies in each. Information bias was minimized by data extracting and interpretation by different observers.

No exclusion for language was used, and no assessment of quality were applied due to the type of studies.

Summary of the evidence: This SR is based on the available evidence of exosomes in CE with histological or microbiological confirmation (Fig. 2). The information was recovered from systematic searches carried out in 6 information sources (Metasearch engines, libraries, and databases), including the last 10 years, and represents a compilation of 36 primary articles that represent slightly more than 200 human and animal samples, treated in different countries, but preferably in China. There are no other related SRs.

DISCUSSION

Exosomes and other extracellular vesicles may be used by parasite and hosts in the outcome of an infection. It can function by transmitting signals between parasites, from parasite to host, or from host to environment for antigen presentation and other aspects of host defense (Coakley *et al*., 2015).

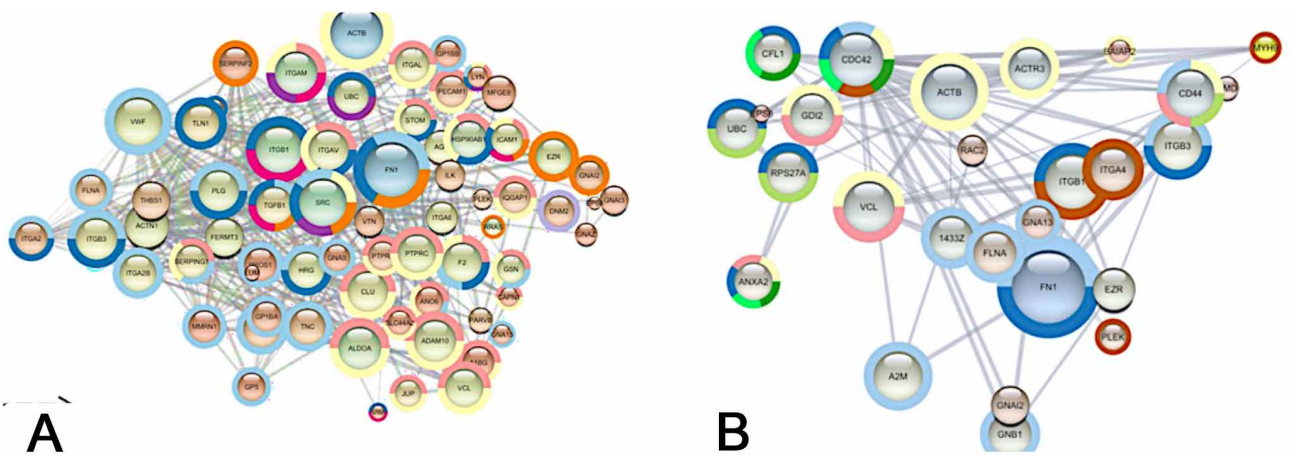

Fig. 2. Exosomes from human samples of CE (adapted from Fratini *et al*., 2020). A) Red Lyn-Src, TGF-b in active CE. B) Red Cdc42/ Racin inactive CE.

Regarding the observed data, it is noteworthy that 70 % of the articles were published in the last three years, which may be associated with the permanent and progressive development of CE research.

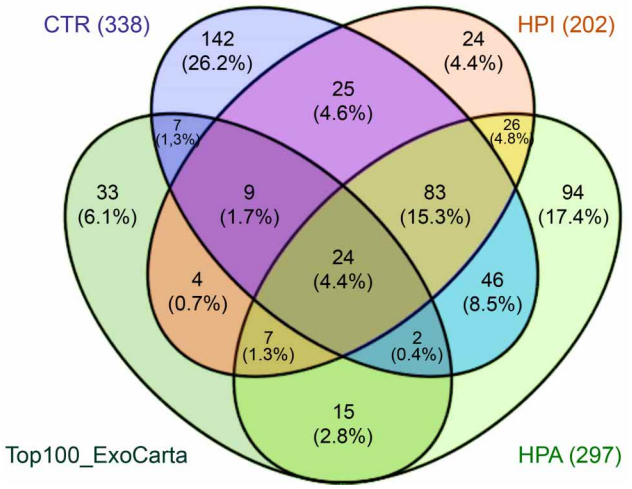

Fig. 3. Venn diagram sum up results of protein data sets (adapted from Fratini *et al*., 2020). HPA: Proteins from exosomes of humans with active CE;HPI: proteins extracted from exosomes of humans with inactive CE; CTR: control; Top100_ExoCarta:100 most usuallyidentified proteins in exosome proteomics studies.

