Consejo Directivo
FACULTAD DE CIENCIAS VETERINARIAS
Universidad Nacional de La Pampa

RESOLUCIÓN Nº 035/2023

GENERAL PICO, 02 de Marzo de 2023.-

VISTO:

La evaluación positiva enviada por las/los integrantes del Comité Científico de la Facultad de Ciencias Veterinarias de la Universidad Nacional de La Pampa, respecto del Proyecto de Investigación: *"Identificación de áreas de riesgo de transmisión de la Equinococosis quística /Hidatidosis en las Provincias de La Pampa y Rio Negro y desarrollo de capacidades diagnósticas en salud animal"*, y

CONSIDERANDO:

Que el Proyecto de Investigación enunciado en el visto estará bajo la dirección del Dr. Edmundo LARRIEU y la co-dirección del Dr. Leonardo MOLINA, participando en carácter de Investigadores/as el M.V. Claudio CALVO, la Mg. Mariela GARCIA CACHAU, la Esp. Cecilia LAPUYADE, la Esp. Tamara CORNEJO, la Esp. Natalia CAZAUX, la Dra. Mónica BOERIS, la Dra. Ana Inés PORTU, la Dra. María Guillermina BILBAO, el M.V. Marcos MORENO, el M.V. Franco LUCERO ARTEAGA, la Dra. Florencia D`FRANCISCO y la M.V. Noelia KAPPES (Ministerio de Salud de La Pampa), en carácter de Asistentes de Investigación las profesionales M.V. Vanina BENITEZ, M.V. Clara CARIATORE y M.V. Daiana FEDERICI y las estudiantes de la carrera Medicina Veterinaria Pilar MEGLIA y Fernanda Candela MONSALVE.

Que tendrá una duración de veinticuatro (24) meses, a partir del 01 de Enero de 2023 y hasta el 31 de Diciembre de 2024.

Que de acuerdo a la presentación el citado proyecto es de Investigación Aplicada.

Que participan en su desarrollo las Cátedras Epidemiología y Salud Pública, Parasitología y Enfermedades Parasitarias, el Instituto de Zoonosis, el Centro de Producción de Animales de Experimentación y el Centro de Investigación y Desarrollo de Fármacos (CIDEF), todos pertenecientes a la Facultad de Ciencias Veterinarias de la Universidad Nacional de La Pampa.

Que también participarán el Instituto Nacional de Microbiología "Anlis-Malbrán", el Ministerio de Salud de la Provincia de La Pampa, el Ministerio de Salud de la Provincia de Rio Negro, el Instituto CONICET "César Milstein" y el Laboratorio de Parasitología de la Facultad de Medicina, UN del Comahue.

Que el citado proyecto ha sido presentado de acuerdo con las normas vigentes y aprobado por el Comité Científico de la Facultad.

Que el Artículo 5º Anexo I de la Resolución Nº 100/99 y su modificatoria Nº 88/02 del Consejo Superior, estipula que: "*Todo Programa y todo Proyecto de Investigación que obtenga dos (2) evaluaciones externas favorables será acreditado mediante resolución del Consejo Directivo de cada Facultad a la que pertenezca"*.

Que cuenta con dos (2) evaluaciones externas satisfactorias, de acuerdo con lo previsto en la Resolución Nº 100/99 y Nº 88/02 del Consejo Superior de la Universidad Nacional de La Pampa.

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Universidad Nacional de La Pampa

Corresponde a Resolución Nº 035/2023

//2.-

Que las evaluaciones fueron realizadas por el Dr. Guillermo Maria DENEGRI y la Dra. Maria Celina ELISSONDO, ambos pertenecientes al Centro Científico Tecnológico Conicet de Mar del Plata.

Que dicho proyecto cuenta con la aprobación del formulario del protocolo institucional para el cuidado y uso de animales de experimentación bajo la responsabilidad el consejo asesor institucional para el uso y cuidado de animales de experimentación (CAICUAE) de la Facultad de Ciencias Veterinarias de la Universidad Nacional de La Pampa.

Que en Sesión Ordinaria del Consejo Directivo del día 02 de Marzo de 2023, puesta la acreditación del Proyecto de Investigación a consideración de los/as Sres/as. Consejeros/as, se aprueba por unanimidad.

POR ELLO:

EL CONSEJO DIRECTIVO DE LA FACULTAD DE CIENCIAS VETERINARIAS

R E S U E L V E:

ARTICULO 1º: Acreditar como Proyecto de Investigación de la Facultad de Ciencias Veterinarias de la Universidad Nacional de La Pampa, el proyecto denominado: *"Identificación de áreas de riesgo de transmisión de la Equinococosis quística /Hidatidosis en las Provincias de La Pampa y Rio Negro y desarrollo de capacidades diagnósticas en salud animal"* bajo la dirección del Dr. Edmundo LARRIEU y la co-dirección del Dr. Leonardo MOLINA, participando en carácter de Investigadores/as el M.V. Claudio CALVO, la Mg. Mariela GARCIA CACHAU, la Esp. Cecilia LAPUYADE, la Esp. Tamara CORNEJO, la Esp. Natalia CAZAUX, la Dra. Mónica BOERIS, la Dra. Ana Inés PORTU, la Dra. María Guillermina BILBAO, el M.V. Marcos MORENO, el M.V. Franco LUCERO ARTEAGA, la Dra. Florencia D`FRANCISCO y la M.V. Noelia KAPPES (Ministerio de Salud de La Pampa), en carácter de Asistentes de Investigación las profesionales M.V. Vanina BENITEZ, M.V. Clara CARIATORE y M.V. Daiana FEDERICI y las estudiantes de la carrera Medicina Veterinaria Pilar MEGLIA y Fernanda Candela MONSALVE, el cual tiene noventa y ocho (98) folios y se adjunta como Anexo I de la presente Resolución.

ARTICULO 2º: Aprobar el protocolo de bioseguridad denominado *"Infección experimental de jerbos (Meriones unguiculatus) con protoescólices provenientes de metacestodes de Echinococcus granulosus. (CePAE – CIDEF-FCV UNLPam)"*, el cual se adjunta en el Anexo II de la presente Resolución e incluye las diferentes reglamentaciones obligatorias para llevar a delante dicho procedimiento, las cuales forman parte de los Anexos III, IV y V del presente acto resolutivo.

ARTÍCULO 3º: Encomendar a la Dra. María Guillermina BILBAO, responsable de llevar a cabo el protocolo de bioseguridad, de formar al equipo del Proyecto de Investigación en cuanto a los procedimientos especificados en el mismo.

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Universidad Nacional de La Pampa

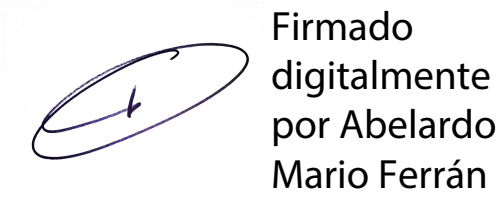
Corresponde a Resolución Nº 035/2023

//3.-

ARTICULO 4º: El proyecto tendrá una duración de veinticuatro (24) meses, a partir del 01 de Enero de 2023 y hasta el 31 de Diciembre de 2024.

ARTICULO 5º: Justificar los gastos que se produzcan de pasajes, viáticos, combustibles, aparatos, material de laboratorio, etc., del citado proyecto.

ARTÍCULO 6º: Regístrese, comuníquese. Tomen conocimiento los/as interesados/as, Secretaría de Investigación y Posgrado. Cumplido, archívese.



Presidente
Consejo Directivo
Facultad de Ciencias Veterinarias
UNLPam

ANEXO

I

TITULO: "Identificación de áreas de riesgo de transmisión de la Equinococosis quistica /Hidatidosis en las Provincias de La Pampa y Rio Negro y desarrollo de capacidades diagnósticas en salud animal".

INTEGRANTES	FIRMA
Larrieu, Edmundo	
Molina, Leonardo	
Calvo, Claudio	
Lapuyade, Cecilia	
Cazaux, Natalia	
Garcia Cachau, Mariela	
Cornejo, Tamara	
Boeris, Mónica	
Portu, Ana	
Bilbao, Guillermina	
Marcos Moreno	
Lucero Arteaga Franco	
Noelia Kappes	
Meglia Pilar	
Monsalve, Fernanda	
Benitez, Vanina	

1

D'Francisco, Florencia

Federici, Daiana

Cariatore, Clara

2

UNIVERSIDAD NACIONAL DE LA PAMPA
Facultad de CIENCIAS VETERINARIAS

1. IDENTIFICACIÓN del PROYECTO

1.1. TÍTULO: Identificación de áreas de riesgo de transmisión de la Equinococosis quistica /Hidatidosis en las Provincias de La Pampa y Rio Negro y desarrollo de capacidades diagnósticas en salud animal

1.2. TIPO de INVESTIGACIÓN: Aplicada

1.3. CAMPO de APLICACIÓN PRINCIPAL: Salud Pública (1206-1212)

1.4. CAMPOS de APLICACIÓN POSIBLES: 1210.

1.5. ÁREA DE CONOCIMIENTO: Agropecuarias y del Ambiente

1.6. SUBÁREA DE CONOCIMIENTO: Ciencias Veterinarias

2. INSTITUCIONES y PERSONAL que INTERVIENEN en el PROYECTO

2.1. ÁREAS: Cátedra Epidemiología y Salud Pública, Facultad Ciencias Veterinarias, UNLPam; Cátedra Parasitología y Enfermedades Parasitarias, Facultad Ciencias Veterinarias, UNLPam; Instituto de Zoonosis Facultad de Ciencias Veterinarias, UNLPam; Centro de Producción de Animales de Experimentación, Facultad de Ciencias Veterinarias, UNLPam; CIDEF, Facultad de Ciencias Veterinarias, UNLPam

2.2. OTRAS INSTITUCIONES

- Cooperación técnica con Instituto Nacional de Microbiología “Anlis-Malbrán”
- Ministerio de Salud, Provincia de La Pampa:
- Ministerio de Salud de la Provincia de Rio Negro
- Instituto CONICET “César Milstein”
- Laboratorio de parasitología, Facultad de Medicina, UN del Comahue

2.3. EQUIPO de TRABAJO:

Apellido y Nombre	CUIL	Título Académico	Categ. Invest.	Respon-sabilidad	Cátedra o Institución	Cargo y Dedicación	Tiempo dedicac. Hs./Sem
Larrieu, Edmundo	20-10704921-6	Dr	II	D	Instituto de Zoonosis, FCV UNLPAM	Prof Consulto	5
Molina, Leonardo	20-25329502-4	Dr	V	CD	Epidemiología y Salud Pública FCV UNLPAM	ADJ EX	4
Calvo, Claudio	20-14295600/5	MV	III	I	Parasitología y Enfermedades Parasitarias FCV UNLPam	ADJ EX	10
Garcia Cachau, Mariela	27-21673895-6	Mg	III	I	Epidemiología y Salud Pública FCV UNLPAM	ADJ EX	4
Lapuyade, Cecilia	23-32073668-4	Esp	-	I	Parasitología y Enfermedades Parasitarias FCV UNLPam	AY1º S	5
Cornejo, Tamara	27-29628068-8	Esp	-	I	Epidemiología y Salud Pública FCV UNLPAM	AY1º S	5
Cazaux, Natalia	27-36314900-1	Esp.	IV	I	Parasitología y Enfermedades Parasitarias FCV UNLPam	JTP S	5
Boeris, Mónica	27-14928079-6	Dra	III	I	Bioterio/Física Biológica FCV UNLPam	ADJ S	2
Portu, Ana	27-31892745-1	Dra	-	I	Bacteriología y micología FCV UNLPam	JTP SE	2
Bilbao, Guillermina	27-28004454-2	Dra	IV	I	Física Biológica/ CIDEF FCV UNLPam/ CONICET	ADJ S	2
Moreno, Marcos	20-26507929-7	MV		I	Epidemiología y Salud Pública FCV UNLPAM	JTP SE	2
Lucero Arteaga, Franco	20-36221849-8	MV		I	Centro de Producción de Animales de Experimentación/Virología e Inmunología Básica FCV UNLPam	AY1º SE	4
Kappes, Noelia	27-27828175-8	MV		I	Ministerio de Salud de La Pampa		2
Meglia, Pilar	27-37556177-3	Estudiante		AI	FCV UNLPAM		2
Monsalve, Fernanda C.	27-40111261-3	Estudiante		AI	FCV UNLPam		2
Benitez, Vanina	23-39378593-4	MV		AI	Graduada		5
D'Francisco, Florencia	27-34536795-6	MV		I	Fisiología Animal FCV UNLPam		5
Cariatore, Clara	27-33532172-9	MV		AI	Graduada/Residente de Salud Pública Veterinaria Rio Negro		5
Federici, Daiana	28-34778104-0	MV (UNR)		AI	Graduada/Residente de Salud Pública Veterinaria Rio Negro		5

D: Director, CD: Co-Director, A: Asesor, I: Investigador, AI: Asistente de Investigación.

3. DURACIÓN ESTIMADA del PROYECTO:

FECHA de INICIO: 01 / 01 /2023 **FINALIZACIÓN:** 31 / 12/ 2024

4. RESUMEN DEL PROYECTO:

La Hidatidosis o Equinococosis quística (EQ) es una zoonosis parasitaria, endémica en Argentina en regiones donde las condiciones sociales, culturales y ambientales generan un ambiente epidemiológico que favorecen el ciclo de *Echinococcus granulosus* En la Provincia de

La Pampa existen reportes de alta prevalencia en bovinos, aunque no existen estudios actualizados de prevalencia en perros, su distribución geográfica y los factores de riesgo que podrían significar transmisión a las personas, especialmente en el oeste pampeano, región históricamente con mayor prevalencia. El objetivo de la investigación es identificar la prevalencia de infección en perros de establecimientos ganaderos y evaluar otros factores de riesgo en la Provincia de La Pampa. y generar capacidades diagnósticas de las que hoy se carece en la Facultad de Ciencias Veterinarias como soporte de la investigación y como apoyo futuro a los programas de control de la Patagonia. La información se obtendrá mediante encuestas con coproELISA en establecimientos ganaderos, mediante ELISA en ovinos y mediante diagnóstico macroscópico en salas de faena de rumiantes menores incluyendo identificación de cepas. El avance del proyecto requiere del desarrollo de capacidades en la UNLPAM de procesamiento de materia fecal canina fresca, copro ELISA y PCR para confirmación, además de generar técnicas para obtención de ejemplares de *E. granulosus* adultos requeridos por los laboratorios de referencia para la producción de antígenos-

4.1 Palabras Clave: equinococosis, diagnóstico, ovejas, perros

4.2 Abstract en Ingles

Hydatidosis or Cystic Echinococcosis (CE) is a parasitic zoonosis, endemic in Argentina in regions where social, cultural and environmental conditions generate an epidemiological environment that favors the cycle of *Echinococcus granulosus* In the Province of La Pampa there are reports of high prevalence in cattle , although there are no updated studies on prevalence in dogs, their geographical distribution and the risk factors that could mean transmission to people, especially in the west of the Pampas, a region historically with the highest prevalence. The objective of the research is to identify the prevalence of infection in dogs from livestock establishments and to evaluate other risk factors in the Province of La Pampa and generate diagnostic capabilities that are currently lacking in the Faculty of Veterinary Sciences as support for research and as future support for control programs in Patagonia. The information will be obtained through surveys with coproELISA in livestock establishments, through ELISA in sheep and through macroscopic diagnosis in small ruminant slaughter rooms, including identification of strains. The progress of the project requires the development of capacities in the UNLPAM for the processing of fresh canine fecal matter, copro ELISA and PCR for confirmation, in addition to generating techniques for obtaining specimens of adult *E. granulosus* required by the reference laboratories for the production of antigens

4.3 Keywords: echinococcosis, diagnoses, sheep, dogs

5. INTRODUCCIÓN y ANTECEDENTES

5.1. INTRODUCCIÓN, MANEJO DE FUENTES BIBLIOGRÁFICAS Y DESCRIPCIÓN DE LA SITUACIÓN ACTUAL DEL PROBLEMA

INTRODUCCION

La equinococosis quística (EQ) o hidatidosis es una zoonosis parasitaria producida por un cestodo de la familia *Taenidae*, género *Echinococcus*, especie *granulosus (EG)*, descripto en 1786. Requiere de dos hospederos mamíferos para completar su ciclo de vida. Un hospedero definitivo, (carnívoro, especialmente el perro) donde se desarrolla la faz adulta o estrobilar y un hospedero intermediario en donde se desarrolla la faz larvaria o metacestode (Craig et al, 2015; 2017).

Cuando huevos de EG son ingeridos por hospederos susceptibles (especialmente el ovino) llegan al estómago en donde sufren la disgregación del embriósfero y la activación de la oncósfera. Esta exhibe intrincados movimientos rítmicos del cuerpo y los ganchos y penetran a través de las microvellosidades intestinales pasando a los sistemas linfáticos y venosos para ir a instalarse definitivamente en alguna víscera, preferentemente hígado o pulmón.
La forma larval o metacestode que se desarrolla es típicamente unilocular, pleomórfica, llena de fluido y con una compleja estructura consistente en una membrana germinal interna, compuesta por células de núcleo circular u ovalado y una membrana cuticular, acelular y elástica (externa), rodeada por una membrana adventicia y fibrosa producida por el hospedador.
Existe una gran diversidad de cepas de EG, algunas de las cuales se encuentran presente en Argentina: cepa ovina G1, cepa ovina tasmania G2, cepa vaca G5, cepa camello G6 y cepa porcina G7 (Cucher et al, 2016).
Es una de las enfermedades zoonóticas de mayor prevalencia en Argentina, Uruguay, Chile, Perú y el sur del Brasil. Produce elevados costos para la ganadería en función del valor de las vísceras decomisadas y pérdidas en la producción de lana, leche y carne; y para los sistemas de salud en razón de los costos de internación y tratamiento de las personas (Larrieu y Zanini, 2012; Pavletic et al, 2017).
Uno de los aspectos más relevantes de la biología de EG es la consideración de su periodo prepatogénico y patogénico en el perro.
Cuando el perro ingiere vísceras crudas con quistes hidatídicos (o si es inoculado experimentalmente), a los 17 a 20 días se encuentra formado el primer proglótide, a los 20 a 28 días es visible el segundo proglótide y, finalmente el primer proglótide maduro (infectante) es eliminado con la materia fecal entre los 47 – 52 días. Ello implica que los primeros 45 días post infección, el perro no transmite EQ.
El perro continuará eliminado proglótides maduras durante aproximadamente 180 días, deponiendo huevos cada 15 días y material parasitario incluyendo antígenos metabólicos, cuticulares y restos parasitarios con la materia fecal.
Un perro puede ser portador de cientos de EG. Sin embargo, no causan enfermedad clínica ni otros efectos, aun en casos de infección severa (Eckert et al, 2001)
En la Provincia de Rio Negro el programa logró fuertes disminuciones en la prevalencia de la infección en perros y humanos, aunque se mantiene un nivel endémico en el ovino que asegura el mantenimiento del ciclo de la enfermedad, con la consiguiente aparición de casos nuevos en niños (3).
Históricamente, la línea sur de Rio Negro ha sido la zona con mayores niveles de transmisión, por sus características de zonas de producción ovina, condiciones sociales y un ambiente favorable a la sobrevida del parasito.
Si bien el programa de control logro avances importantes con una fuerte disminución en el número de casos, fue en forma disímil en distintos parajes y áreas nucleadas del área endémica. Por ello, en los parajes rurales en donde los riesgos para las personas se mantenían elevados, se ajustaron las estrategias para aumentar sobre ellas la presión de control incluyendo nuevas herramientas, tal como la vacunación de lanares, cuya potencialidad para cortar el ciclo de la enfermedad ha sido evaluado en un proyecto anterior (Mujica et al, 2021).
Por su parte, en diciembre de 2019 comenzó un brote de un novel coronavirus de origen zoonótico, denominado SARS-COV.2 o COVID-19, que alcanzo dimensiones de pandemia el 11 de marzo de 2020. Ya el 3 de marzo había ingresado a la Argentina, mientras que el 9 de marzo se produjo el primer caso en la Provincia de Rio Negro. En esta provincia, al 30 de junio de 2020 ya se identifican 823 casos con 41 defunciones y para el 10 de diciembre se mantenían 2122 casos activos con 30079 casos recuperados, habiéndose producido 848 fallecidos. La ocupación

de camas en terapia intensiva era del 76%, luego de haberse mantenido una gran cantidad de días con cifras del 95% (Crowley et al, 2020).
La ocurrencia de casos dio lugar a sucesivas estrategias de intervención. Entre todas las medidas, las vinculadas a Intervenciones no farmacéuticas o INF basadas en Atención Primaria de la Salud y Una Salud con participación integrada entre agentes sanitarios del Primer Nivel de Atención, salud pública veterinaria y áreas medicas de los Departamentos de Actividades para el área y sus Centros de Atención Primaria de la Salud y servicios de epidemiologia resultaron centrales para la contención de la epidemia, hasta la disponibilidad de vacunas que se encuentra actualmente en fase de amplio desarrollo y cobertura. Es un interrogante el efecto que tiene el desarrollo de la pandemia en la prevalencia de otras enfermedades.