On the other hand, these parasitic worms release some components such as microRNAs and extracellular vesicles as mediators which participate in host-parasite interaction, immune regulation/evasion, and governing processes associated with host infection (Mu *et al.*, 2021).

Even though studies of helminths extracellular vesicles have expanded our knowledge of host-pathogen interaction and for roles of this vesicles in biogenesis and pathogenesis of helminths. Characterizing extracellular vesicles functions of may result in the identification of new biomarkers and therapeutic strategies (like vaccines develop), against neglected tropical diseases such CE (Figs. 3a and 4) (Riaz & Cheng).

Limitations: The available evidence on exosomes and SE is limited, since it was obtained only from level 4 studies (report and retrospective case series with reduced casuistry).

CONCLUSIONS

The available evidence of exosomes and CE is scarce and is scattered in few articles with reduced casuistry, which makes it difficult to sustain it, based on an adequate level of evidence and the generalization of the conclusions.

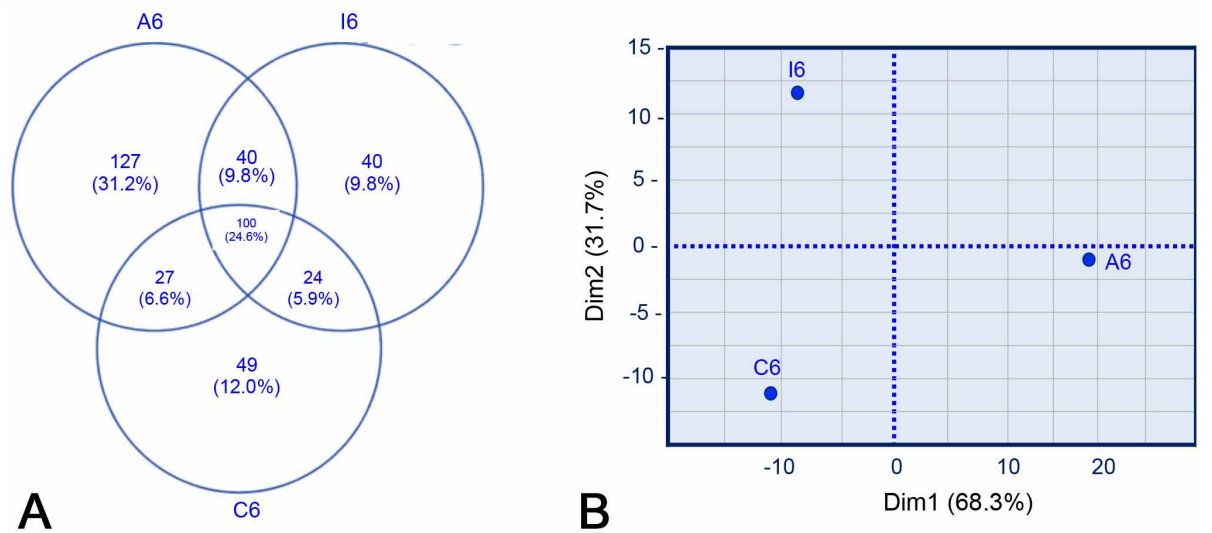

Fig. 4. Analysis of proteins detected in fraction 6 of active, inactive and control human samples(adapted from Fratini *et al*., 2020). A)Venn diagram with proteins distribution detected in fraction 6 data sets. B) Principal component plot of samples of fraction 6 data sets. Variability explained by first and second components of PCA is indicated in parentheses.