BIOSEGURIDAD

EQ es una zoonosis parasitaria. Por ende, el manejo de la enfermedad, con especial referencia a la manipulación de parásitos adultos, manejo y contacto con animales infectados con la forma adulta de EG y manipulación y manejo de materia fecal proveniente de dichos animales es un punto clave en cualquier trabajo con EG debiendo mantenerse estrictas precauciones de seguridad.
- Inactivación de huevos de EG adultos: los huevos de EG son inactivados a temperaturas de 65° durante 30 minutos y en 1 minuto a 100°. En frío, son inactivados en freezer a -80° en 2 días o a -70° 4 días o a -20° durante 90 días. Ello incluye su aplicación a materia fecal o trozos de intestino delgado o carcasas de animales como zorros (Eckert et al, 2001).
- Descontaminación ambiental: Después de pruebas con test de arecolina o para el confinamiento de animales infectados se deben aplicar medidas específicas de bioseguridad. El test de arecolina debe ser aplicado en áreas cerradas con piso de concreto que pueda ser fácilmente limpiada y desinfectada o sobre carpetas plásticas que puedan ser incineradas (Eckert et al, 2001).
- Precaución en laboratorio o el afectado a tareas de campo: El personal de laboratorio en contacto con material no esterilizado o inactivado debe asegurar el uso de ropas descartables (mamelucos, guantes, botas) evitando el uso de ropas que requieran envío a lavadero. En caso de necropsias de animales de laboratorio o de otro tipo, el procesado de los intestinos deberá efectuarse sobre material plástico que pueda ser eliminado mediante incineración. Los instrumentos utilizados deberán ser esterilizados en autoclave. Los animales experimentalmente infectados deberán mantenerse idealmente solo hasta el periodo prepatente, aunque con fines de obtención de especímenes para la elaboración de antígenos para copro ELISA o PCR se requiere su mantenimiento hasta periodo patente, el cual deberá ser el mínimo necesario que permita la obtención de huevos de EG.

DESCRIPCION DE LA SITUACION ACTUAL:

Para la caracterización de la infección en ovinos y caprinos el método utilizado tradicionalmente para el diagnóstico es la identificación post mortem de la presencia de quistes hidatídicos, siendo importante conocer la edad de los animales para la interpretación epidemiológica de los datos. Sus limitaciones incluyen no ser sensible para la detección de quistes en animales jóvenes (que son los de mayor interés para la vigilancia en un programa de control) y errores diagnósticos en animales adultos (quistes supurados, degenerados y calcificados). Otra limitación importante es que en muchas zonas endémicas no existen mataderos donde se puedan llevar a cabo estos estudios o el beneficio casero (de traspatio) es una práctica muy común en la población, particularmente en las crianzas de ovinos y caprinos de manera familiar o en pequeña escala (pequeños ganaderos); por lo tanto, no es posible obtener en todos los casos una información representativa y real de la situación de la enfermedad en ovinos.

Alternativamente, se pueden utilizar técnicas serológicas que están disponibles con una sensibilidad y especificidad aceptable (Gatti y col, 2007), siendo en especial útiles en animales recientemente infectados (la respuesta humoral en los corderos se detecta en los 10 días posteriores a la infección (Lamberti y col, 2014). Si se aplica a corderos puede ser útil para evaluar si la transmisión está presente o ausente: un diagnóstico positivo en al menos un cordero significa que hay perros infectados con EG en el sitio y por lo tanto el medio ambiente está contaminado. Actualmente el Instituto Cesar Milstein ha ajustado una técnica en base a anticuerpos monoclonales (Poggio, comunicación personal, en revisión en One Health) que puede estar disponible.

Desde la aparición de coproELISA (Allan et al, 1992; 2006), es la técnica de elección para diagnóstico de situación de EQ en el huésped definitivo y para la vigilancia epidemiológica en programas de control (Craig et al, 2017). La sensibilidad (78%/100%) y especificidad (85%) es razonable con fines de diagnóstico de situación (Craig et al, 2017), pudiendo arrojar resultados falsos negativos cuando la carga de parásitos es baja, dando además falsos positivos por reacciones cruzadas con otras tenias tal como *Taenia hydatigena* (Allan and Craig, 2006; Craig et al, 2017).

En los últimos años varios coproELISA han sido estandarizados para su uso en los programas de control vigentes en las zonas endémicas de América del Sur (Guarnera et al, 2000; Pierangeli et al, 2010; Morel et al, 2013) e incluidos en los sistemas de vigilancia de los países (Larrieu and Zanini, 2012; Pavletic et al, 2017).

Eventualmente, puede mejorarse la especificidad del sistema incorporando pruebas de confirmación, tal como WB o PCR (Guarnera et al, 2000; Cabrera et al, 2002; Abassi et al, 2003; Stefanic et al, 2004; Craig et al, 2017) lo que permite ajustar la estimación de la prevalencia

Los sistemas de vigilancia de la EQ en humanos, por su parte, no solamente permiten diagnosticar la situación en las personas, sino que, en la medida en que se utilizan estudios transversales sistemáticos en población asintomática, permiten un diagnóstico precoz y un tratamiento oportuno de los casos, resultando por ende medidas costo efectivas (Larrieu et al, 2011; 2019).

La ultrasonografía (US) es actualmente la prueba de elección. Con una elevada sensibilidad (100%) y especificidad (95%) (Del Carpio et al, 2000) en su uso como prueba de tamizaje, ha demostrado que puede ser aplicada a la vigilancia epidemiológica en los programas de control, pudiendo detectar transmisión en el pasado reciente si es utilizada en población joven, como por ejemplo escolares de 6 a 14 años de edad (Larrieu et al, 2011, 2019).

En este contexto, la Facultad e Ciencias Veterinarias ha generado proyectos de investigación como soporte a actividades de vigilancia y control en la Patagonia. Sin embargo carece de capacidades actuales de diagnóstico que permitan dar soporte a los programas de control.

5.2. RESULTADOS ALCANZADOS POR LOS INTEGRANTES DEL PROYECTO DENTRO DEL ÁREA DE CONOCIMIENTO DE ESTE y INVESTIGACIÓNES DE LOS INTEGRANTES RELACIONADOS AL PROYECTO (desde el año 2000):

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6. DESCRIPCIÓN del PROYECTO

6.1. PROBLEMA CIENTÍFICO, OBJETIVOS, HIPÓTESIS Y RESULTADOS ESPERADOS DEL PROYECTO

En la Provincia de La Pampa existen evidencias que EQ es endémica (Larrieu et al, 1996; Lamberti et al, 1999), con especial referencia a la zona oeste. Sin embargo, los informes científicos son de vieja data y no se cuenta con reportes modernos de prevalencia y distribución de EQ en distintos ambientes de la Provincia de La Pampa.

Es reconocido que EQ como enfermedad zoonótica en Argentina está especialmente asociada a sistemas de producción ovina o caprina, en tanto en estos sistemas productivos se favorecen prácticas que permiten la transmisión de EQ tal como la faena de animales pequeños para consumo con entrega de vísceras a los perros.

Estudios recientes efectuados por SENASA muestran elevada prevalencia en ganado bovino, pero no asociado a casos en las personas.

Asimismo, la Provincia de La Pampa y la Facultad en particular carecen de tecnologías y profesionales formados para el diagnóstico de la equinococosis animal tanto para fines de investigación como de soporte para el programa de control de La Pampa. Un problema agregado es que actualmente no hay en Argentina elaboración de antígenos para diagnóstico, por limitaciones en la obtención de ejemplares *de E granulosus* que se requieren para para la elaboración.

En relación a COVID 19, no se conoce el impacto de la pandemia en áreas con programas sistemáticos de control como el caso de Rio Negro.

HIPÓTESIS

En ciertas áreas de la provincia de La Pampa relacionadas con la producción ovina y caprina EQ es endémica.

La aparición de COVID 19 llevo a aplicar todos los recursos humanos y materiales disponibles en la red hospitalaria y de atención primaria a su contención, ello podría haber generado el abandono de otros programas de control de enfermedades produciendo un aumento en la prevalencia de la infección por *Echinococcus granulosus* en el perro.

La Provincia de La Pampa carece de capacidad diagnostica en EQ, en un contexto nacional de limitaciones severas para la elaboración de los antígenos requeridos para las pruebas “in house”.

OBJETIVO GENERAL

Contribuir a la vigilancia epidemiológica y el control de la EQ en la región patagónica mediante actividades de campo y del desarrollo de un centro de diagnóstico de EQ en animales como soporte estratégico de programas de vigilancia y control de la Provincia de La Pampa y de otras provincias como la de Rio Negro, donde la EQ es endémica, estableciendo asociaciones con

otros laboratorios e institutos especializados del país tal como el Laboratorio de Parasitología de la Universidad Nacional del Comahue, el Centro CONICET Cesar Milstein y el Instituto Nacional de Microbiología Carlos Malbrán.

OBJETIVOS ESPECIFICOS

1. Generar la capacidad de producción de EG adultos requeridos para la elaboración de antígenos que puedan ser utilizados en el diagnóstico de la EQ en perros.
2. Generar la capacidad de procesar muestras de materia fecal canina para la obtención de sueros sobrenadantes que puedan ser posteriormente procesados mediante la técnica de coproELISA.
3. Generar la capacidad de efectuar análisis de coproELISA en muestras de la provincia de La Pampa o de otras provincias endémicas.
4. Generar la capacidad de efectuar análisis de coproPCR para la confirmación de muestras positivas a coproELISA.
5. Utilizar las capacidades desarrolladas como apoyo a la educación de grado, posgrado y a líneas de investigación operativa.
6. Identificar la prevalencia de la infección y la dispersión de EQ en cabras, ovinos y perros, con especial referencia al oeste pampeano.
7. Identificar la prevalencia de la infección de EQ en perros y establecimientos ganaderos de áreas programa de hospitales con programa de control identificando a) Coberturas de desparasitación 2020/21; b) Ocurrencia de casos de COVID 19; c) Asociación posterior con casos de EQ en niños de 0 a 14 años; d) Información de registros de años anteriores del programa de prevalencia canina y en niños.

METODOLOGÍA, MODELOS y TÉCNICAS.

Los protocolos siguientes están planteados secuencialmente en función de los pasos requeridos para alcanzar las capacidades planteadas.

PROTOCOLO DE OBTENCION DE PROTOESCOLICES A PARTIR DE QUISTES HIDATIDICOS

Normalmente los quistes se encuentran en los órganos filtro, hígado y pulmón (vísceras rojas) son de color blanquecinos de tamaño entre 1 hasta 5 o más centímetros de diámetro con forma de pelota. Generalmente se encuentran en animales adultos.

Estos quistes serán destinados para elaboración de conjugados para realizar técnicas diagnósticas para vigilancia.

Toma de muestra: colocar los órganos o la parte de los órganos con quistes en continentes plásticos (en lo posible) evitando la ruptura de los mismos, importante es que no queden al alcance de las mascotas.

Los continentes ideales pueden ser baldes o frascos plásticos con tapa y que se puedan sellar (tipo envases de miel grandes).

Aquellos que cuenten con solución fisiológica los pueden colocar en la misma hasta ser recogidos o enviados al receptor.

Rotular Envase: colocando fecha de extracción, establecimiento de origen y órgano que contiene el quiste (aclarar si es ovino, caprino o bovino).

Avisar inmediatamente a los receptores involucrados para coordinar el retiro o la remisión.

En caso de que sean transportados, tratar de garantizar el cerramiento de los frascos (se puede reforzar el cierre de la tapa con cinta adhesiva) para evitar el vuelco y/o ruptura de los quistes, envolver el frasco con papel absorbente tipo servilleta de papel o diario.

Se recomienda mantener en lugares frescos, ideal mantener y transportar refrigerados y sin contacto con luz solar.

PROTOCOLO DE INFECCION EXPERIMENTAL DE MERIONES (*Meriones unguiculatus*) PARA OBTENCION DE EQ

Se utilizarán 6 meriones de 4 semanas de edad.

Los animales serán experimentalmente infectados por vía oral con aproximadamente 3000 protoescólices concentrados en 0.5 ml de solución fisiológica y bajo una leve anestesia con éter.

Los protoescólices se obtendrán de necropsias de ovinos o caprinos naturalmente infectados faenados en mataderos de la Provincia de La Pampa o de la Provincia de Río Negro.

Una alícuota de protoescólices y de la membrana germinal se conservará en alcohol 70 para su envío al Instituto Nacional de Microbiología Carlos Malbrán o al Laboratorio de parasitología de la Universidad Nacional del Comahue.

Los meriones serán alojados en jaulas ubicadas en el bioterio de la Facultad de Ciencias Veterinarias de la UNLPAM.

Serán alimentados con alimento concentrado comercial y agua *ad libitum.*

Los animales serán inyectados en forma subcutánea con una dosis de 2 mg / metilprednisolona acetato (MTPA) cada tercer día desde dos días antes de la infección a 42 días después de la infección.

Para la infección experimental, quistes hidatídicos en estado hialino serán punzados para la extracción del líquido hidatídico con una jeringa de 10 ml y aguja 20 x 1". Las membranas serán lavadas con solución fisiológica y raspadas para despegar los protoescólices restantes.

El líquido se colocará en un tubo Falcon de 50 ml, donde se espera que sedimente para retirar el sobrenadante.

Se evaluará la viabilidad de los protoescólices con el colorante vital eosina en solución acuosa al 0.1% y también por observación al microscopio de las células flamígeras y de los movimientos contráctiles de los protoescólices.

Desde la ubicación de los meriones en jaulas la materia fecal será recogida y guardada en bolsas rojas para su disposición final a cargo de la empresa que retira los residuos patológicos de la Facultad.

Desde la llegada de los meriones a las jaulas, hasta el día 30 post infección el manejo general de la bioseguridad será el normal para el manejo de animales de laboratorio, no requiriendo de medidas especiales de bioseguridad. El personal responsable del manejo utilizara las medidas normales de bioseguridad en lo referente a ropas de protección e higiene general.

Desde el día 30 hasta el día 50 las medidas de bioseguridad deberán asegurar la protección de los operadores, la esterilización de los materiales usados para la recolección de la materia fecal y la desinfección de las jaulas.

El día 50 se procederá al sacrificio de los 2 primeros meriones de acuerdo a normas.

En el sacrificio, se extirpará el intestino delgado, dividiéndose en tres porciones iguales (proximal, media y distal), abierto longitudinalmente y colocado en placas de Petri con solución salina. Se raspará la mucosa y el contenido será examinado bajo el microscopio en cámara con flujo laminar. Los parásitos serán contados y examinados cuidadosamente para determinar su movilidad y etapas de desarrollo. De resultar los EG maduros se procederá al sacrificio de los restantes.

Los EG serán guardados en solución fisiológica para su envío a los laboratorios del Instituto Nacional de Microbiología y el Laboratorio de parasitología de la Universidad Nacional del Comahue.

PROTOCOLO DE OBTENCION DE MUESTRAS EN PERROS

Los Procedimientos para la toma de muestras de heces caninas incluyen:

Muestra: la unidad muestral es una porción de heces de canes que está dispersa en el suelo de una unidad epidemiológica (UE=vivienda rural).

Obtención de la muestra: la muestra puede ser indistintamente materia fecal recién emitida, líquida, sólida o semisólida, la cual deberá ser recogida evitando la contaminación excesiva con tierra, pastos u otros contaminantes del suelo. Si no hay heces frescas, se recogerán muestras sólidas emitidas en los días anteriores al día de la visita de recolección.
Volumen de la muestra: cuando se recogen heces frescas se toma el equivalente a dos cucharas soperas colmadas. Si se toman heces secas se recoge toda la deposición. En caso de que fuera muy voluminosa es necesario fraccionarla, tomando partes de diferentes sitios del conjunto.
Envase de la muestra: las muestras se recogen en envases de plástico secos y limpios con tapa a rosca. No se adiciona ningún conservante.
Transporte de la muestra: las muestras se colectarán siempre individualmente. Todas las muestras se incluyen en una bolsa mayor que las contenga. La identificación de cada bolsa debe ser completa. Para el transporte se coloca la bolsa en una caja de telgopor. Con el fin de cumplir con las normas de bioseguridad en el transporte de muestras, esta caja debe estar incluida dentro de otra de mayor tamaño, separadas por abundante papel absorbente. La última caja debe tener claramente identificada la parte de arriba y la indicación de no volcarla.
Conservación de las muestras: si las muestras no pueden ser enviadas inmediatamente al laboratorio de referencia, se mantendrán refrigeradas a la menor temperatura disponible. Se pondrán en un freezer (una semana a -70ºC o dos semanas a -20ºC) afuera de las cajas de telgopor pero contenidas adentro de las bolsas que reúnen independientemente a cada unidad epidemiológica. Es importante considerar la correcta conservación de las muestras ya que el frío es crítico, puesto que evita o interrumpe la actividad enzimática de la materia fecal que degrada a los antígenos parasitarios.
Los lugares de muestreo serán georreferenciados.

PROTOCOLO DE PROCESADO DE MATERIA FECAL PARA COPROELISA

Tomar muestras de materia fecal de perros recién emitida o seca (de varios días de deposición, aunque preferentemente lo más fresca posible). El número de muestras a obtenerse de cada establecimiento ganadero o unidad epidemiológica deberá ser protocolizado en relación al número de perros existentes en el predio.
Colocar las muestras en doble bolsa de nylon o en frascos tipo recolector de orina. Rotular las muestras. Colocar el rótulo entre ambas bolsas si se usa este método. Completar una planilla global de datos de recolección. Conservar en la heladera o en lugar fresco hasta que se envían al laboratorio.
Una vez recibidas en el laboratorio, congelar a – 80º C durante 48 hs, o a – 70º C por 4 días.
En laboratorio 1) colocar una porción de aproximadamente 1.5 a 2.0 ml de MF con una cuchara de plástico descartable para cada muestra, en un tubo de centrífuga de plástico con tapa a rosca. En caso de que la muestra sea demasiado seca, ésta puede dejarse hidratando con el buffer de extracción desde la noche anterior. 2) Agregar al tubo con MF igual volumen de buffer PBS 0.15 M con 0.3% de Tween 20 a temperatura ambiente. Agitar enérgicamente o bien con vórtex durante 30 segundos como mínimo. Centrifugar los tubos a 3500 rpm durante 30 minutos a temperatura ambiente. Separar el sobrenadante en tubos tipo Eppendorf, descartando el pellet. Rotular con el número de muestra y guardar a –20ºC hasta su procesamiento. Los extractos pueden conservarse hasta 4 a 6 meses en estas condiciones. 3) En todo momento respetar normas de bioseguridad por tratarse de muestras potencialmente patógenas.

TEST DE COPROANTÍGENO POR ELISA:

Se utilizará la técnica descripta por Allan *et al* (1992) con algunas modificaciones de Pierangeli et al., según se describe a continuación.

Día 1:

1) Preparar la dilución del antisuero de conejo anti *E. granulosus* en la concentración óptima establecida en buffer carbonato/ bicarbonato 0.05 M pH 9.6. (Dilución para LOTE 6 en uso 1/200: 55 µl + 11 ml buffer carbonato para 1 placa).

2) Sensibilizar cada pocillo de fondo plano de la placa de microelisa con 100 µl de la dilución del antisuero. Cubrir con papel film y llevar en cámara húmeda a 4ºC toda la noche.

Día 2:

3) Rotular las placas, si es necesario remarcar algunos números de columna.

4) Volcar el contenido de la placa y hacer 3 lavados con **200 µl** por pocillo de buffer PBS 0.15 M pH 7.2 con 0.1% de Tween 20 cada vez (pipeta multicanal). Cada lavado dura 5 minutos, volcar la placa y absorber el exceso de líquido con papel absorbente cada vez.

5) Sacar del freezer del box de procesamiento de muestras el suero fetal bovino (SFB), los controles positivos y negativos y los extractos de las muestras a analizar.

6) Bloquear la placa con 100 microlitros por pocillo de buffer PBS 0.15 M con 0.3% de Tween 20. Cubrir con papel film y dejar reposar 1 hora a temperatura ambiente.

7) Armar el cuadrito con la disposición de las muestras en cada placa.

8) Lavar como en el paso 4.

9) A cada pocillo agregar 50 microlitros de SFB inactivado (alicuotado en freezer a –20ºC) y 50 microlitros del extracto de MF canina (micropipeta individual violeta). En cada placa incluir blancos (SFB sin muestra), controles negativos y positivos, por duplicado. Agregar 50 microlitros de buffer PBS 0.15 M con 0.3% de Tween 20 a los blancos. Cubrir con papel film e incubar durante 1 hora a 37ºC en cámara húmeda. 10) Lavar como en el paso 4.

11) Sacar del freezer a -20°C el eppendorf en uso del conjugado.

12) Realizar la dilución del conjugado marcado con peroxidasa en la concentración óptima establecida, con buffer PBS 0.15 M Tween 0.1%. Agregar 100 microlitros por celda. Incubar 1 hora en cámara húmeda a 37ºC.

13) 15 minutos antes de finalizar la incubación con el conjugado preparar la solución de trabajo del sustrato ABTS y conservar en la oscuridad hasta su utilización.

14) Pegar la planilla de la placa en el cuaderno de registro de Coproantígeno.

15) Lavar como en el paso 4.

16) Agregar 200 µl por pocillo de la solución de trabajo del sustrato ABTS. Incubar por 10 a 15 minutos a 37ºC en cámara húmeda en la oscuridad. Al cabo de este tiempo, verificar el desarrollo de color en la placa. Puede dejarse más tiempo en caso de observase color verde muy tenue.

17) Frenar la reacción de color agregando 100 µl por pocillo de ácido fluorhídrico 0.1 M pH 3.5.

18) Leer y registrar las absorbancias en lector para ELISA a 405 nm

- Los resultados negativos no requieren confirmación
- Todos los resultados positivos deben ser confirmados, repitiendo la determinación en una nueva placa.
- Dos resultados POSITIVOS confirman la presencia de coproantígeno.
- En caso de discordancia (POS/NEG), realizar una 3ª determinación (desempate).

PROTOCOLO DIAGNOSTICO EN OVINOS Y CAPRINOS

En animales vivos de zonas de producción ovina y caprina se obtendrá 10 cc. de sangre de la vena yugular, de animales sujetados en posición de pie y con la cabeza fijada lateralmente, utilizándose agujas 25/8 y jeringas plásticas descartables.

Los tubos serán rotulados con el número de identificación del animal y la fecha de obtención.

El suero se extraerá mediante centrifugación, se conservará refrigerado a 5º/8 ºC hasta su remisión a laboratorio (48 hs. máximo) en donde se mantendrá en freezer a –20 ºC hasta su procesado.
Las muestras de suero serán procesadas mediante ELISA con antígenos recombinantes en el Laboratorio del Instituto Cesar Milstein.
También se efectuarán necropsias en caprinos y ovinos faenados en mataderos provinciales. Los quistes hidatídicos detectados serán conservados en alcohol 70 para determinación de cepas en el Laboratorio de Parasitología de la Facultad de Medicina de la UNCOMA.

PROTOCOLO DE PROCESADO MEDIANTE PCR PARA LA CONFIRMACION DE COPROELISA POSITIVOS

Las técnicas en uso en la Argentina se encuentran estandarizadas y validadas tanto en el Instituto Nacional de Microbiología Carlos Malbrán (Cabrera et al, 2002; Jercic et al, 2019) como en el laboratorio de parasitología de la Universidad del Comahue.

ÁREA DE TRABAJO Y PROCEDIMIENTOS ESTADISTICOS

Las tareas se efectuarán en la Provincia de La Pampa, en áreas de producción ovina y caprina con especial referencia al oeste pampeano y en áreas de la Provincia de Rio Negro con programa sistemático de control, con especial referencia al este de la Provincia.9.
El análisis estadístico de los resultados se efectuará con EPIDAT 3.1 estimándose proporciones y sus intervalos de confianza del 95%. Chi cuadrado de asociación con un nivel de significación de p = 0.05 para comparar prevalencias, así como se estimarán los OR.
Los lugares de muestreo serán georreferenciados, incorporándose la información a un Sistema de Información Geográfico (SIG) desarrollado en QGIS 3.4.6, asociándose la geolocalización al área programa de salud.

6.3 CONTRIBUCIÓN AL CONOCIMIENTO CIENTÍFICO Y/O TECNOLÓGICO Y A LA RESOLUCIÓN DE LOS PROBLEMAS

La identificación de la dispersión y la prevalencia de EQ, permitirá a las autoridades sanitarias direccionar programas de vigilancia, prevención y control de la EQ con un enfoque racional, ajustando las acciones en función de la identificación de áreas de riesgo.
Asimismo, la producción de EG adultos permitirá a los laboratorios nacionales de referencia la producción de reactivos para diagnóstico.

FORMACION DE RECURSOS HUMANOS

El proyecto contribuirá a la formación de los RRHH de los componentes del equipo de investigación, en aspectos de metodología de investigación en general y en particular en el desarrollo de procesos de inoculación experimental y manejo en bioterio.
Muy especialmente se formarán RRHH de laboratorio para el diagnóstico de la hidatidosis mediante PCR y ELISA y en parasitología para el manejo y procesado de muestras ambientales y de quistes hidatídicos.
En particular se espera la formación de a) un profesional de la Cátedra de Epidemiología con el título de doctorado y b) una estudiante de la especialización en SPVET desarrolle su Tesina a partir de este proyecto.

6.4 CRONOGRAMA ANUAL de ACTIVIDADES

Obtención de quistes y meriones	enero a marzo 2023
Inoculación y recuperación de parásitos	abril a julio 2023
Capacitación de laboratoristas	marzo a junio 2023

Muestreo en el ambiente de MF	marzo a noviembre 2023 marzo a noviembre 2024
Muestreo serológico ovino y caprino	marzo a noviembre 2023 marzo a noviembre 2024
Procesado de MF y coproELISA	marzo a noviembre 2023, marzo a noviembre 2024
PCR	marzo a noviembre 2023 marzo a noviembre 2024
Informe final	diciembre 2024
Publicación de resultados	diciembre 2024

7 INFRAESTRUCTURA Y PRESUPUESTO

7.1 INFRAESTRUCTURA, EQUIPAMIENTO, SERVICIOS Y OTROS BIENES REQUERIDOS POR EL PROYECTO YA EXISTENTES EN ESTA INSTITUCIÓN:

Infraestructura de la Cátedra Parasitología y Enfermedades Parasitarias, del Centro de Producción de Animales de Experimentación y del Laboratorio de Biología Molecular de la Facultad de Ciencias Veterinarias de la UNLPam.

7.2 INFRAESTRUCTURA, EQUIPAMIENTO, SERVICIOS Y OTROS BIENES NECESARIOS PARA EL PROYECTO Y NO DISPONIBLES EN ESTA FACULTAD

- Laboratorio de Zoonosis del Ministerio de Salud de Rio Negro
- Laboratorios de la Cátedra de Parasitología de la Facultad de Medicina de la Universidad Nacional del Comahue

7.3 JUSTIFICACIÓN DE LA ADQUISICIÓN O FACTIBILIDAD DE ACCESO EN CONDICIONES DE PRESTAMO O USO DE LOS BIENES NO EXISTENTES EN ESTA INSTITUCIÓN

7.4 ESPECIFICAR OTRAS FUENTES DE FINANCIACIÓN:

Programa de control de Zoonosis de la Provincia de La Pampa y de la Provincia de Rio Negro

7.5 PRESUPUESTO ESTIMADO PARA EL PROYECTO PRESENTADO

Básicamente el presupuesto está dirigido a material para toma de muestras, financiar la capacidad analítica para los análisis serológicos de ELISA (drogas y reactivos) y el trabajo en terreno de investigadores (considerando que el área de trabajo incluye zonas rurales de La Pampa y de Rio Negromataderos ubicados en Santa Isabel, lo que requiere de varias salidas a terreno para toma de muestras, además de la publicación de resultados y presentación a congresos).
También el mantenimiento de los animales de experimentación y equipamiento de bioseguridad

	Total, solicitado: $ 91.000,00
Insumos (reactivos Lab., agujas y jeringas, material recolección materia fecal)	$ 70.000,00
Bibliografía	$ 1.000,00
Viajes y viáticos salidas a campo y muestreo	$ 15.000,00

Viajes y viáticos congresos:	$ 5.000,00

El 50% se requerirá el primer año, 25% los siguientes dos años

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ANEXO II

Infección experimental de jerbos (*Meriones unguiculatus*) con protoescólices provenientes de metacestodes de *Echinococcus granulosus.*
CePAE – CIDEF - FCV UNLPam

Proyecto de investigación: "Identificación de áreas de riesgo de transmisión de la Equinococosis quística /Hidatidosis en las Provincias de La Pampa y Rio Negro y desarrollo de capacidades diagnósticas en salud animal"

Protocolo de bioseguridad

Para la elaboración de este protocolo, se siguieron las recomendaciones de la Organización Mundial de la Salud (*WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern*, ISBN 92-9044-522-X), publicado en 2002, y las Ordenanzas municipales Nro. 246/96 y Nro. 58/97. Consideramos su lectura un requisito indispensable para las personas profesionales en áreas de la salud que llevarán adelante el trabajo de laboratorio y que están implicadas en este proyecto.

Materiales e insumos:

- ✓ Autoclave para esterilización por calor húmedo.
- ✓ Lavarropas con temperatura y tiempo programable.
- ✓ Lupa binocular.
- ✓ Freezer de -20 °C.
- ✓ Heladera.
- ✓ Material de cirugía.
- ✓ Recipientes para esterilizar por calor húmedo.
- ✓ Bolsas rojas, 60 cm x 75 cm, diámetro ≥ 100 µm.
- ✓ Precintos de seguridad, resistentes y combustibles.
- ✓ Hipoclorito de sodio (NaOCl 100 %).
- ✓ Etanol 96 %.
- ✓ Etanol 70%.
- ✓ Formaldehído 38 %.
- ✓ Solución salina al 0,9 %.
- ✓ Guantes.
- ✓ Barbijos.
- ✓ Gafas de seguridad.
- ✓ Máscaras de seguridad.
- ✓ Camisolines.
- ✓ Cofias.
- ✓ Cubrecalzados.
- ✓ Jeringas y agujas.
- ✓ Droga inmunosupresora.
- ✓ Jaulas para animales de experimentación.
- ✓ Viruta.
- ✓ Alimento balanceado.
- ✓ Bebederos.
- ✓ Cánulas de inoculación.

- ✓ Contenedores rígidos para descartes corto-punzantes.

Recepción de metacestodes obtenidos de frigoríficos.

1) Los quistes hidatídicos deberán llegar a las instalaciones del CePAE en un recipiente primario debidamente rotulado e identificado, detallando los siguientes aspectos: Remitente, lugar y fecha de obtención, origen de la muestra, temperatura de traslado. El recipiente primario deberá estar contenido en un segundo recipiente para evitar derrames.
2) Se desinfectarán las paredes externas del recipiente primario con etanol 70 % y se almacenará a 2 - 8 °C hasta su ulterior procesamiento.
3) Previo a la manipulación de los metacestodes, las mesadas de acero inoxidable, el material de cirugía, y demás superficies serán desinfectadas con etanol 70 %.
4) Al momento de la manipulación de los metacestodes para la extracción de protoescólices, las personas profesionales de laboratorio deberán emplear guantes, gafas de seguridad, barbijo, cofia, guardapolvo, camisolín y cubrecalzados.
5) Luego de realizar la infección experimental de roedores, el remanente del material biológico proveniente de los metacestodes se inactivará en etanol 40 % o superior, y se almacenará a -20 °C por al menos 1 semana. Posteriormente, se enviará a incinerar con un seguimiento estricto (ver "Gestión de residuos patológicos", Tipo A).
6) Sobre la mesada de trabajo se dispondrán recipientes con solución de hipoclorito de sodio (NaOCl) 3,75 % para colocar el instrumental de acero inoxidable y material de vidrio al finalizar su uso. Se dejarán sumergidos al menos 5 min antes de proceder a la esterilización por calor húmedo para su posterior reutilización.
7) El material de plástico descartable se colocará en solución de NaOCl 3,75 % y se enviará a incinerar (ver "Gestión de residuos patológicos", Tipo A).
8) Las bandejas y las mesadas de acero inoxidable se desinfectarán con solución de NaOCl 3,75 % por 1 h.
9) Los pisos pueden recubrirse con láminas plásticas desechables, cuya desinfección con NaOCl 3,75 % o superior se realizará por al menos 2 h y luego se enviará a incineración (ver "Gestión de residuos patológicos", Tipo A).
10) Los elementos de protección individuales deberán ser esterilizados por calor húmedo en autoclave y luego desechados para su incineración, en caso de ser descartables, o lavados en lavadora a 60°C durante 1h en caso de ser reutilizables.

Manipulación de jerbos post-infección:

Los jerbos experimentalmente infectados serán confinados en cajas en grupos de 3 individuos prepúberes al inicio del protocolo, machos y hembras indistintamente. El desarrollo de los portoescólices a tenias sexualmente maduros conlleva 4-6 semanas. Si bien durante las primeras 3 semanas no se requiere de un manejo diferencial, para garantizar la seguridad de las personas y del medio, la reposición de las camas se llevará a cabo con una frecuencia semanal. Se individualizará la materia fecal por jaula y se inspeccionará la presencia de huevos. El descarte se almacenará en doble bolsa roja precintada y se enviará a incineración con un seguimiento estricto (ver "Gestión de residuos patológicos", Tipo B). Las personas profesionales responsables que llevarán adelante esta tarea utilizarán guardapolvo, cofia, gafas de seguridad, barbijo, camisolín y cubre-calzados. En el mismo recinto se dispondrá de una batea plástica con

solución de NaOCl 3,75 % para decontaminación semanal de jaulas y bebederos. Durante el período de postinfección experimental de jerbos, toda manipulación de animales, viruta y materia fecal, quedará reducido a un único recinto. Las superficies de trabajo y el piso se limpiarán previamente con NaOCl 3,75 %, y al terminar la manipulación con una solución de NaOCl más concentrada durante 2 a 3 h. En la mesada se dispondrán recipientes con NaOCl 3,75 % para decontaminación de material en contacto con animales, que se esterilizarán por calor húmedo en caso de ser reutilizables, o se enviarán a incinerar en caso de ser descartables.

Necropsia de jerbos presuntamente infectados para obtención de parásitos maduros de *E. granulosus*

1) Sobre la mesada de trabajo se dispondrán recipientes con solución de NaOCl 3,75 % para colocar el instrumental de acero inoxidable y material de vidrio al finalizar su uso. Se dejarán sumergidos al menos 5 min antes de proceder a la esterilización por calor húmedo para su posterior reutilización.
2) El material de plástico descartable se colocará en solución de NaOCl 3,75 % y se enviará a incinerar (ver "Gestión de residuos patológicos", Tipo A).
3) Las bandejas y las mesadas de acero inoxidable se desinfectarán con solución de NaOCl 3,75 % por 1 h.
4) Los pisos pueden recubrirse con láminas plásticas desechables, cuya desinfección con NaOCl 3,75 % o superior se realizará por al menos 2 h y luego se enviará a incineración (ver "Gestión de residuos patológicos", Tipo A).
5) Los elementos de protección individuales deberán ser esterilizados por calor húmedo en autoclave y luego desechados para su incineración, en caso de ser descartables, o lavados en lavadora a 60°C durante 1 h en caso de ser reutilizables.
6) Las carcasas e intestinos de los jerbos se enviarán a incinerar con seguimiento estricto (ver "Gestión de residuos patológicos", Tipo B).

Gestión de residuos patológicos

La gestión de residuos patológicos se encuentra bajo la supervisión del Departamento de Saneamiento Ambiental, Dirección de Gestión Ambiental, Secretaría de Ambiente y Servicios Públicos, Municipalidad de General Pico (Teléfono corporativo de contacto: 02302 - 53 - 4133). Las disposiciones se rigen por las Ordenanzas municipales Nro. 246/96 y Nro. 58/97.

La Facultad de Ciencias Veterinarias se encuentra inscripta en un Registro Municipal de Generadores de Residuos Patológicos con el N° 8. Posee una frecuencia de recolección de residuos semanal. Para ello, los residuos deben almacenarse en doble bolsa roja con precinto de seguridad inviolable, resistente y combustible y rotuladas con la leyenda "Residuos Patológicos".

Las actividades realizadas en este proyecto generarán dos tipos de residuos patológicos: los que previamente serán inactivados/esterilizados antes de descartarse en bolsa roja (Tipo A), y los que, hasta que no se adquieran instrumentos específicos, deberán descartarse sin esterilización previa (Tipo B). Este último tipo de residuos patológicos (Tipo B) serán almacenados en doble bolsa roja desde el momento de su generación. Por instrumentos específicos, nos referimos a autoclave para esterilizar viruta de descarte y freezer (-20 °C) para almacenamiento prolongado de residuos patológicos.

ANEXO III

WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern

World Health Organization

World Organisation for Animal Health

WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern

Edited by

J. Eckert, M.A. Gemmell, F.-X. Meslin and Z.S. Pawłowski

- Aetiology
- Echinococcosis in humans
- Echinococcosis in animals
- Diagnosis
- Treatment
- Ethical aspects
- Geographic distribution
- Surveillance
- Epidemiology
- Control
- Prevention
- Methods

Cover image: *Echinococcus granulosus*
Courtesy of the Institute of Parasitology, University of Zurich

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Chapter 2

Echinococcosis in humans: clinical aspects, diagnosis and treatment

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Summary

In humans, three forms of echinococcosis are known to occur: cystic echinococcosis (CE), caused by Echinococcus granulosus, *alveolar echinococcosis (AE), caused by* E. multilocularis, *and polycystic echinococcosis (PE), due to* E. vogeli *or* E. oligarthrus. *In this Chapter, the natural history, clinical presentation, diagnosis and treatment of these diseases are described. The diagnostic repertoire includes imaging techniques, mainly ultrasound (US) and computed tomography (CT) examination for abdominal echinococcosis and X-ray for lung echinococcosis, and immunodiagnostic tests. The US examination can be used under field conditions for population screening. Today, treatment options for CE include: surgery, PAIR (puncture, aspiration, injection, reaspiration) and chemotherapy. For AE, the first choice of treatment in all operable cases is radical surgical resection of the entire parasitic lesion from the liver and all affected organs. After radical surgery, chemotherapy is indicated for at least two years. Long-term chemotherapy is mandatory after incomplete resection of lesions, in inoperable patients (including patients after interventional procedures) and in AE patients after liver transplantation. Ethical aspects related to research, novel diagnostic or therapeutic approaches and population-based studies are discussed.*

2.1. Forms of echinococcosis in humans

Echinococcosis in humans is an infection which is caused by a larval stage, the metacestode, of *Echinococcus* species and may result in asymptomatic infection to severe disease; it may even be fatal. The metacestodes of all four recognised *Echinococcus* species can infect humans and cause various forms of echinococcosis (Table 2.1.). Among these forms cystic and alveolar echinococcosis are of special medical importance.