MANTEROLA, C. & LEAL, P. Exosomas y equinococosis quística. Revisión sistemática. *Int. J. Morphol., 40(1)*:115-121, 2022.

RESUMEN: Los exosomas son orgánulos pequeños y secretados por una sola membrana, de hasta 200 nm de diámetro que tienen la misma topología que la célula, pero están enriquecidos en proteínas, ácidos nucleicos, lípidos y glicoconjugados. Se puede encontrar en cualquier tipo de fluidos corporales como plasma, orina, saliva, esperma, bilis, etc. Por otro lado, la equinococosis quística (CE) ha sido estudiada desde diferentes puntos de vista, entre otros, desde la genómica y proteómica, y la presencia de exosomas de CE en humanos y otros huéspedes intermediarios se ha informado en muy pocos artículos. El objetivo de esta revisión fue informar la evidencia disponible con respecto a los exosomas y la CE. Revisión sistemática. Fuentes de datos: Trip Database, SciELO, WoS, MEDLINE, EMBASE y SCOPUS. Criterios de elegibilidad: Estudios relacionados con la EC en cualquier tipo de hospedador y estado del parásito, sin restricción de idioma, publicados entre 1966-2021. Variables: año de publicación, origen geográfico, especie aislada, ubicación de exosomas. Se identificaron inicialmente 42 estudios. Después de examinar los títulos y resúmenes, verificar las duplicaciones y analizar en profundidad los estudios seleccionados, se incluyeron 12 artículos que incluían muestras de humanos, bovinos, ovinos y caninos. Todos fueron informes de casos o series de casos. La mayor proporción de artículos se publicó entre 2019 y 2021 (58,3 %). Las publicaciones fueron predominantemente de China (58,3 %). La evidencia sobre exosomas y CE es escasa y la gama de artículos y casos es reducida.

PALABRAS CLAVE: Exosomas; Vesículas extracelulares; Equinococosis; Quiste hidatídico; Hidatidosis; Equinococosis quística.

REFERENCES

Ancarola, M.E.; Lichtenstein, G.; Herbig, J.; Holroyd, N.; Mariconti, M.; Brunetti, E.; *et al*. Extracellular non-coding RNA signatures of the metacestode stage of *Echinococcus multilocularis*. PLoS Negl. Trop. Dis., 14(11):e0008890, 2020.

Booth, M. Climate change and the neglected tropical diseases. *Adv. Parasitol., 100*:39-126, 2018.

Coakley, G.; Maizels, R. M. & Buck, A.H. Exosomes and other extracellular vesicles: the new communicators in parasite infections. *Trends Parasitol., 31(10)*:477-89, 2015.

Cui, S. J.; Xu, L. L.; Zhang, T.; Xu, M.; Yao, J.; Fang, C. Y.; Feng, Z.; Yang, P. Y.; Hu, W. & Liu, F. Proteomic characterization of larval and adult developmental stages in *Echinococcus granulosus* reveals novel insight into host-parasite interactions. J. Proteomics, 84:158-75, 2013.

Ding, J.; He, G.; Wu, J.; Yang, J.; Guo, X.; Yang, X. *et al.* miRNA-seq of *Echinococcus multilocularis* Extracellular Vesicles and Immunomodulatory Effects of miR-4989. *Front. Microbiol., 10*:2707, 2019.

Fratini, F.; Tamarozzi, F.; Macchia, G.; Bertuccini, L.; Mariconti, M.; Birago, C.; Iriarte, A.; Brunetti, E.; Cretu, C. M.; Akhan, O.; *et al*. Proteomic analysis of plasma exosomes from Cystic Echinococcosis patients provides in vivo support for distinct immune response profiles in active vs inactive infection and suggests potential biomarkers. *PLoS Negl. Trop. Dis., 14(10)*:e0008586, 2020.

Kalluri, R. & LeBleu, V. S. The biology, function, and biomedical applications of exosomes. *Science, 367(6478)*:eaau6977, 2020.