Table 2.1.
Forms of echinococcosis in humans (3, 84)

Form of echinococcosis	Causative agent	Disease synonyms
Cystic echinococcosis	*Echinococcus granulosus*	Hydatid disease, hydatidosis, *E. granulosus* echinococcosis
Alveolar echinococcosis	*Echinococcus multilocularis*	Alveolar hydatid disease, *E. multilocularis* echinococcosis
Polycystic echinococcosis	*Echinococcus vogeli*	*E. vogeli* echinococcosis
Polycystic echinococcosis	*Echinococcus oligarthrus*	*E. oligarthrus* echinococcosis

Although *Echinococcus granulosus* and *E. multilocularis* occur simultaneously in large endemic areas, mixed infections of cystic echinococcosis (CE) and alveolar echinococcosis (AE) in humans are apparently rare (125).

With regard to the mode of infection the following two entities have to be distinguished.

Primary echinococcosis

Metacestodes develop in various sites of the human body from oncospheres liberated from ingested eggs of *Echinococcus* spp. In CE, parasite cysts may establish in virtually all anatomic sites, but the liver and the lung are the most frequently affected organs. In AE, the liver is involved in 98% to 100% of the cases as primary site of metacestode development, but in later phases metastases may establish in other organs (see below).

Secondary echinococcosis

Metacestode material spreads from the primary site to adjacent or distant organs and proliferates. In CE, this form occurs after release of viable parasite material (protoscoleces, small daughter cysts) during invasive treatment procedures or after spontaneous or trauma-induced cyst rupture (129). Secondary echinococcosis in AE is caused by the tumour-like proliferation of the metacestode with direct infiltration of adjacent organs or by metastasis formation in distant organs due to spreading of parasite cells via lymph and blood vessels (3, 32, 69).

A uniform terminology related to *Echinococcus* and echinococcosis has been recently proposed and is used in this document (84).

2.2. Cystic echinococcosis

Several review papers or monographs on human CE have been published in recent years (2, 3, 5, 6, 45, 74, 83, 85, 129). For further references of original papers the reader is referred to these sources.

2.2.1. Causative agent and course of infection

Causative agent

The causative agent of CE is the metacestode of *Echinococcus granulosus*. The metacestode develops from the oncosphere and is a cystic structure typically filled with a clear fluid (hydatid fluid) (Chapter 1). The post-oncospheral development takes 10-14 days. By this time, the bladder (measuring 60 µm-70 µm in diameter) consists of a nucleated germinal layer and a thin laminated layer which lacks nuclei. Most of the cysts grow slowly in size and become surrounded by host tissue (pericyst) encompassing the endocyst of metacestode origin. The endocyst consists of the outer laminated layer and the inner cellular germinal layer, which may form brood capsules and protoscoleces. The minimum time required for the development of protoscoleces in cysts in humans is not exactly known, but based on data from animals, it is expected to be 10 months or longer after infection (2, 85). Protoscoleces can be already formed in small cysts of 0.5 cm-2.0 cm diameter. In the same patient, fertile (with protoscoleces) and sterile (without protoscoleces) cysts may coexist. Quite frequently, smaller daughter cysts are formed within a larger mother cyst (see below). Several small single cysts growing in close proximity to each other may form clusters, thus presenting a 'polycystic' or 'multivesicular' appearance which has to be distinguished from AE and PE.

Echinococcus granulosus cysts have a variable natural course of development. According to an ultrasound study in 66 human patients in Turkana area of Kenya, about 30% of cysts grew slowly (1 mm to 5 mm per year), 43% showed a moderate growth (6 mm to 15 mm per year), 11% exhibited a more rapid increase (average: 31 mm, maximum: 160 mm per year), and 16% of cysts did not expand or had collapsed (96, 97). Partially or totally calcified cysts are not uncommon. The size of cysts is variable and ranges usually between 1 cm and 15 cm, but much larger cysts containing 48 l of cyst fluid have been noted (2). Spillage of viable protoscoleces or small daughter cysts after cyst rupture may result in secondary echinococcosis.

Course of infection

The natural history of *E. granulosus* cysts and its clinical implications are presented in Figure 2.1. The initial phase of primary infection is always asymptomatic, and small (<5 cm) well-encapsulated cysts located in organ sites, where they do not induce major pathology, may remain asymptomatic for many years or permanently (3,

83, 85). In two Italian series with 420 and 424 patients, 38% and 60% of all CE cases were asymptomatic (14, 50), but this rate may be lower in other regions.

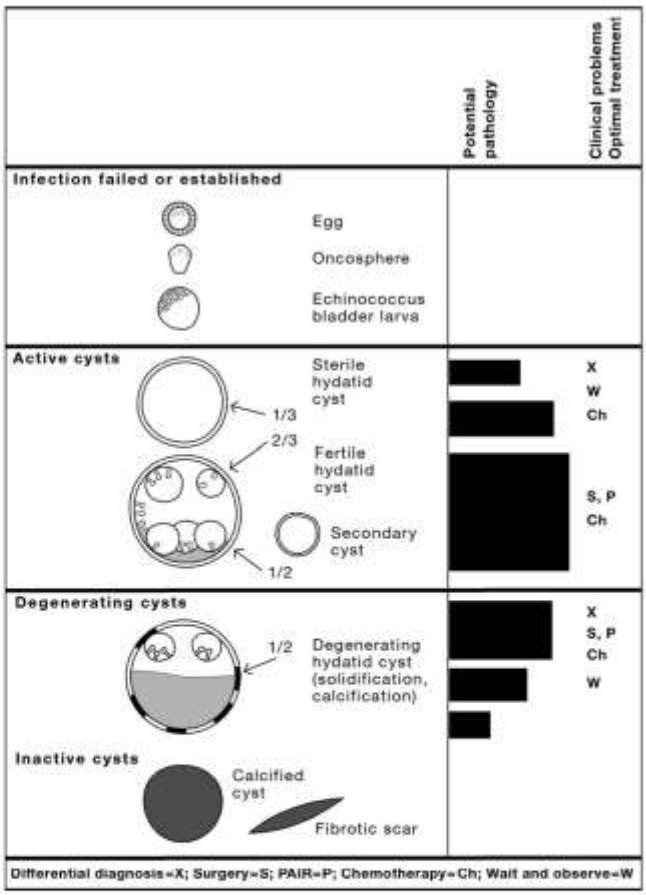

Fig. 2.1.
Natural history of *Echinococcus granulosus* liver cysts
The numbers (1/3 etc.) indicate approximate frequencies of cyst types
Reproduced from (85) with permission from F.L. Andersen (ed.)

After an undefined incubation period of several months or years, the infection may become symptomatic if cysts exert pressure on adjacent tissue and induce other pathological events. Sudden symptomatology may be due to spontaneous or traumatic cyst rupture. Spontaneous cure is possible, due to collapse and resolution of cysts, cyst calcification or cyst rupture into the bile duct or the bronchial tree with discharge of the cyst content. Recurrence of the disease may occur after operation on primary cysts (see surgery).

It is difficult to present exact data from recent years on the rates of morbidity, mortality and fatality. One of several reasons is that the terms mortality (= rate of fatal cases per 100,000 of the total population in a defined area) and fatality (= fatal cases related to the number of confirmed CE cases) are often not clearly differentiated. Therefore, only some examples are given here.

Up to 60% of the CE cases may be asymptomatic (see above), but it is assumed that some may become symptomatic with the time. In the Regional Hospital of Valdivia, Chile, a total of 137 new cases of CE was registered in 1987-1991; the mortality rate was 0.2 per 100,000 population, and the fatality rate 2.2% (34). The fatality rate is highly dependent on the severity of the infection and on facilities for treatment. For example, the fatality rate in 98 cases of CE of the heart was 23% (33), whereas this rate is around 2% (3) or less in cases of uncomplicated CE of the liver if adequate surgical facilities are available.

2.2.2. Clinical presentation

Age and sex of patients

Cystic echinococcosis may reach medical attention in almost all ages, from below 1 year of age to over 75 years old, and in both sexes. Among 1,473 patients admitted to a children's hospital in Madrid (Spain), 2% were <1 year old, 21% between 1 and 4 years and 77% between 5 and 14 years (116). In a Chinese series of 15,289 surgical cases, 49% were in males and 51% in females (70). In both sexes, case numbers reached a peak between 6 and 15 years and then decreased with successive age (Fig. 2.2.).

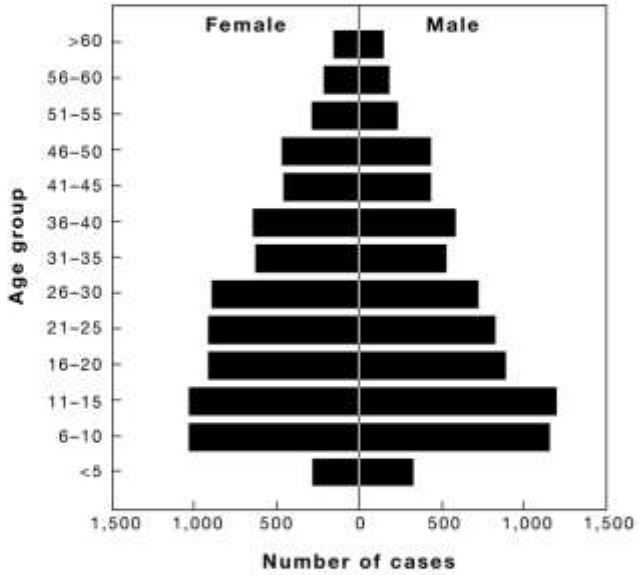

Fig. 2.2.
Age and sex distribution of surgical cases of cystic echinococcosis in Xinjiang, People's Republic of China, 1951-1990
Reproduced from (70) with permission from F.L. Andersen (ed.)

In other regions, the highest numbers of CE cases were recorded in older age groups, e.g. between 21 and 30 years (Kenya) or 21 to 40 years (Libya) (17). Further, it should be noted that the patterns of age distribution of the cases may vary with the mode of selection of patients and the technique of examination. In series of surgical patients, the frequency of interventions declines in older age groups, but it increases with age when populations are screened by ultrasound (17).

Occupation of patients

The occupational distribution of patients may vary widely from country to country depending on epidemiological and socio-economic circumstances. One example from the People's Republic of China is given in Figure 2.3.

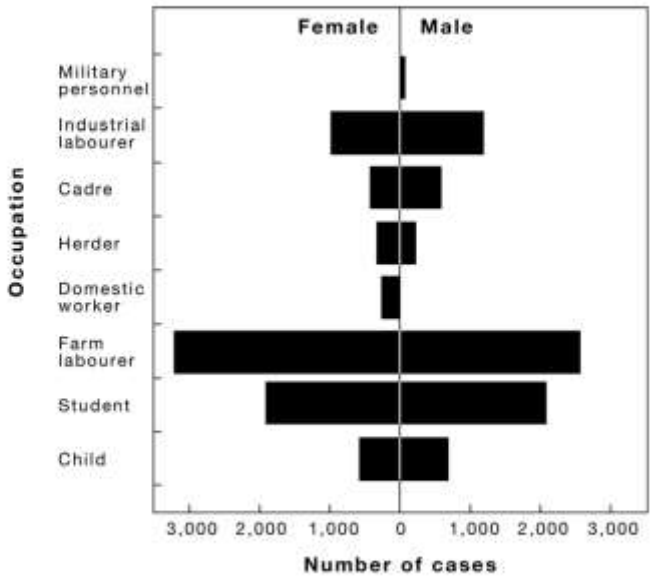

Fig. 2.3.
Occupational distribution of surgical cases of cystic echinococcosis in Xinjiang, People's Republic of China, 1951-1990
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Organ sites of cysts

Many patients (about 40% up to 80%) with CE have a single organ involved and harbour a solitary cyst. Examples of sites of the cysts in cases with single organ involvement and with single and multiple organ sites are presented in Table 2.2. Relative percentages of liver and lung locations, which together account for at least 90% of the cysts, may vary depending on the country.

Symptoms

The clinical symptomatology of CE is variable and never pathognomonic (2, 3, 74, 83, 85). The spectrum depends primarily on:

a) the organ(s) involved
b) the size of the cysts and their site within the affected organ
c) the interaction between the expanding cysts and the adjacent organ structures
d) the complications related to cyst rupture, spread of protoscoleces, and bacterial infection.

In addition, systemic immunological reactions may be observed like urticaria, asthma, anaphylaxis or membranous nephropathy (3). Presenting symptoms and signs are listed in Table 2.3. Asymptomatic liver CE is quite common and may remain symptom-free for more than ten years (37).

The course of CE may be associated with a wide spectrum of complications. Some examples of complications which may occur in cases of liver echinococcosis are presented in Table 2.4.

Table 2.2.
Organ sites of *Echinococcus granulosus* cysts in humans
A Single organ involvement in 459 patients[a] (*Source*: 30, 42)
B Single and multiple organ involvement in 15,289 Chinese surgical cases (modified from 70)

Organ	A		B	
	Number of cases	Percentage of cases	Number of cases[b]	Percentage of cases
Liver	316	68.8	11,499	75.2
Lung	79	17.2	3,432	22.4
Kidney	17	3.7	68	0.4
Spleen	15	3.3	160	1.0
Muscles and skin	10	2.2	29	0.2
Abdominal and pelvic cavity	9	2.0	794	5.2
Mediastinum, heart	5	1.1	4	0.03
Brain	4	0.9	61	0.4
Bones	3	0.6	30	0.2
Ovarium	1	0.2	9	0.06
Other organs: skin, eye, spinal cord, pancreas, urinary bladder, testis, etc.	–	–	–	Each <0.1

a) single organ involvement is indicative for cyst development after primary infection
b) the number of cases in this column exceeds the total of 15,289 since many patients had multiple organ involvement. The same applies to the percentages

2.2.3. Diagnosis

2.2.3.1. General aspects

The process of diagnosis of CE in individual patients goes through various steps, as follows:

- suspicion on clinical grounds or upon screening
- confirmation by imaging (US, CT, X-ray, etc.) and identification of characteristic or suspicious cyst structures
- confirmation by detection of specific antibodies with immunodiagnostic tests (ELISA, IFAT, immunoblot, detection of arc 5 antibodies, etc.) (Chapter 2.2.3.8.)
- in doubtful cases diagnostic puncture may be considered, if it is not contraindicated (Chapter 2.2.3.6.)
- material obtained by biopsy puncture or surgery can be examined: hydatid fluid for *Echinococcus* protoscoleces or hooks; protoscoleces for DNA by PCR; antigen from sterile cysts, and cyst wall material for characteristic structures by histology.

In many cases, a diagnosis can be made by detecting the characteristic structure and size of *E. granulosus* cysts visualised by various imaging techniques, including ultrasonography (US), computed tomography (CT) standard radiology (X-ray), and magnetic resonance imaging (MRI) in specialised centres. Introduction of US has improved both the diagnosis of CE and the understanding of the natural history of the disease (2, 3, 13, 14, 67, 68, 74, 83, 88, 93, 120, 121). The US examination is a suitable technique for population studies aimed at detecting cases and determining prevalence of CE. In this indication, US has achieved great significance in recent years since portable US units allow the application of this technique in field situations (67, 68). Immunodiagnostic tests for detecting specific antibodies are commonly used for the aetiological confirmation of the findings of imaging examinations (Chapter 2.2.3.8.).

Table 2.3.
Presenting symptoms and signs of cystic echinococcosis (3)

Organ	Symptoms and signs
Liver	'Tumour' – hepatomegaly, ± cholestasis ± jaundice
	Secondary biliary cirrhosis
	Biliary colic-like symptoms ± cholangitis or pancreatitis (elimination of fragments of the cyst via biliary tract)
	Liver abscess
	Calcified lesions in liver or spleen
	Portal hypertension ± ascites
	Inferior vena cava compression or thrombosis
	Budd-Chiari syndrome
	Cyst rupture, peritoneal spread, biliary peritonitis
	Haemobilia
	Biliary fistula to skin, bronchial system or gastrointestinal tract
Lung	Lung 'tumour' ± chest pain
	Chronic cough, expectoration, dyspnea
	Haemoptysis
	Biliptysis
	Pneumothorax
	Pleuritis
	Lung abscess
	Eosinophilic pneumonitis
	Parasitic lung embolism
Cyst rupture into biliary tree	Biliary colic
	Cholestatic jaundice
	Cholangitis
	Symptoms of pancreatitis
	Symptoms of anaphylaxis
	Fever
Cyst rupture into bronchial tree	Asthma-like symptoms
	Cough, expectoration, dyspnea
	Haemoptysis
	Symptoms of anaphylaxis
	Fever
Heart	Pain
	'Tumour'
	Cardiac insufficiency
	Embolism
	Pericardial effusion
Bone and muscles	Pain
	Bone 'outgrowth'
	Bone fragility
	Disturbances of motility
	Muscle cyst
Brain and spine	Headache
	'Tumour' with neurological symptoms
	Back pain
Eyes	Pain
	Protrusio bulbi
	Ptosis
	Visual disturbances

± : with or without

Table 2.4.
Complications in 221 patients with cystic echinococcosis

Complication and site of involvement	Number of cases	Percentage of total[a]
Biliary tract	47	21.3
Cystic rupture into bile ducts	36	
Gallbladder or common duct obstruction	9	
Fibrosis of the papilla	4	
External bile fistulas	4	
Bacterial infection	27	12.2
Intracystic	26	
Subphrenic	21	
Intraperitoneal rupture	23	10.4
Acute (anaphylactic shock)	2	
Multiple intraperitoneal cysts	21	
Hepatopulmonary cysts	20	9.0
Lung involvement, intact cyst	11	
Pericystobronchial fistula[b]	3	
Biliptysis[c]	4	
Rupture into pleural cavity	2	
Portal hypertension and gastro-intestinal bleeding	1	0.5

a) percentages refer to total number of cases; many patients had more than one complication

b) expectoration of cysts

c) vomiting of bile, in two cases in association with haemoptysis

Source: Barros, cited in (3)

A direct method of diagnosis is finding characteristic protoscoleces or hooks of *E. granulosus* in aspirated hydatid fluid specimens (Chapter 1). The method requires only a simple microscope and very basic laboratory training. This examination is not performed frequently as the material for such a direct examination can only be available after a surgical intervention, therapeutic puncture (PAIR) or diagnostic puncture. Rarely characteristic hooks or protoscoleces may be found in sputum, bile, stool or urine after a spontaneous rupture of the cysts in lungs, liver or kidneys.

The direct diagnosis can also be made by macroscopic identification of the structure and size of *E. granulosus* cysts obtained by surgery and/or by histological examination of the parasite tissue, available after surgery or biopsy (Figs 2.4. and 2.5.). More sophisticated techniques in direct diagnosis include finding of specific *E. granulosus* antigen (antigen 5) in the fluid from sterile cysts (79, 103) or DNA markers in the cysts fluid or parasite tissue (e.g. by PCR).

In view of the availability of different diagnostic methods, it is necessary to proceed rationally in selecting techniques, taking their contribution to diagnosis into account. In some cases, performing an additional imaging examination adds nothing to the diagnosis, but may well provide guidance concerning surgical procedure. It is important to select the simplest and most cost-effective method, and one that is most valid and least harmful.

Fig. 2.4.
Intraoperative situs of an opened large liver cyst with daughter cysts of *Echinococcus granulosus*
Photograph: courtesy of Professor R. Ammann, University Hospital, University of Zurich

2.2.3.2. Standard radiology

Chest radiography

This is still the technique of choice for the diagnosis of pulmonary cysts of *E. granulosus* which may display various features (2, 74, 93, 120, 121).

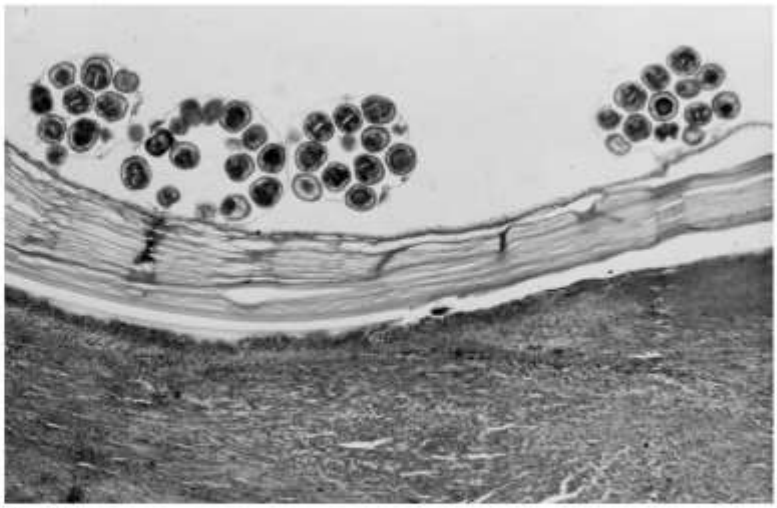


Fig. 2.5.
***Echinococcus granulosus*, histological section through cyst wall**
Reproduced from (120) with permission from Elsevier Science

- **Uncomplicated cysts**

Uncomplicated cysts are clearly defined, usually round or oval structures with diameters between 1 cm and >20 cm, displaying a homogeneous shadow indicating a fluid-filled space. They may also occur as thin-walled 'empty' cysts. The cysts may be located anywhere in the lung as solitary or multiple cysts. Pulmonary cysts usually do not calcify, and daughter cyst formation is rare.

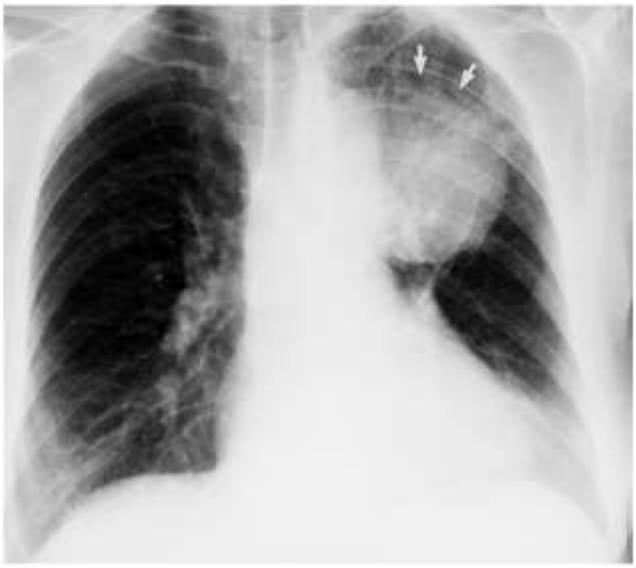


Fig. 2.6.
Radiograph of *Echinococcus granulosus* lung cyst (diameter: 6 cm)
Arrows indicate a small 'meniscus sign' suggesting the presence of a hydatid cyst
Reproduced from (120) with permission from Elsevier Science

- **Complicated cysts**

Complicated cysts may exhibit the following:

a) the 'air meniscus sign' caused by air entering the space between ecto- and endocyst producing a radiolucent shadow (= pneumocyst)

b) the 'double arch sign' caused by the ectocyst (outer arch) and the detached wall of the endocyst (inner arch); and

c) the 'water-lily sign' indicative of collapsed wavy endocyst membranes floating on top of the remaining cyst fluid.

Following rupture of the cyst, the endocyst may be ejected completely, leaving a cavity that may retract or become infected with bacteria. Radiography may also show lobar homogenous consolidation of lung parenchyma.

- **Other findings**

Chest X-ray images may also show upward displacement of the diaphragm, possibly indicative for a hydatid cyst of the liver; asymmetry of the heart outline, which may be a sign of a hydatid cyst of the heart to be confirmed by US or CT; pleurisy and pneumothorax in the event of rupture of a hydatid cyst into the pleura; a costal subpleural cyst.

- **Differential diagnosis**

Cysts filled with clear fluid, with an air shadow or with water-lily sign are pathognomonic. If a rounded parenchymatous opacity is seen, it is necessary to consider tuberculoma, a tumour or pulmonary sequestration. A fluid and air shadow will lead to consideration of a bacterial, fungal or amoebic abscess.

Plain abdomen radiography

In case of an abdominal cyst site, a fluid-type shadow may be seen, displacing the air-filled radiolucent areas of the digestive tube. The best indicator for a hydatid origin is the presence of calcifications, which may be crescent-shaped, or like homogenous or heterogenous globules, or ring-shaped. This examination should be supplemented by US or CT.

Bone radiography

Bone localisations of cysts are not common (<1% of CE cases). In about 50% of such cases, the site is the spine. At the initial stage, one or more lacunae are seen in the body or posterior arch of the vertebra. At a more advanced stage, an extension is seen to the adjacent vertebral bodies, with involvement of the neighbouring bones (ribs and iliac bone) (93).

2.2.3.3. Ultrasonography

General aspects

Abdominal US has overturned the hierarchy of diagnostic methods. It can be used not only to detect abdominal cysts and determine their number, site and dimensions (cyst >1 cm), but also to identify whether they are hydatid in nature and their relationships with other organs. Schemes for classification of *E. granulosus* cysts have been proposed by various authors, including Gharbi *et al.* (40), Caremani *et al.* (13), and Perdomo *et al.* (88, 89). Recently, an expert committee of the WHO Working Group on echinococcosis has presented a proposal for an internationally agreed classification of US images in hepatic CE (Table 2.5.).

- **Hepatic cysts**

The classification system proposed by the WHO Informal Working Group on Echinococcosis is presented in Table 2.5. For more details see the Working Group document (130).

Cysts in other abdominal sites

Cysts in other abdominal sites are less common and are located in spleen, kidney, uterus and other organs. Their images are essentially similar to those observed at hepatic sites.

Differential diagnosis

Differential diagnosis poses various problems. It is difficult to differentiate simple hydatid cysts (Table 2.5., Type CL) from simple hepatic cysts, renal, ovarian, mesenteric or pancreatic cysts, from a non-organised haematoma, amoebic liver abscess or necrotic tumour. In such cases, serological examination for specific *E. granulosus* antibodies may bring an important hint to verification or exclusion of CE. Type CE 4 cysts may be difficult to distinguish from abscesses, neoplasms, AE lesions, cavernous haemangiomas and other structures. On the other hand, cysts of Types 1, 2 and 3 are usually pathognomonic and can be diagnosed with a high degree of accuracy.

Pathognomonic features of *Echinococcus granulosus* cysts

The following US images of space occupying lesions in the liver are considered to be pathognomonic for cysts of *E. granulosus*:

a) unilocular anechoic lesions which are round or oval with a clearly visible cyst wall (laminated layer) with snowflake-like inclusions or floating laminated membranes

b) multivesicular or multiseptate cysts with a wheel-like appearance

c) unilocular cysts with daughter cysts with honeycomb appearance.

- **Pulmonary cysts**

For pulmonary sites, US examination is unhelpful in most cases, but it can sometimes confirm the cystic nature of a parenchymatous mass that is juxtaparietal. It will display an anechoic area with posterior strengthening.

- **Cardiac cysts**

In the cardiac site, two-dimensional US displays a mass that is echo-free or has a mixed echo structure.

- **Ultrasonography for field use**

Ultrasonography is the only imaging technique that can be used in the field. It has a number of characteristics that make it an excellent screening tool, as follows:

a) acceptability by the population

b) can explore the abdominal sites, which are most commonly infected

c) can evaluate a broad spectrum of the disease, i.e. number and location of the parasite, its stage (active, degenerating and inactive), some complications

d) can be performed by less qualified but easily trainable staff

e) it is easy to be performed in the field at low cost (Chapter 6.1.2).

2.2.3.4. Computed tomography

General aspects

Computed tomography (CT) can detect small cysts (≥1 cm in diameter), it has the potential to inspect any organ, it allows the measurement of cysts and facilitates differential diagnosis of lesions caused by *Echinococcus* metacestodes from non-parasitic lesions (2, 3, 28, 83, 119, 120, 121). It also allows the determination of the liver volume from CT transverse sections by the point-integrating method (3).

On CT, round or spherical cysts with contents near water density are easily recognised. Measurement of the cyst density is a useful diagnostic parameter, particularly for follow-up examinations during and after chemotherapy. The information obtained from CT varies depending on the organ systems to be inspected.

In one study, CT findings alone allowed a correct diagnosis in 61% of 120 patients with CE of the liver, lung, kidney, spleen and some other sites, and in 94% if CT was combined with serology (28). In another study, CT provided a correct diagnosis in 96% of 157 patients with CE of the liver and other visceral organs (3).

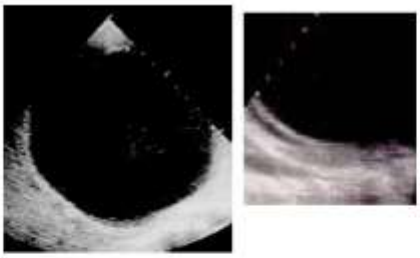
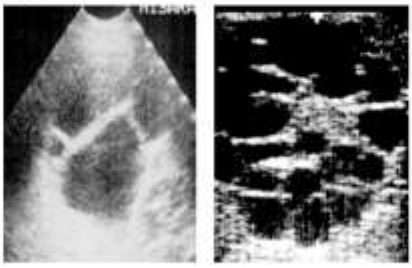
Hepatic cysts (Figs 2.7. and 2.8.)

Hepatic cysts can be diagnosed by US in the majority of cases, but CT is indicated when US diagnosis is uncertain, mainly in cysts of types CE 4 and CE 5 (Table 2.5.). Differential diagnosis of CL lesions is, however, not made easier by the use of CT. CT can detect small-sized cysts, study their content (univesicular or multivesicular), visualise membrane detachment, and provide information on the condition of the liver parenchyma and bile ducts. Pathognomonic images are membrane detachment and daughter cysts (spherical formations within a larger 'mother cyst' scattered or located at the periphery of the cyst). Completely calcified cysts that are difficult to explore by US can be studied by CT or X-ray, which usually reveal the typical 'egg-shell' pattern of calcification (1).

Pulmonary cysts

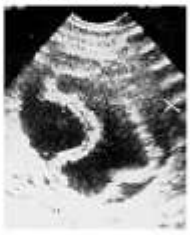
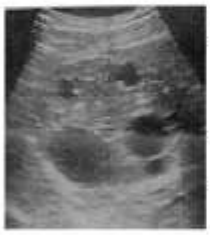
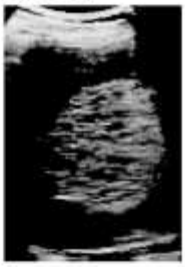
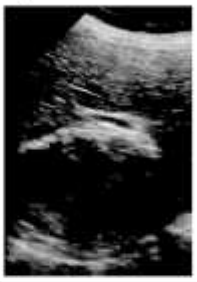
In the case of pulmonary sites, CT may add some additional information to plain X-ray examinations. It can confirm the liquid nature of a 'shadow' and visualise signs of the onset of complications, such as incipient membrane detachment or small bubbles located in the cyst wall.

Table 2.5.
Types of cystic lesions (CL) and *E. granulosus* cysts (CE) which may be found on ultrasound (US) examination of the liver *
Classification proposed by the WHO Informal Working Group on Echinococcosis (130)

Type of cyst	Imaging features and remarks (s): small; (m): medium; (l): large
Type CL 	• Status: active (if CE) • Unilocular, cystic lesion(s) (CL) with uniform anechoic content, not clearly delimited by an hyperechoic rim (=cyst wall not visible) • Normally round but may be oval • Size variable: but usually small. CL(s): <5.0 cm CL(m): 5-10 cm, CL(l): >10 cm **Remarks** If these cystic lesions are due to CE then these cysts are usually at an early stage of development and are not fertile US does not detect any pathognomonic signs Differential diagnosis of these cystic lesions require further diagnostic techniques
Type CE 1 	• Status: active • Unilocular, simple cyst with uniform anechoic content. Cyst may exhibit fine echoes due to shifting of brood capsules which is often called hydatid sand ('snowflake sign') • Cyst wall is visible • Normally round or oval • Size variable: Type CE1(s): <5.0 cm Type CE1(m): 5-10 cm, Type CE1(l): >10 cm **Remarks** Usually fertile Pathognomonic signs include visible cyst wall and snowflake sign
Type CE 2 	• Status: active • Multivesicular, multiseptated cysts; cysts septations produce 'wheel-like' structures, and presence of daughter cysts is indicated by 'rosette-like' or 'honeycomb-like' structures. Daughter cysts may partly or completely fill the unilocular mother cyst • Cyst wall normally visible • Normally round or oval • Size variable: Type CE2(s): <5.0 cm Type CE2(m): 5-10 cm, Type CE2(l): >10 cm **Remarks** Usually fertile US features are pathognomonic

* **Important note:** Schemes of classification should be used with caution because of great variability of cyst appearance, and in cyst recognition by different observers

Table 2.5. (contd)
Types of cystic lesions (CL) and *E. granulosus* cysts (CE) which may be found on ultrasound (US) examination of the liver *
Classification proposed by the WHO Informal Working Group on Echinococcosis (130)

Type of cyst	Imaging features and remarks (s): small; (m): medium; (l): large
Type CE 3  	• Status: transitional • Unilocular cyst which may contain daughter cysts • Anechoic content with detachment of laminated membrane from the cyst wall visible as floating membrane or as 'water-lily sign' which is indicative of wavy membranes floating on top of remaining cyst fluid • Cyst form may be less rounded due to decrease of intra-cystic fluid pressure • Size variable: Type CE3(s): <5.0 cm Type CE3(m): 5-10 cm, Type CE3(l): >10 cm **Remarks** Transitional stage: cyst is usually starting to degenerate but may sometimes also produce daughter cysts US features are pathognomonic
Type CE 4 	• Status: inactive • Heterogenous hypoechoic or hyperechoic degenerative contents. No daughter cysts • May show a 'ball of wool' sign which is indicative of degenerating membranes • Size variable: Type CE4(s): <5.0 cm Type CE4(m): 5-10 cm, Type CE4(l): >10 cm **Remarks** Most cysts of this type do not contain living protoscoleces US features are not pathognomonic and further diagnostic tests are required to ascertain a diagnosis
Type CE 5 	• Status: inactive • Cysts characterised by thick calcified wall which is arch-shaped, producing a cone shaped shadow. Degree of calcification varies from partial to complete • Size variable: Type CE5(s): <5.0 cm Type CE5(m): 5-10 cm, Type CE5(l): >10 cm **Remarks** The majority of cysts does not contain living protoscoleces Diagnosis is uncertain. Features are not pathognomonic but highly suggestive of *E. granulosus*

* **Important note:** Schemes of classification should be used with caution because of great variability of cyst appearance, and in cyst recognition by different observers

Brain cysts

Computed tomography is the principal method for the diagnosis of cerebral cysts (120, 121). It shows a spherical cyst with a thin wall, not enhanced after injection of contrast medium, without perilesional oedema displacing the adjacent structures.

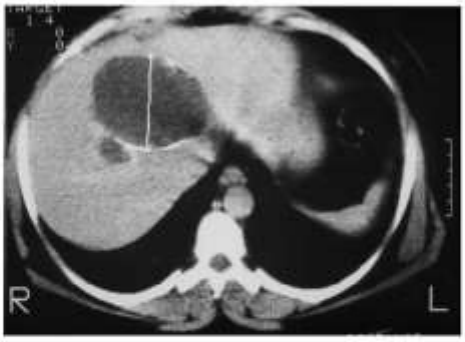


Fig. 2.7.
Computed tomography scan of a liver cyst of *Echinococcus granulosus* with partial wall calcification and a small bulging cyst
Photograph: courtesy of Professor R. Ammann, University Hospital, University of Zurich

Cysts in the other sites

Computed tomography is of little additional value at splenic and renal sites, except in doubtful cases, such as type CE 4 cysts (Table 2.5.). In the case of bone involvement, CT displays areas of osteolysis with localised bone expansion and fluid formations of cyst-like appearance developing in the soft tissue.

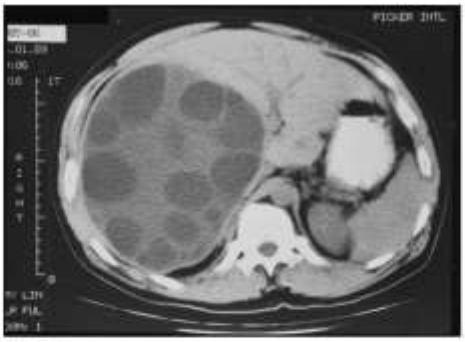


Fig. 2.8.
Computed tomography scan of the liver with a large *Echinococcus granulosus* cyst containing daughter cysts
Photograph: courtesy of Professor R. Ammann, University Hospital, University of Zurich

2.2.3.5. Other exploratory methods

Magnetic resonance imaging (MRI) is indicated only for certain sites, particularly the diagnosis of cerebral cysts (Fig. 2.9.). It supplies not much additional information for the pleuropulmonary and abdominal sites, but is useful in identifying changes of the intrahepatic and extrahepatic vascular system, due to intrinsic contrast of vascular structures (3, 120, 121).

Endoscopic retrograde (or percutaneous transhepatic) cholangiography (ERC) may be indicated in patients with cholestatic jaundice. This technique can be combined with therapeutic drainage procedures (2).

Angiography and scintigraphy have now been replaced by other imaging methods.

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Intravenous urography may be useful in the case of renal cysts in order to assess the quality of the renal parenchyma, particularly if the excretory ducts are compressed.

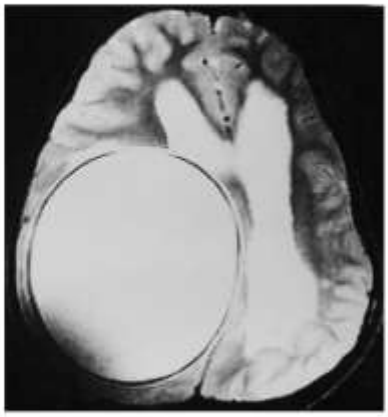

Fig. 2.9.
Magnetic resonance imaging of *Echinococcus granulosus* cyst in brain
Reproduced from (120) with permission from Elsevier Science

2.2.3.6. Diagnostic puncture

Traditionally the diagnostic puncture of *E. granulosus* cysts of the liver was discouraged, as it was regarded as carrying the risk of dangerous anaphylactic reactions or spillage of viable cyst material inducing secondary echinococcosis. Recently, some studies have shown that fine-needle puncture of cysts performed under US-guidance, by transhepatic routes and under anthelmintic cover can be regarded as a rather safe technique (107, 108, 122). Thus, ultrasound-guided fine-needle puncture has been used as a diagnostic procedure in doubtful cases, i.e. in absence of detectable anti-*Echinococcus* serum antibodies, with small lesions resembling simple hepatic cysts, and with lesions which cannot be distinguished from liver abscesses, neoplasms or other conditions by any of the non-invasive techniques (85, 107). Diagnostic puncture is the only technique which helps to diagnose pre-surgically sterile *E. granulosus* cysts by finding the specific antigen 5 in the aspirated hydatid fluid (82). However, the use of fine-needle biopsy is still controversial and it is definitely contraindicated, when diagnosis can be made by standard methods or when the risk of anaphylactic shock is high, i.e. in patients with a high level of total IgE antibodies and/or with allergy history, and patients with larger cysts superficially situated and/or under a high hydatid fluid pressure. In order to prevent secondary echinococcosis, chemotherapy with albendazole is recommended for four days before puncture and for at least one month after puncturing a lesion that was diagnosed as *E. granulosus* cyst (Chapter 2.2.4.3.). It has to be mentioned that puncture is now used as part of a new treatment procedure of CE (PAIR, Chapter 2.2.4.2.).