Kern, P.; Menezes da Silva, A.; Akhan, O.; Müllhaupt, B.; Vizcaychipi, K. A.; Budke, C. & Vuitton, D. A. The Echinococcoses: diagnosis, clinical management and burden of disease. *Adv. Parasitol., 96*:259-369, 2017.

Macchiaroli, N.;Preza, M.; Pérez, M.G.; Kamenetzky, L.;Cucher, M.; Koziol, U. *et al*. Expression profiling of *Echinococcus multilocularis* miRNAs throughout metacestode development *in vitro. PLoS Negl. Trop. Dis., 15(3)*:e0009297, 2021.

Manterola, C.; García-Méndez, N. & Rojas, C. General aspects of the proteomic profile of *Echinococcus granulosus*. *Int. J. Morphol., 37(2)*:773-9, 2019.

Manterola, C.; Rojas, C.; Totomoch-Serra, A.; García-Méndez, N. & Riffo-Campos, Á. L. *Echinococcus granulosus* genotypes verified in human hydatid disease around the world. Systematic Review. *Rev. Chil. Infectol., 37(5)*:541-9, 2020.

Manterola, C.; Totomoch-Serra, A.; Rojas, C.; Riffo-Campos, Á. L. & García-Méndez, N. *Echinococcus granulosus* sensu lato genotypes in different hosts worldwide: a systematic review. *Acta Parasitol*., 2021. DOI: https://www.doi.org/10.1007/s11686-021-00439-8

Marcilla, A.; Martin-Jaular, L.;Trelis, M.; de Menezes-Neto, A.; Osuna, A.; Bernal, D.; Fernandez-Becerra, C.; Almeida, I. C. & Del Portillo, H. A. Extracellular vesicles in parasitic diseases. *J. Extracell. Vesicles, 3*:25040, 2014.

Mathivanan, S.; Ji, H. & Simpson, R. J. Exosomes: extracellular organelles important in intercellular communication. *J. Proteomics, 73(10)*:1907-20, 2010.

Mu, Y.; McManus, D. P.; Gordon, C. A. & Cai, P. Parasitic helminth-derived microRNAs and extracellular vesicle cargos as biomarkers for helminthic infections. *Front. Cell. Infect. Microbiol., 11*:708952, 2021.

Nicolao M. C.; Rodriguez Rodrigues, C. & Cumino, A. C. Extracellular vesicles from *Echinococcus granulosus* larval stage: Isolation, characterization and uptake by dendritic cells. *PLoS Negl. Trop. Dis., 13(1)*:e0007032, 2019.

Pegtel, D. M. & Gould, S. J. Exosomes. *Annu. Rev. Biochem., 88*:487-514, 2019.

Riaz, F. & Cheng, G. Exosome-like vesicles of helminths: implication of pathogenesis and vaccine development. *Ann. Transl. Med., 5*:175, 2017.

Santos, G. B.; Monteiro, K. M.; da Silva, E. D.; Battistella, M. E.; Ferreira, H. B. & Zaha, A. Excretory/secretory products in the *Echinococcus granulosus* metacestode: is the intermediate host complacent with infection caused by the larval form of the parasite? *Int. J. Parasitol., 46(13-14)*:843-56, 2016.

Siles-Lucas, M.; Sánchez-Ovejero, C.; González-Sánchez, M.; González, E.; Falcón-Pérez, J. M.; Boufana, B.; Fratini, F.; Casulli, A. & Manzano-Román, R. Isolation and characterization of exosomes derived from fertile sheep hydatid cysts. *Vet. Parasitol., 236*:22-33, 2017.

Stroup, D. F.; Berlin, J. A.; Morton, S. C.; Olkin, I.; Williamson, G. D.; Rennie, D.; Moher, D.; Becker, B. J.; Sipe, T. A. & Thacker, S. B. Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA, 283(15)*:2008-12, 2000.