2.2.3.7. Laboratory findings

Haematology and blood chemistry

As a rule, the routine laboratory tests show non-specific results. The biochemical profile in patients with liver involvement may be normal or exhibit evidence of cholestasis with or without hyperbilirubinaemia and/or elevation of transaminases and/or gamma-glutamyl transferase (γ-GT). In patients with rupture of a cyst into the biliary tree, marked transient elevations of γ-GT and alkaline phosphatase concentrations may occur, often in association with hyperamylasaemia and eosinophilia (>500/µl). However, in most instances, eosinophilia is moderate (500/µl-1,000/µl) or absent. Hypergammaglobulinaemia is observed in about 30% of the CE cases. Marked eosinophilia usually occurs in cases of cyst rupture.

2.2.3.8. Immunodiagnosis

The current status of immunodiagnosis of human CE has been discussed in several review articles (17, 19, 51, 64, 105). A summary of practical aspects is presented in the following section. For determination of performance characteristics for immunodiagnostic assays see Annex 2.1.

2.2.3.8.1. Immunodiagnosis in individual patients

In the procedure for diagnosing human CE imaging methods for detecting space occupying lesions (US, CT, MRI, X-ray, etc.) are commonly the primary approaches. Immunodiagnostic procedures for serum antibody detection are used for the aetiological confirmation of imaging structures suggestive for CE or for diagnosis or differential diagnosis in cases of uncharacteristic imaging findings.

In clinical practice tests for detecting specific serum antibodies are of particular importance in the diagnosis of CE, whereas detection of circulating antigens is less relevant. Even if highly sensitive tests are used, such as the IgG-ELISA, antibodies may not be detectable in a certain proportion of patients with echinococcosis (false-negative results; see below). Cysts in the brain or eye and calcified cysts often induce low or no antibody titres. Antibody response may also be low in certain human population groups and in young children. False-positive results may also occur, especially in patients with other helminthic diseases.

The following approach can be used for immunodiagnosis of human CE:

Primary antibody test: test for serum antibody detection: IgG-ELISA with *E. granulosus* antigen or another adequate system (Table 2.6.).

Table 2.6.
Approaches for immunodiagnosis of cystic echinococcosis in humans

First step: Primary antibody test
Test for serum antibody detection: IgG-ELISA with *E. granulosus* antigen or another adequate system (Table 2.7.). A combination of two or more primary tests may increase sensitivity
Subsequent steps

↓	↓	↓
Seronegative samples	**Seronegative samples**	**Seropositive samples**
People without imaging structures or other signs suggestive for CE	**People with imaging structures suggestive for CE**	**People with or without imaging structures suggestive for CE**
No further serological follow-up or further steps of differential diagnosis	**Asymptomatic cases** Extended and/or advanced imaging and repeated serological examinations, including differential diagnosis for AE* 'Wait and observe' approach with repeated serological examinations **Symptomatic cases** Consideration of cyst puncture (Chapter 2.2.3.6.) Consideration of surgical intervention and/or chemotherapy without further serological examinations	**Asymptomatic and symptomatic cases** Secondary antibody test (Tables 2.8. and 2.9.) Arc 5 test IgG4-ELISA Immunoblot for antibodies reactive with subunits of *E. granulosus* antigens Serological differential diagnosis for AE (ELISA-Em2plus, immunoblot) (Chapter 2.3.3.4.)*

* differential diagnosis for AE and in certain cases (for example brain cyst) for cysticercosis may be necessary in patients from areas with endemic occurrence of these diseases

Primary tests for antibody detection

Of the serological tests for detecting anti-*Echinococcus* serum antibodies, the enzyme-linked immunosorbent assay (for detecting of IgG) (IgG-ELISA), the indirect hemagglutination antibody test (IHAT), and the latex agglutination test (LAT) are commonly used in laboratories (51); less frequently, the immunofluorescence antibody test (IFAT), immunoelectrophoresis (IEP) and some other tests are employed. In many countries, the materials, reagents and equipment to perform the IgG-ELISA are readily available, and this technique is probably the best overall choice for use in immunodiagnosis for human CE. However, there is still no standard, highly sensitive, and specific serological test for antibody detection in cases of human CE (17).

Therefore, for clinical practice, it should be noted that the results of serological tests depend on multiple factors, such as antigen quality, test system, organ site and number of hydatid cysts, individual variability of immune responses, etc. One example is presented in Table 2.7., which shows that in more than 20% of patients with hepatic cysts and more than 40% of patients with pulmonary cysts specific antibodies may not be detectable with some of the test systems. As shown in Table 2.7., the IgG-ELISA is one of the most sensitive tests presently available. The IFAT has a sensitivity similar to that of the ELISA-IgG. Because of the variable sensitivities of the various tests, many laboratories employ at least two different primary tests for routine diagnosis of CE which usually increases the sensitivity.

Table 2.7.
Sensitivities of various assays for antibody detection in patients with confirmed cystic echinococcosis*

Test	Organ sites of cysts and number of patients (N)			
	Liver (N: 41)	Lung (N: 79)	Liver and lung (N: 49)	Others (N: 7)
	Sensitivity (%)			
Latex agglutination (LA)	80	58	88	57
Indirect haemagglutination (IHA)	80	61	90	57
Immunoelectrophoresis (IEP)	68	51	71	50
IgG-ELISA	93	83	96	93

* of 165 patients 79 (48%) patients had one cyst and 86 (52%) had more than one cyst
Source: Orduna *et al.* (80)

Most of the routine laboratory test systems or commercialised test kits are based on crude or semi-purified preparations of *E. granulosus* antigens (i.e. hydatid fluid or protoscolex antigen for IFAT). The use of the two major hydatid cyst fluid antigens, antigen 5 (thermolabile) and antigen B (thermostable), is predominantly restricted to scientific applications, and these antigens are not generally available. Both antigens are lipoproteins which are composed of subunits. In antigen 5 subunits of 52 kDa-67 kDa have been identified under non-reducing conditions, while subunits of 20-24 and 38 kDa were detected under reducing conditions. Antigen B consists of 8-12, 16 and 24 kDa subunits detectable under both non-reducing and reducing conditions (27, 104). It has been shown that antigen B, purified from human hydatid cyst fluid by the method of Oriol *et al.* (81) exhibited a high sensitivity of 94% and a high specificity (excluding 60% cross-reactivity in AE cases) in the ELISA (17, 95). There are few reports on the use of antigen 5 in various types of ELISAs (17), and definite conclusions cannot be drawn. Antigen B is currently considered to be more specific to *E. granulosus* than antigen 5 (105). A recombinant antigen B had comparable diagnostic sensitivity and specificity compared to native antigen B (17). There is a need to provide purified native and recombinant antigens 5 and B in large quantities for further large-scale evaluation.

Secondary tests for antibody detection

Tests using crude *E. granulosus* antigens are reasonably sensitive (Table 2.7.) (51), but specificity is not always satisfactory. Specificity may be expressed as specificity 1 (Sp1) and specificity 2 (Sp2) indicating the percentage

of correct negative testing results in non-infected and in parasite-infected individuals, respectively (Annex 2.1.); both may be combined to overall specificity (Spo). In various studies, Sp1 in the IgG-ELISA was generally high at 96%-100%, while Sp 2 varied between 2% and 49% (49). Cross-reactivity (causing low Sp2) is especially high in cases of AE, PE, cysticercosis, fasciolosis, filariosis and other helminthic infections, whereas protozoan infections normally do not induce cross-reactions. Therefore, positive serological results should be confirmed by a more specific secondary test, except in cases in that imaging structures are clearly suggestive for CE.

In recent years, several secondary test systems have been used in specialised laboratories, such as the detection of a precipitation line designated as arc 5, the identification of IgG subclasses, and immunoblotting which demonstrates the reactivity of serum antibodies with subunits of *E. granulosus* antigens (17, 27, 54, 62, 63, 64, 91, 104, 105, 124). Generally, these tests are less sensitive, but more specific than primary test systems. Examples are presented in Tables 2.8. and 2.9.

Table 2.8.
Sensitivities of secondary tests for antibody detection in cases of human cystic echinococcosis (examples)

Antibody type detected (test system)	Number of CE cases tested	Percentage of sensitivity (= percentage of cases seropositive)	Ref.
Arc 5 (DD)	Not given	50-60	94
Arc 5 (DD and IEP with antigen 5)	166	78[a]	106
IgG4 (ELISA)	Symptomatic[b]: 58 Asymptomatic[c]: 133	71 31	102
IgG4 (IB with antigen B)	30	87	54
IgG4 (ELISA)	56	62	49
IgG1 (ELISA)	56	96	49
39 kDa (IB)[d]	65 166	94 90	17 106
10, 16, 20 kDa (IB)	65	57	17
16 kDa (IB)	166	46	106
16 kDa (IB with antigen B)	30	50	54
12 kDa (IB)	166	34	106
12 kDa (IB)	55	91	63
12 kDa (IB with antigen B)	30	80	54

DD : double diffusion
IB : immunoblot
IEP : immunoelectrophoresis
a) positive by one or both tests
b) clinical, hospitalised cases
c) asymptomatic cases diagnosed by ultrasound examination
d) reactivity of human sera to *E. granulosus* antigen subunits

Of the various secondary test systems, the arc 5 precipitation test has mostly a low sensitivity of 50%-60%, but it is taeniid specific, and this includes cross-reactivity in cases of AE and in approximately 15%-20% of cysticercosis cases (17). Detection of IgG4 is more sensitive, but can be low in asymptomatic cases of CE (Table 2.8.). Cross-reactivity occurs in cases of AE and in a low percentage of cysticercosis cases, but not in cases of schistosomosis, onchocercosis, and some other helminthic infections (Table 2.9.). Identification of specific IgE antibodies has a sensitivity of approximately 60%-80% and a Sp2 of 80%-100%. Immunoblotting for the detection of antibody reactivity with certain subunits of *E. granulosus* antigens, predominantly 39 kDa, 16 kDa, and 12 kDa subunits, is of diagnostic value as sensitivity and specificity are quite high (Tables 2.8. and

2.9.). However, cross-reactivity is not completely excluded. For example, cross-reactivity has been observed between the 12 kDa subunit and 40% of sera from AE patients and 5% of cysticercosis patients (73) (Table 2.9.). In some studies, a combination of subunit bands have been used for diagnosis of CE cases. Interpretation of immunoblots requires experience; therefore, such tests should be performed in specialised laboratories.

Table 2.9
Specificities of secondary tests for antibody detection using *Echinococcus granulosus* antigens

Antibody type detected (test system)	Percent specificity and number of cases tested (N)						Ref.
	Alveolar echinococcosis or polycystic echinococcosis	Cysticercosis	Schistosomosis	Onchocercosis /filariosis	Other helminthoses	Healthy controls or non-parasitic diseases	
IgG4 (ELISA)	–	95 (N: 38)	100 (N: 17)	100 (N: 28)	–	100 (N: 50)	102
IgG4 (ELISA)	48 (N: 54)	100 (N: 8)	100 (N: 8)	100 (N: 8)	100 (N: 32)[a]	99 (N: 253)	49
39 kDa (IB)	–	–	100 (N: 15)	–	100 (N: 7)[b]	100 (N: 20)[c]	106
16 kDa (IB)	–	–	100 (N: 15)	–	100 (N: 7)[b]	100 (N: 20)[c]	106
12 kDa (IB)	60 (N: 60)	95 (N: 55)	100 (N: 3)	–	–	100 (N: 15)	63

IB : immunoblot
a) each eight cases of fasciolosis, strongyloidosis, toxocarosis and trichinellosis
b) cases of trichinellosis
c) non-parasitic diseases

Antibody response and assessment of treatment

Antibody assays for IgG generally have poor value in assessment of the results of surgery or chemotherapy. Analysis of IgG subclasses may better reflect qualitative changes in serum parameters after surgery or chemotherapy (17). However, there are neither conclusive results nor reproducible tests system which could be generally recommended (17).

Antibody response and puncture – aspiration – injection – reaspiration (PAIR)

Antibody detection in serum samples is also used for confirmation of the ultrasound diagnosis during the PAIR procedure (35) (Chapter 2.2.4.2). A new test, the hydatid antigen dot immunobinding assay (HA-DIA), was developed which allows a quick diagnosis and is particularly suited for application in medical units where laboratory facilities are not readily available (72). So far, follow-up of PAIR is based on ultrasound or other imaging techniques. Apparently, long-term observations on the course of antibody titres after PAIR have not yet been published.

Detection of circulating antigens

Detection of circulating *E. granulosus* antigens in serum samples is less sensitive than antibody detection and therefore, it is not recommended for routine purposes. The sensitivity of antigen detection was only 43% in 116 patients with confirmed CE (17).

Detection of antigens in cyst fluid

Putative hydatid cyst fluid samples obtained by puncture or after surgical intervention can be tested for the presence or absence of *Echinococcus* antigen through binding of enzyme-labelled anti-*Echinococcus* (hydatid cyst

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fluid) antibodies in an ELISA (18). The sensitivity of this test was 100% in nine proven human hydatid cyst fluids (18). In a recent Polish study, an ELISA with a monoclonal antibody against antigen 5 (Ag5) was used for the same purpose (82). In all fluids of fertile liver cysts obtained by puncture from 6 CE patients Ag5 was detected, and also in the cyst fluids of 9 out of 81 patients harbouring sterile cysts. These data indicate that detection of Ag5 may be useful in confirmation of the *Echinococcus* nature of the fluid.

2.2.3.8.2. Immunodiagnosis of cystic echinococcosis in human populations

Mass-screening programmes for human CE have been conducted using serological tests in a number of endemic countries including Argentina, China, Israel, Kenya, Tunisia, Uruguay and others. To date, three approaches exist for mass-screening of human populations for CE (17, 19), as follows:

a) application of a sensitive serological test (for example ELISA) to blood samples from the target population as a primary test and follow-up of all seropositives by ultrasound screening and, if possible, by X-ray examination for CE of the lung

b) application of ultrasound screening to the population using portable units as a primary test and use of serology to confirm image positives

c) combination of approaches (*a*) and (*b*).

Ethical rules have to be followed in all mass-screening programmes (Chapter 2.5.).

In approach (*a*), the test should be highly sensitive and specific. Before seropositive individuals are examined clinically, they should be tested by a secondary serological test. The relative low specificity of most of the primary test systems (see above) can lead to a rather high number of false-positive reactors. For example, in a recent study in Libya, the population of a village was screened for CE using portable ultrasound equipment and IgG-ELISA for serum antibody detection (103). Abdominal CE was detected in 4.5% of 485 individuals, but 13.2% were seropositive. Part of the seropositives could have been attributed to extra-abdominal CE or to abortive *Echinococcus* infections, but a relatively high proportion had to be classified as false-positive. In a hypothetical mass-screening programme carried out under the same conditions with 40,000 individuals, 5,280 seropositives have to be expected and would need serological and/or clinical follow-up.

Using the IgG-ELISA or a similar test, the probability to obtain a correct positive result is relatively low (positive predictive value). This is illustrated by a hypothetical example published by Craig (17) in which an immunodiagnostic test with 70% sensitivity and 90% specificity was applied to a population with a CE prevalence of 2% (Table 2.10.). In this case, the positive predictive value is only 12.5%, whereas the negative predictive values is high.

Therefore, approach (*b*) might be more appropriate with application of serology as secondary test for confirmation of positive images.

Table 2.10.
Hypothetical data for predictive value calculation with a 2% cystic echinococcosis prevalence and an immunodiagnostic test with 70% sensitivity and 90% specificity (17)

Result of serological test	CE present	CE absent	Total number of cases
Positive	14	98	112
Negative	6	882	888
Total	20	980	1,000
Positive predictive value: 12.5%	Calculation: 14/(14 + 98) = 14/112 = 0.125 × 100 = 12.5%*		
Negative predictive value: 99.3%	Calculation: 882/888 = 0.993 × 100 = 99.3%*		

* for details for calculating predictive values, see Annex 2.1.

A combination of approaches (*a*) and (*b*) has been suggested, in which venous blood samples (3 ml-5 ml) be taken from ultrasound positive cases or cases with images suggestive for CE and finger (or ear) prick blood samples be collected onto filter paper from every individual examined. Filter paper blood samples are reliable for CE antibody testing if stored at –20°C. The sensitivity of ultrasound scanning of the liver for space occupying lesions (with minimum resolution around 1 cm-2 cm) is high (approximately 70%), but lower (approximately 30%) if only CE characteristic lesions are included. A small number of strongly seropositive, but ultrasound negative (and X-ray negative) will be identified. These individuals should be followed-up at 12-24 months intervals by clinical examination, preferably including CT examination.

It should be noted that the general level of antibody seroreactivity (IgG), and therefore, test sensitivity, is likely to be lower in CE cases identified by US from an endemic community. This is because most cases will be asymptomatic and with greater probability of presentation either with early pathology, i.e. small, unilocular, vesicular cysts, or with calcified cysts, that are known to be less seroreactive (19).

2.2.4. Treatment

The following chapter refers mainly to special guidelines published by the WHO Working Group on Echinococcosis (129).

General considerations

Currently, surgery is still the treatment that has the potential to remove *E. granulosus* cysts and lead to complete cure (129). It can be performed successfully in up to 90% of patients if a cyst does not have a risky localisation or if the disease is not too far advanced. However, surgery may be impractical in patients with multiple cysts localised in several organs and if surgical facilities are inadequate. The introduction of chemotherapy and of the PAIR technique (puncture – aspiration – injection – respiration) offers an alternative treatment, especially in inoperable patients and for cases with a high surgical risk (129). Cysts with homogeneously calcified cyst walls need probably no surgery, but only a 'wait and observe' approach (86, 89). The choice of an optimal treatment should be carefully assessed in each case.

Treatment options for CE are as follows (85, 86, 89, 129):

- surgery
- PAIR
- chemotherapy
- 'wait and observe' approach.

2.2.4.1. Surgery

Indications

Surgery is indicated for large liver cysts with multiple daughter cysts; single liver cysts, situated superficially that may rupture spontaneously or as a result of trauma; cysts that are infected; cysts communicating with biliary tree and/or exerting pressure on adjacent vital organs; cysts in the lung, brain and kidney, bones and other organs.

Contraindications

Surgery of CE is contraindicated as defined for surgical procedures in general, i.e. patients refusing surgery, patients at the extremes of age, pregnant women, patients with concomitant severe diseases (i.e. cardiac, renal or hepatic diseases, diabetes and hypertension). In addition, surgery is contraindicated in patients with multiple cysts or cysts difficult to access, dead cysts either partly or totally calcified, and in patients with very small cysts.

Choice of surgical technique

Surgical procedures include several main options that are summarised in Table 2.11. and described in more detail by Morris and Richards (74).

Table 2.11.
Surgical techniques for cystic echinococcosis (CE) of the liver and lung (74, 129)

Surgical techniques for CE of the liver	Surgical techniques for CE of the lung
Partial hepatectomy	Lobectomy
Pericystectomy	Extrusion of cysts (Barrett's technique)
Open cystectomy with or without omentoplasty	Pericystectomy
Palliative surgery (tube drainage of infected cysts)	

Usually, the more radical the intervention, the higher the intraoperative risk but the lower likelihood of relapses, and vice versa. With the inclusion of chemotherapy before surgery the aggressive surgical procedures are less commonly performed (see below).

Use of protoscolicides

The use of protoscolicidal substances for intraoperative killing of protoscoleces is questionable, as there is no ideal agent that is both effective and safe (129). The lethal action observed *in vitro* may be hampered *in vivo* by instability of the substance used (e.g. hydrogen peroxide), or by an unpredictable dilution by hydatid fluid, and difficulties in penetrating daughter cysts. Potential communication between the hydatid cyst and the biliary tree substantially increases the safety requirements for using protoscolicides, which can cause chemical cholangitis leading to frequently fatal subsequent sclerosing cholangitis. Therefore, formalin should never be used.

The following protoscolicides, which appear to be effective, have a relatively low risk of toxicity: 70%-95% ethanol (both protoscoleces and germinal layer of the cyst are damaged), 15%-20% hypertonic saline solution, and 0.5% cetrimide solution. For optimal efficacy, the substances have to be left in the cyst cavity for at least 15 min (129). More experimental studies and clinical observations are urgently needed in evaluating the efficacy and safety of protoscolicides.

Peri-interventional chemotherapy

Preoperative treatment with benzimidazoles has been reported to soften the cysts and to reduce intracystic pressure, enabling the surgeon to remove the endocyst more easily. However, neither the required duration of such treatment, nor its efficacy has been adequately determined. There are hints from several studies that postoperative treatment of patients can reduce the rate of recurrences (2). In rodents, the number of *E. granulosus* cysts developing from intraperitoneally inoculated protoscoleces could be reduced by 80%-90% if albendazole treatment (10 mg/kg body weight [bw] per day) for a duration of 1 week was initiated immediately after inoculation; when treatment was delayed for 15 days, it was ineffective (75). Based on these hints, it is recommended for cases in which spillage of protoscoleces may have occurred during surgery to initiate postoperative chemotherapy with albendazole (ABZ) or mebendazole (MBZ) (for dosages, see below) immediately after operation for at least 1 month (ABZ) or 3 months (MBZ).

Benefits

Radical surgery has the potential to cure completely the patient, but involves some perioperative risks.

Risks

The risks include those associated with any surgical intervention (anaesthesia, stress, infections including those transmitted by blood transfusion e.g. viral hepatitis, HIV). Despite progress in surgical techniques, secondary echinococcosis owing to spillage of viable parasite material during the intervention may occur. The prevalence of long-term recurrence is in the range of 2% to 25% (3). In a Chinese series (1950-1990), with 15,289 surgical cases, 92% of the patients had one operation, 7% two, 0.8% three and 0.2% four to eight operations (70). Recurrence can be due to incomplete cyst removal or to previously undetected cysts. Anaphylactic reactions represent a further risk on rare occasions. Postoperative fatality is about 2% or less, but may be higher in the second or further operations or if medical facilities are inadequate.

2.2.4.2. Puncture, aspiration, injection, reaspiration (PAIR)

General considerations and technique

Ultrasound-guided cyst puncture for treatment of CE was introduced in the mid-1980s (9, 35, 39) and includes the following steps:

- percutaneus puncture of cysts under ultrasonic guidance
- aspiration of a substantial amount of cyst fluid
- injection of protoscolicidal substance (preferably 95% ethanol)
- re-aspiration of the fluid cyst content after 15 min to 20 min.

Favourable results have been reported from PAIR interventions in several hundred patients with the follow-up periods of up to 5 years (35, 87, 129). However, the efficacy and potential risks have not yet been fully evaluated and require further properly controlled long-term studies. The PAIR should be accompanied by a chemotherapeutic coverage to minimise risks of secondary echinococcosis (see below).

This minimal-invasive technique should be reserved for use by skilled and well experienced physicians and with a surgical and intensive care back-up team well prepared to deal immediately with complications. Ultrasound-guided transhepatic puncture is the essential technique. The WHO scheme for US-classification of *E. granulosus* cysts (Table 2.5.) can be used as a rough guideline for judging their suitability for PAIR procedure. It is essential that aspirates of liver cysts are analysed immediately for traces of bilirubin and protoscoleces or hooks. PAIR should only be performed under chemotherapeutic coverage, except in early pregnant patients (35).

Indications

PAIR is indicated for inoperable patients with CE (see contraindications for surgery) and those who refuse surgery. It has been used in treatment of cysts in the liver, the abdominal cavity, spleen, kidney and bones, but it should not be used for lung cysts (129). Various types of liver cysts CL, CE 1, CE 2 and CE 3 may be selected for PAIR (Table 2.5.); especially anechoic lesions >5 cm in diameter; cysts with a regular double laminated layer; cysts of >5 cm diameter with multiple septal divisions; multiple cysts (>5 cm in diameter) in different liver segments. PAIR can also be used in cases of a relapse after surgery or in failure to respond to chemotherapy.

Experience using PAIR in pregnant women and children aged <3 years is still limited. The application of PAIR might be indicated in pregnant women with symptomatic CE, but the potential risk associated with peri-interventional chemotherapy (see below) has to be carefully assessed since the benzimidazoles are contraindicated during pregnancy, especially during the first 3 months.

Contraindications

PAIR is contraindicated for inaccessible or superficially located cysts in the liver (for the latter, there is a risk of spillage of cyst content into the abdominal cavity); for cysts with multiple septal divisions (honeycomb-like cysts); for cysts with hyperechogenic solid patterns or calcified cysts; cysts communicating with bile ducts, and cysts in the lung. In order to avoid the induction of chemical cholangitis, aspirates from liver cysts should be

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analysed for traces of bilirubin prior to injection of protoscolicides. Contamination of cyst content with bilirubin indicates that there is a communication with biliary ducts.

Peri-interventional chemotherapy

Peri-interventional treatment with benzimidazoles is highly recommended for four days before PAIR and at least for 1 month (albendazole) or 3 months (mebendazole) thereafter (61, 129). The duration of chemotherapy should be adapted according to the cyst size and US appearance (35).

Benefits

PAIR is minimally invasive and less risky than surgery. It confirms the diagnosis and removes large numbers of protoscoleces and antigens with the aspirated cyst fluid. The costs of PAIR with concomitant chemotherapy is less than that of surgery. Fewer days of hospitalisation are needed (35). For example, in a series of 33 PAIR-treated patients in Argentina the mean hospitalisation time was 1.8 days (range: 0-15 days) (87).

Risks

Risks include those associated with any puncture (haemorrhage, mechanical damage of other tissues and infections); anaphylactic shock or allergic reaction caused by leakage of cyst fluid and secondary echinococcosis due to spillage. Transhepatic puncture is strongly advised, since puncture of superficially located cysts involves a higher risk of spillage. Other potential risks or failures are chemical sclerosing cholangitis, sudden intracystic decompression leading to biliary fistulas, and persistence of satellite daughter cysts.

2.2.4.3. Chemotherapy

General considerations

Over 2,000 well documented cases of CE have been treated with benzimidazoles, to date (2, 3, 22, 25, 41, 53, 110, 111, 112, 113, 119, 126, 129). When evaluated up to 12 months after initiation of chemotherapy, 10% to 30% of patients show cyst disappearance (cure), 50%-70% show degeneration of cysts and/or significant size reductions (improvement) (Fig. 2.10.), but 20%-30% exhibit no morphological changes in cysts (i.e., failure). Chemotherapy is apparently more effective among young rather than older patients. Small cysts that have thin walls without infection or communication, as well as secondary cysts (even when multiple) are most susceptible to chemotherapy. Chemotherapy may, however, be less effective for thin-walled daughter cysts within a mother cyst. Some of the treated patients exhibit relapses, but these are usually sensitive to retreatment in a high proportion of cases (up to 90%). The rate of relapses after chemotherapy is relatively high (14%-25%) (53, 111).

Indications

Chemotherapy is indicated for inoperable patients with primary liver echinococcosis and for patients with multiple cysts in two or more organs. Cysts localised in bones are less susceptible to chemotherapy. Since radial surgery is often impossible (e.g. cyst localisation in spine or pelvis), long-term chemotherapy may be needed. Another important indication for chemotherapy is the prevention of secondary echinococcosis. The pre-surgical use of benzimidazoles (ABZ or MBZ) may reduce the risk of recurrence of CE and/or facilitate the operation by reduction of intracystic pressure, but this is not well documented. Peri-interventional chemotherapy is also recommended for PAIR (Chapter 2.2.4.2.).

Contraindications

Chemotherapy is contraindicated for large cysts with a risk of rupture (notably superficially situated, infected cysts) or for inactive or calcified cysts. Patients with severe chronic hepatic diseases and with bone marrow depression should not be treated. Early pregnancy is a contraindication. Chemotherapy during later stages of pregnancy might better be postponed until after delivery.

Choice of drugs (see also alveolar echinococcosis and Annex 2.2)

Two benzimidazoles have been extensively evaluated using animal models and used on over 2,000 patients:

- Albendazole (ABZ) (Eskazole®, Zentel®; 400 mg tablets and 4% suspension, SmithKline Beecham, England)
- Mebendazole (MBZ) (Vermox®; 500 mg tablets, Janssen Pharmaceutica, Belgium).

These drugs show definite efficacy against CE, and are generally well tolerated. Studies with different groups of CE patients, summarised by Horton (53), have shown that 48% of 665 cysts disappeared, and further 24% improved after chemotherapy with ABZ, compared to 28% of 516 cysts disappeared and 30% improved after treatment with MBZ. MBZ is apparently more effective against cysts in the lungs than in the liver, whereas such a difference was not observed for ABZ. Exact comparative efficacy of the drug is difficult to assess, as treatment protocols were variable in the different groups of patients.

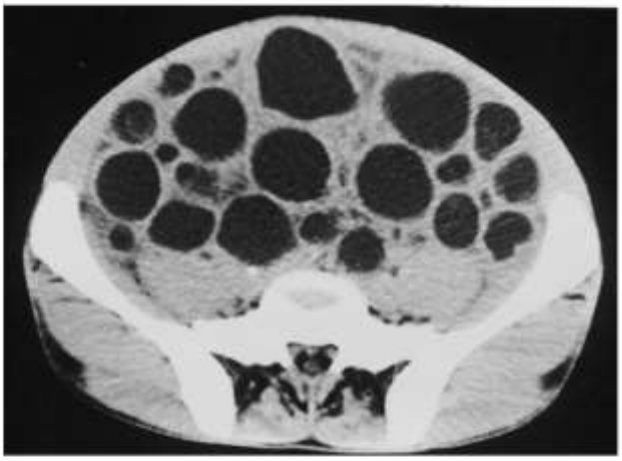

Fig. 2.10.a
Computed tomography scan of the pelvis of a patient with disseminated *Echinococcus granulosus* cysts: before treatment
Photograph: courtesy of Professor W. von Sinner, King Faisal Specialist Hospital and Research Centre, Riyadh

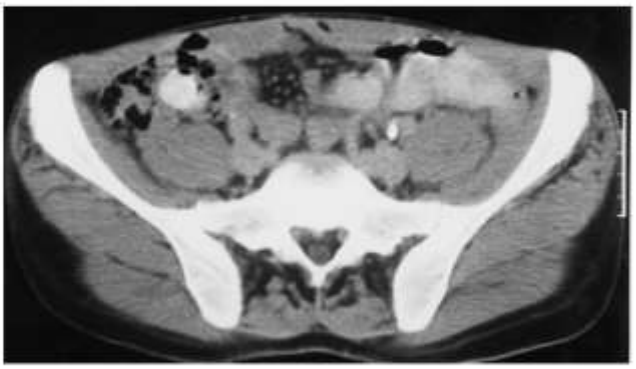

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Fig. 2.10.b
Computed tomography scan of the pelvis of a patient with disseminated *Echinococcus granulosus* cysts: after 3 months of albendazole treatment
Photograph: courtesy of Professor W. von Sinner, King Faisal Specialist Hospital and Research Centre, Riyadh

For treatment of CE the following oral dosages are recommended:

- Albendazole: 10 mg/kg-15 mg/kg bw per day in two divided doses postprandially. In practice, adults receive 800 mg/day in two single doses of 400 mg each (53). The division of the daily dose is supported by pharmacokinetic data (58).

Cyclic treatment with intervals of 14 days was originally recommended by the manufacturer, and 3- to more than 6-monthly courses have been regarded as necessary for treating patients with single or multiple cysts (53, 126). However, recent data have shown equal or improved efficacy of continuous treatment for 3 to 6 months or longer without an increase of adverse effects (36, 65). In a recent comparative study, this type of treatment was more effective than chemotherapy with mebendazole (36). Therefore, cyclical albendazole treatment seems to be no longer advisable.

- Mebendazole: the usual oral dosage of mebendazole is 40 mg/kg-50 mg/kg bw per day in three divided doses for at least 3-6 months.

In animal experiments, it has been shown that efficacy of mebendazole against *Echinococcus* metacestodes was positively correlated with drug concentration in the serum and duration of treatment (31). In human patients, serum drug levels of MBZ and ABZ may vary widely in individual patients, and correlation with oral doses and drug efficacy is inconsistent. Drug dosing in conjunction with a fatty meal improves intestinal absorption of benzimidazoles (3, 53).

The use of praziquantel (PZQ) (Biltricide®, Bayer, Germany), a heterocyclic pyrazinoisoquinoline derivative, has been proposed at a dose of 40 mg/kg bw once a week concomitantly with benzimidazoles. The PZQ might also be useful in cases of cyst content spillage during surgery. A recent study has shown that a combined treatment with albendazole (10 mg/kg/day) and praziquantel (25 mg/kg/day) given during the month prior to surgery increased the number of patients with nonviable protoscoleces as compared to monotherapy with albendazole (16). However, further studies are needed for evaluating the efficacy of the combined treatment. According to the manufactuer, the plasma levels of albendazole metabolites (sulphoxide) are increased 4.5 times if praziquantel is given simultaneously, and this may increase the rate of side effects (133).

Benefits

Chemotherapy is a non-invasive treatment that can be used on patients of any age, although there is little experience with children under 6 years old, and is less limited by the patient's status (except pregnancy) than surgery.

Risks

The adverse effects of benzimidazoles include neutropaenia, proteinuria, mild hepatotoxicity (transient increase of aminotransferases), gastrointestinal disturbances and transient alopecia (Annex 2.2.). The potential risks of benzimidazoles include embryotoxicity and teratogenicity which, however, have only been observed in some laboratory animals during the early stages of pregnancy. For special precautions see Annex 2.2.

Medical requirements

Hospitalisation is usually not necessary, but regular follow-up examinations are required. Costs of anthelmintics and repeated medical examinations may be considerable.

Monitoring of patients

Medical and laboratory examinations for adverse reactions are necessary initially every 2 weeks then monthly (129). Leukocyte counts should be checked at 2-week intervals during the first 3 months because in rare instances severe and not always reversible leukopaenia has been observed in early phases of chemotherapy. Serum drug concentrations (ABZ-sulfoxide or MBZ parent compound) should be monitored after 2 and 4 weeks of chemotherapy, respectively, in order to identify levels too high (possibly toxic) or too low (ineffective). For MBZ, it has been recommended to determine serum or plasma levels 4 h after the morning dose. Oral drug doses can be adapted to individual patients in order to achieve adequate serum levels (Annex 2.2.), but such attempts are not always effective. Unfortunately, only few laboratories have the capability to measure ABZ-sulfoxide or MBZ serum drug levels (see also section on AE). Follow-up examinations, including imaging if needed, should be carried out at intervals of about 3 to 6 months for 1 to 3 years after termination of chemotherapy because of the relatively high rate of relapses.

2.3. Alveolar echinococcosis

For detailed information, the reader is referred to several recent monographs or reviews (2, 3, 21, 32, 45, 114, 115, 123, 129).

2.3.1. Causative agent and course of infection

Causative agent

Alveolar echinococcosis is an infection caused by the metacestode stage of *E. multilocularis*, which is characterised by a tumour-like, infiltrative and destructive growth with the potential to induce serious disease with a high fatality rate.

Course of infection

After oral infection with eggs of *E. multilocularis*, metacestodes develop primarily almost exclusively in the liver. This can be concluded from findings in patients with single organ involvement (Table 2.12.). Parasitic lesions in the liver can vary from small foci of a few millimetres in size to large areas of infiltration (15 cm-20 cm in diameter). Primary extrahepatic localisations of the *E. multilocularis* metacestodes are extremely rare. From the liver, the metacestode tends to spread to both the adjacent and distant organs by infiltration or metastasis formation (Table 2.12.). Metastasis formation is due to spreading of germinal cells via lymph or blood vessels (32, 69).

Cases of AE are characterised by an initial asymptomatic incubation period of 5 to 15 years duration and a subsequent chronic course. The fatality rate in untreated or inadequately treated persons is high. In a series of 66 individuals with AE from Germany (period 1960-1972), 70% died within 5 years and 94% within 10 years after diagnosis of the disease (2, 3). According to data from Alaska, in 21 untreated persons, the average survival time after diagnosis was 5.3 years, and all patients died within 14 years (131). However, data obtained from more recent series (diagnosis after 1983), show an improvement of the survival rate which may depend on early diagnosis and other factors.

Until recently, it was believed that the metacestode of *E. multilocularis* usually retains an unlimited proliferative capacity until the death of the patient. However, under the influence of the host's defence mechanisms, the metacestode can degenerate, calcify, and finally die. Therefore, spontaneous cure of AE is possible, but the frequency of such an event is unknown (92).

Table 2.12.
Organ sites of *Echinococcus multilocularis* metacestodes in patients (3, 99)

Single organ involvement* N = 199		Single and multiple organ involvement N = 152	
Organ	**Percentage of cases**	**Organ**	**Percentage of cases**

Liver only	99.0	Liver only	88.7
Skin or muscle only	0.5	Liver and lungs	8.5
Bones only	0.5	Liver and spleen	1.4
		Liver and brain	0.7
		Liver, lungs, brain	0.7

* single organ involvement is indicative for metacestode development after primary infection

2.3.2. Clinical presentation

Age and sex of patients

The age at the time of diagnosis of AE is significantly higher than for CE. In Europe, the peak age group is 50-70 years, range 10-89 years; in Japan, 40-60 years and 7-81 years respectively. The sex distribution of AE is about equal.

Organ sites of metacestodes

The primary site of metacestode development is almost exclusively in the liver (Table 2.12.). The right lobe is predominantly infected, but the liver hilus together with one or two lobes may also be involved. Extrahepatic primarily locations are rare. During the infection, secondary echinococcosis (= metastasis formation) may occur in variety of adjacent or distant organs (Table 2.12.).

Symptoms

Symptoms of AE are primarily cholestatic jaundice (about a third of the cases) and/or epigastric pain (about a third of the cases). In the remaining third of patients, AE is detected incidentally during medical examination for symptoms such as fatigue, weight loss, hepatomegaly, or abnormal routine laboratory findings (3, 123).

Classification and staging of alveolar echinococcosis cases

The European Network for Concerted Surveillance of Alveolar Echinococcosis has recently proposed a classification system for human cases of AE which should:

a) aid the clinician in planning of treatment

b) give some indications for prognosis

c) assist in evaluating the results of treatment

d) facilitate the exchange of information between treatment centres

e) contribute to continued investigation of AE.

The system, denominated as PNM, can be used for describing the anatomical extent of AE and is based on the assessment and ranking of three components at the time of diagnosis (Table 2.13.).

The PNM system is used for staging of AE cases as shown in Table 2.14.

2.3.3. Diagnosis

The diagnosis of AE is based on similar findings and criteria as in CE (2, 3, 129).

Diagnosis of AE in individual patients:

- case history, including epidemiological hints
- clinical findings
- morphological lesions detected by imaging techniques
- immunodiagnostic tests.

2.3.3.1. Imaging

General aspects

This subject has been discussed in various publications (2, 3, 38, 60, 71, 78, 79, 114, 115). In the majority of patients with AE, the liver is involved as the primary focus of metacestode development. Lesions caused by the parasite in the liver can be best visualised by the US and CT techniques. Some cases may benefit from the use of other imaging techniques, such as magnetic resonance imaging (MRI), angiography (AG) cholangiography (CAG), endoscopic retrograde cholangiography (ERC), percutaneous transhepatic cholangiography (PTC) or MRI-cholangiography (MRIC).