Virginio, V. G.; Monteiro, K. M.; Drumond, F.; de Carvalho, M. O.; Vargas, D. M.; Zaha, A. & Ferreira, H. B. Excretory/secretory products from in vitro-cultured *Echinococcus granulosus* protoscoleces. *Mol. Biochem. Parasitol., 183(1)*:15-22, 2012.

Wang, W.; Zhou, X.; Cui, F.; Shi, C.; Wang, Y.; Men, Y.; Zhao, W. & Zhao, J. Proteomic analysis on exosomes derived from patients' sera infected with *Echinococcus granulosus*. *Korean J. Parasitol., 57(5)*:489-97, 2019.

Wang, Y.; Xiao, D.; Shen, Y.; Han, X.; Zhao, F.; Li, X.; Wu, W.; Zhou, H.; Zhang, J. & Cao, J. Proteomic analysis of the excretory/secretory products and antigenic proteins of *Echinococcus granulosus* adult worms from infected dogs. *BMC Vet. Res., 11*:119, 2015.

Wen, H.; Vuitton, L.; Tuxun, T.; Li, J.; Vuitton, D.A.; Zhang, W. & McManus, D. P. Echinococcosis: advances in the 21st Century. *Clin. Microbiol. Rev., 32(2)*:e00075-18, 2019.

WHO Informal Working Group. International classification of ultrasound images in cystic Echinococcosis for application in clinical and field epidemiological settings. *Acta Trop., 85(2)*:253-61, 2003.

Wu, J.; Cai, M.; Yang, J.; Li, Y.; Ding, J.; Kandil, O. M.; Kutyrev, I.; Ayaz, M. & Zheng, Y. Comparative analysis of different extracellular vesicles secreted by *Echinococcus granulosus* protoscoleces. *Acta Trop., 213*:105756, 2021.

Yang J.; Wu J.; Fu Y.; Yan L.; Li Y.; Guo X.; Zhang, Y.; Wang, W.; Shen, Y.; Cho, W. C.; *et al*. Identification of different extracellular vesicles in the hydatid fluid of *Echinococcus granulosus* and immunomodulatory effects of 110 K EVs on sheep PBMCs. *Front. Immunol., 12*:602717, 2021.

Zhang, W.; Wang, S. & McManus, D. P. *Echinococcus granulosus* genomics: a new dawn for improved diagnosis, treatment, and control of Echinococcosis. *Parasite, 21*:66, 2014.

Zhang, X.; Gong, W.; Cao, S.; Yin, J.; Zhang, J.; Cao, J. & Shen, Y. Comprehensive Analysis of Non-coding RNA Profiles of Exosome-Like Vesicles From the Protoscoleces and Hydatid Cyst Fluid of *Echinococcus granulosus. Front. Cell. Infect. Microbiol., 10*:316, 2020.

Zheng, H.; Zhang, W.; Zhang, L.; Zhang, Z.; Li, J.; Lu, G.; Zhu, Y.; Wang, Y.; Huang, Y.; Liu, J.; *et al*. The genome of the hydatid tapeworm *Echinococcus granulosus*. *Nat. Genet., 45(10)*:1168-75, 2013.

Zhou, X.; Wang, W.; Cui, F.; Shi, C.; Ma, Y.; Yu, Y.; Zhao, W. & Zhao, J. Extracellular vesicles derived from *Echinococcus granulosus* hydatid cyst fluid from patients: isolation, characterization and evaluation of immunomodulatory functions on T cells. *Int. J. Parasitol., 49(13-14)*:1029-37, 2019.

Zheng, Y.; Guo, X.; Su, M.; Guo, A.; Ding, J.; Yang, J. *et al*. Regulatory effects of *Echinococcus multilocularis* extracellular vesicles on RAW264.7 macrophages. *Vet. Parasitol., 235*:29-36, 2017.

Corresponding author:
Dra. Pamela Leal
Center of Excellence in Translational Medicine (CEMET)
Universidad de La Frontera
Av. Francisco Salazar 1145
Temuco
CHILE

E-mail: pamela.leal@ufrontera.cl