Table 2.13.
PNM system for classification of human alveolar echinococcosis

Classification of findings
P: Hepatic localisation of the parasite
PX: Primary lesion cannot be assessed
P0: No detectable lesion in the liver
P1: Peripheral lesions without proximal vascular and/or biliar involvement
P2: Central lesions with proximal vascular and/or biliar involvement of one lobe[a]
P3: Central lesions with hilar vascular and biliar involvement of both lobes and/or with involvement of two hepatic veins
P4: Any liver lesion with extension along the vessels[b] and the biliary tree
N: Extrahepatic involvement of neighbouring organs
Diaphragm, lung, pleura, pericardium, heart, gastric and duodenal wall, adrenal glands, peritoneum, retroperitoneum, parietal wall (muscles, skin, bone), pancreas, regional lymph nodes, liver ligaments, kidney
NX: Not evaluable
N0: No regional involvement (see above)
N1: Regional involvement of contiguous organs or tissues
M: Absence or presence of distant metastases
Lung, distant lymph nodes, spleen, CNS, orbital, bone, skin, muscle, distant peritoneum and retroperitoneum]
MX: Not completely evaluated
M0: No metastasis[c]
M1: Metastasis

a) for classification, the plane projecting between the bed of the gallbladder and the inferior vena cava divides the liver in two lobes
b) vessels means inferior vena cava, portal vein and arteries
c) chest X-ray and cerebral CT negative

Source: European Network for Concerted Surveillance of Alveolar Echinococcosis: PNM system for the classification of human cases of alveolar echinococcosis
Data kindly provided by Professor P. Kern, Ulm

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Hepatic lesions

Ultrasonography and computed tomography

In AE, the liver is usually enlarged. In the US and CT, lesions are characterised by heterogenous hypodense masses, often associated with necrotic cavities. The lesion contours are irregular and there is lack of a well-defined wall (Fig. 2.11.). Calcifications are often found and exhibit a typical pattern in regard to shape and distribution: clusters of microcalcifications or irregular plaque-like calcified foci are located in the central or peripheral parts of the lesions.

There may be discrepancies between US and CT patterns, since the two methods yield identical results in only 42% of the cases (78). Hyperechoic haemangioma-like nodules could represent early forms of AE lesions. Quite frequently an extension of the lesions beyond the liver is found toward diaphragm, lungs, pericardium, retroperitoneum, hepatoduodenal ligament and pancreas.

Table 2.14.
Staging of alveolar echinococcosis cases based on PNM classification

Stage of alveolar echinococcosis	PNM classification		
Stage I	P1	N0	M0
Stage II	P2	N0	M0
Stage IIIa	P3	N0	M0
Stage IIIb	P1-3	N1	M0
	P4	N0	M0
Stage IV	P4	N1	M0
	Any P	Any N	M1

Source: European Network for Concerted Surveillance of Alveolar Echinococcosis: PNM system for the classification of human cases of alveolar echinococcosis

Magnetic resonance imaging

Compression or obstruction of inferior vena cava, the hepatic veins or the portal branches (with splenomegaly) may be observed. Pathological changes of the intrahepatic and extrahepatic venous system and of adjacent organs are best visualised by MR imaging. However, calcified lesions are not easily detected. Pathognomonic aspects are represented by multicystic honeycomb-like images. In recent years, angiography has been much less frequently performed, because non-invasive methods, such as CT and MR imaging have become available.

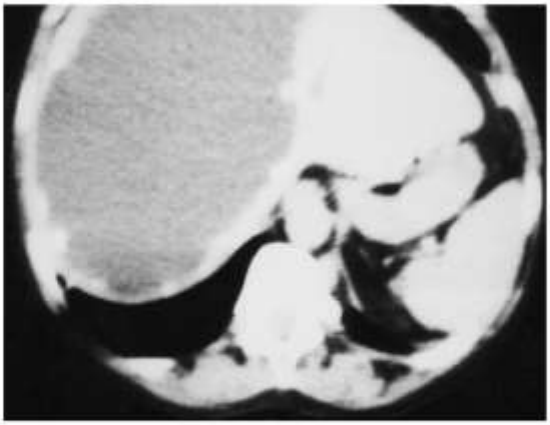

Fig. 2.11.
Computed tomography of liver with lesion caused by *Echinococcus multilocularis*
Photograph: courtesy of Professor R. Ammann, University Hospital, University of Zurich

Cholangiography and endoscopic retrograde or percutaneous cholangiography

Dilated intrahepatic bile ducts are typical findings in cases with involvement of the liver hilus. Displacement of intrahepatic bile ducts, obstructions and other changes can be observed by CAG, ERC or PTC. The non-invasive MRIC will probably become the method of choice in diagnostic cholangiography. An analysis of 18 cholangiograms performed in patients with advanced AE revealed occlusion and/or obstruction of bile ducts in 61%, stretching in 44% and mural irregularities in 18%. In 9 of the 18 patients only intrahepatic bile ducts were involved (78, 118).

2.3.3.2. Diagnostic puncture

Ultrasound-guided fine-needle puncture of liver lesions has recently been used for diagnosing AE, using the biopsy sample for RNA detection by PCR (59). However, the sensitivity of this technique may not be high, since the chances of obtaining sufficient amounts of material are low. More importantly puncture may include the risk of disseminating metacestode cells with subsequent formation of metastases as demonstrated in experimental animals (J. Eckert *et al.*, unpublished findings).

2.3.3.3. Laboratory findings

Haematology and blood chemistry

The routine laboratory tests do not yield specific findings. The blood sedimentation rate is elevated in most of the cases. The numbers of leucocytes and platelets may be depressed in patients with splenomegaly. Lymphopaenia is frequent in advanced cases, and eosinophilia is usually absent. Cholestasis with or without jaundice is observed in patients with intrahepatic bile duct compression or obstruction. Cholangitis and/or liver abscesses, which usually result from bile duct obstruction, are associated with typical alterations of the laboratory parameters. Hypergammaglobulinaemia is present in most of the patients and reflects the specific and polyclonal antibody response. In about one-half of the patients, the presence of specific anti-*E. multilocularis* – IgE can be demonstrated.

2.3.3.4. Immunodiagnosis

Immunodiagnosis of AE is based on similar principles as those for CE (Table 2.15.) (Chapter 2.2.3.8.). However, serological tests for antibody detection are generally more reliable in the specific diagnosis of AE than of CE (Annex 2.1.).

Table 2.15.
Approaches for immunodiagnosis of alveolar echinococcosis in humans

First step: Primary antibody test Usually, for primary testing assays are preferred which exhibit high sensitivity, but may be less specific, whereas in secondary testing assays are employed which have high specificity but may be less sensitive **Subsequent steps**		
↓	↓	↓
Seronegative samples	**Seronegative samples**	**Seropositive samples**
People without imaging structures or other signs suggestive for AE	**People with imaging structures suggestive for AE**	**People with or without imaging structures suggestive for AE**
No further serological follow-up In persons with suspected infection risk: Repeated serological examinations after 3 and 6 months, and US imaging if indicated	**Asymptomatic cases** Extended and/or advanced imaging and repeated serological examinations Fine needle biopsy for PCR or immunohistology may be considered in rare cases If lesions are fully calcified, serological and imaging follow-up after 6 months to confirm parasite abortion **Symptomatic cases** Consideration of surgical intervention and/or chemotherapy without further serological examinations	**Asymptomatic and symptomatic cases** Secondary antibody test: for assessment of primary test and exclusion of cross-reactions (Table 2.16.) Em2Plus-ELISA Em alkaline phosphatase-antigen-ELISA Immunoblot for specific bands or similar test (Table 2.16.) Serological differential diagnosis for CE (see text)

2.3.3.4.1. Immunodiagnosis in individual patients

Primary tests for antibody detection

ELISAs with crude *E. multilocularis* antigens achieve high levels of sensitivity, which may exceed that of tests with purified or recombinant antigens, but specificity is mostly lower (Table 2.16.). Due to cross-reactivity, antibodies against *E. multilocularis* antigens can also be detected with assays using *E. granulosus* antigens, such as ELISA or IHAT (hydatid fluid antigen) or IFAT (protoscolex antigen) (7, 64) (Table 2.16.).

Probably the best overall choice for detecting serum antibodies (IgG) in AE cases is the use of an ELISA based on purified antigens of *E. multilocularis*, such as Em2-antigen (48), the Em18-antigen (57), the Em-alkaline phosphatase-antigen (98, the C-antigen (100) or the recombinant antigens II/3-10 (48) and Em10 (52). Tests using these antigens exhibit diagnostic sensitivities approximating 90%-100% (Table 2.16.).

The following approach can be used for immunodiagnosis of human AE.

Primary antibody test: two types of tests are commonly used: **type A tests** which are highly sensitive and specific assays using purified *E. multilocularis* antigens (Table 2.14.); or **type B tests** which are assays with crude *E. granulosus* or *E. multilocularis* antigens. In practice, Type A tests should be preferred as primary tests.

The specificity of the tests in healthy persons is generally very high (data not shown), and also in cases of parasitoses other than CE the specificities are high. However, in some of these assays cross-reactivity occurs in cases of CE (Table 2.16.). The ELISA using purified *E. multilocularis* phosphatase as antigen has apparently outstanding characteristics with a very high sensitivity combined with high specificity, also in cases of CE (Table 2.16.). So far, only one of the assays, which are highly specific for AE, has been made commercially available. This test is based on a mixture of the Em2 and the II/3-10 antigens (Em2PlusELISA™, Bordier

Affinity Products, Crissier, Switzerland). The use of this assay allows to discriminate between AE and CE with a reliability of approximately 95% (48).

Secondary tests for antibody detection

Like in immunodiagnosis of CE, secondary tests may be used for assessment of the results of primary tests, especially when *E. granulosus* antigens or crude *E. multilocularis* antigens have been used for primary antibody screening. Secondary tests may also be needed for excluding cross-reactivity in positive sera. Several test systems have been used in this indication, such as Western blot analysis (56, 57, 77, 127), an enzyme immune test with *E. multilocularis* protoscolex-antigen (6), and the IgG4 determination in ELISA (29, 49, 124, 128) (Table 2.16.). A Western blot test has recently been made available commercially (Echinococcus WB IgG, LDBIO Diagnostics, Lyons, France) which enables discrimination between AE and CE with a reliability of approximately 76% (90).

Antibody response and post-treatment follow-up

For assessing the efficacy of surgical and chemotherapeutical treatment, and of metacestode viability, serological tests are of limited value. However, it has been shown that in part of the treated patients, particularly those with a cured or regressive form of AE, antibody levels detected by the Em2-ELISA, Em2Plus-ELISA, Western blotting, Ig-isotype-ELISA or alkaline phosphatase-antigen-ELISA (29, 47, 66, 98) tend to decline by the time, but only after long periods of one to several years after the therapeutic intervention.

Cellular immune tests show that the *in vitro* lymphoproliferative response to *E. multilocularis* antigen stimulation is high in cured patients who had radical surgery or in patients with dead metacestodes, and is significantly lower in patients that has partial surgical resection or no resection (43, 76). Such assays can be used in scientific studies.

Detection of parasite antigens or DNA in biopsy specimen

Metacestode tissue samples obtained by surgery or fine needle biopsy of organ lesions can be species-specifically identified by the use of PCR (26, 44) or direct immunofluorescence or immunohistochemistry (26).

Table 2.16.
Sensitivities and specificities of assays for antibody detection in human alveolar echinococcosis (AE) (examples)

Antigen	Assay	Percentage sensitivity in cases of AE (cases tested)	Percentage specificity in cases of cystic echinococcosis (cases tested)	Percentage specificity in cases of other parasitoses (cases tested)	Ref.
E. granulosus					
Hydatid fluid	ELISA	97 (140)	–	51 (144)[a]	48
Hydatid fluid	IgG4-ELISA	52 (54)	62 (56)	100 (80)[b]	49
E. multilocularis (Em)					
CH-10: crude	ELISA	96 (140)	39 (124)	97 (144)[a]	48
Em2: partially purified	ELISA	89 (140)	94 (124)	100 (144)[a]	48
Em 10: recombinant[c]	ELISA	86 (140)	93 (124)	98 (144)[a]	48
		93 (74)	89 (64)	100 (30)[d]	52

Em2Plus: mixture of Em2 & Em II/3-10[c]	ELISA	97 (140)	74 (124)	98 (144)[e]	48
Em alkaline phosphatase: purified	ELISA	100 (37)	100 (44)	100 (34)[e]	98
Em C: 30-35 kDa fraction of crude antigen	WB	95 (60)	100 (10)	100 (24)[f]	100
Em 18/16: partially purified	ELISA	91(79)	67 (48)	100 (35)[g]	57

ELISA : enzyme-linked immunosorbent assay
WB : Western blot

a) cases of fasciolosis (20), schistosomosis (17), cysticercosis (20), taeniosis (17), intestinal and tissue nematode infections (70)
b) cases of infections with protozoa (24), trematodes (16), nematodes (32) and of cysticercosis (8)
c) the recombinant antigens Em10 and II/3-10 are functionally identical
d) cases of amoebosis (2), fasciolosis (2), schistosomosis (4), paragonimosis (3), neurocysticercosis (17), and filariosis (3)
e) cases of liver amoebosis (5), malaria (3), schistosomosis (11), trichinellosis (8), toxocarosis (7)
f) cases of schistosomosis (1), paragonimosis (2), diphyllobothriosis (19), toxocarosis (1), and filariosis (1)
g) cases of cysticercosis (28), sparganosis (2), paragonimosis (5)

2.3.3.4.2. Immunodiagnosis in human populations

Early diagnosis of patients with AE is considered to be a prerequisite for efficient management and treatment of the disease (101). Consequently, serological screenings may be offered to populations and communities at risk. Test operating characteristics allow to perform reliable seroepidemiological studies, and thus, to detect asymptomatic cases of AE as well as cases, in which the metacestode lesion has died out at an apparently early stage of the infection. However, it is still difficult to detect liver lesions below 10 mm in diameter either by US examination or by immunodiagnosis. In a Japanese study, 64% of liver lesions detected by US were small, ranging from 8 mm to 50 mm in diameter (109). Cases with lesions below 10 mm in diameter were seronegative (K. Suzuki and N. Sato, personal communication, 1998). Mass screening programmes have used specific immunodiagnostic assays for primary screening followed by ultrasound and other imaging examinations of suspected cases or US examination has been employed as primary screening alternatively complemented by antibody detection. Additional details are described in Chapter 6.2.

2.3.3.5. Pathological and histological examination

In macroscopic sections of the human liver, the metacestode of *E. multilocularis* typically exhibits an alveolar structure composed of numerous irregular cysts with diameters between less than 1 mm and 30 mm (Fig. 2.12.). Due to necrosis of the lesion, cavities filled with liquid and necrotic material may be formed in the central parts of the parasite (32). Microscopically, the cysts consist of a relatively thin PAS-positive laminated layer and a delicate germinal layer often with only a few nuclei; quite frequently the germinal layer is not discernible (Fig. 2.13.). Brood capsules and protoscoleces are rarely formed in the human host (32). The cysts are surrounded by an inner zone of necrotic tissue and outer layers of histiocytes and lymphocytes. In later phases, tissue reactions of chronic inflammation, often with giant cell foreign body reaction, fibrous tissue and calcifications are seen around cysts. Often fibrous tissue proliferation is so intense that cysts are embedded in a very dense and hard stroma, however, the metacestode as a whole is not demarcated at its outer limits by a fibrous capsule like cysts of *E. granulosus*, except in abortive lesions (32). These are characterised by a fibrous wall, which may be partially calcified, and a cavity filled with amorphous necrotic material, in some cases also with folded parasite layers (32, 92).

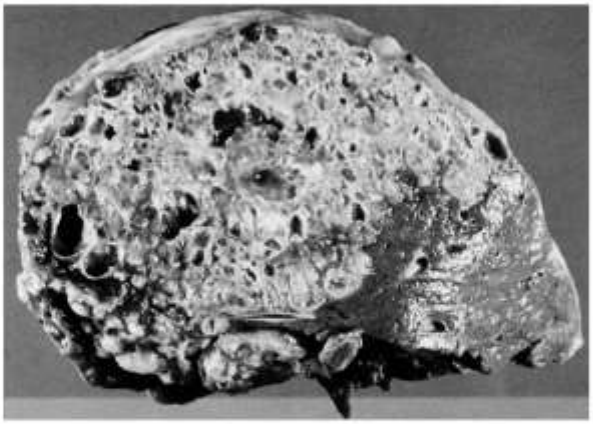

Fig. 2.12.
Macroscopic appearance of human liver with alveolar echinococcosis: multiple small and larger cysts (maximum diameter of a single cyst: 3 cm)
Photograph: J. Eckert, courtesy of the Institute of Parasitology, Zurich

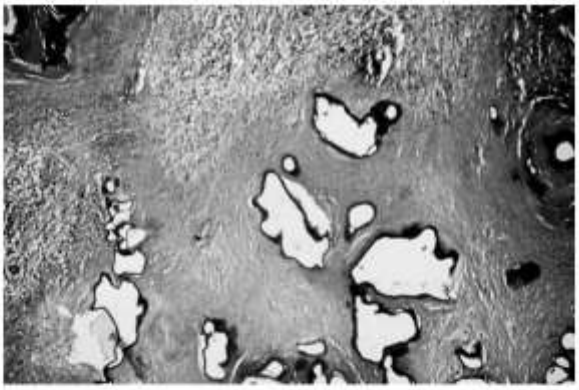

Fig. 2.13.
Histological section of *Echinococcus multilocularis* metacestode in human liver: cysts without brood capsules and protoscoleces
Photograph: J. Eckert, courtesy of the Institute of Parasitology, Zurich

2.3.4. Treatment

General considerations

Treatment of AE involves a variety of options, including surgery and chemotherapy, and requires a specific clinical experience. Therefore, patients should be referred to the recognised national/regional AE treatment centres. As the parasite lesion is comparable to a malignant tumour, early diagnosis of AE is of special importance for successful treatment. Population screening programms for AE in endemic areas of Japan and Europe have clearly shown that early diagnosis reduces morbidity and mortality, as well as costs of the disease (11, 109) (Chapter 6.2.).

The following principles for the treatment of AE are now commonly accepted (129):

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- the first choice treatment in all operable cases is radical surgical resection of the entire parasitic lesion from the liver and other affected organs
- in AE patients after radical surgery chemotherapy is indicated for a limited period of time
- long-term chemotherapy is mandatory after incomplete resection of the lesions, in inoperable patients (including cases after interventional procedures) and in AE patients after liver transplantation (further details see below).

2.3.4.1. Surgery

Excision of the parasitic lesion has to be carried out using the procedures of radical tumour surgery (114, 115). Radical or non-radical surgery and liver transplantation require concomitant chemotherapy (see below).

Indications

Resectability of the parasitic lesion in the liver is a prerequisite for radical surgery and must be assessed by imaging techniques before the operation.

Contraindications

Inoperable lesions, extensive lesions, lesions not confined to the liver and diaphragm, but extending to other organs must be managed by alternative therapies after an interdisciplinary consultation.

Benefits

Radical surgery may eliminate the parasites and cure the patient. An early diagnosis of AE can improve prospects for complete cure. Nonradical surgery for reducing the parasite mass and for increasing chances of effective chemotherapy is debatable.

Risks

Lesions cannot always be clearly defined by imaging techniques; incomplete resection leaves invisible remnants of parasitic tissue with a potential for regrowth and dissemination into other organs, even after some years. General risks may be associated with surgical intervention (anaesthesia, stress, etc.), infections (including those transmitted by blood transfusion) or other factors.

Medical requirements

Hospitalisation in a surgical ward is mandatory. The surgical team should be experienced in major liver surgery and in treating AE.

2.3.4.2. Chemotherapy

Extensive studies in animals showed significant parasitostatic efficacy of benzimidazoles against the metacestode stage of *E. multilocularis* and based on this, chemotherapy of AE in human patients has been practiced since 1975 (31). Carefullly controlled clinical studies have revealed that the 10-year survival rate in inoperable or non-radically operated AE patients (including severe forms) on long-term chemotherapy increased to 80%-83%, compared to 6%-25% in untreated historical control patients (3, 4, 55, 131). In addition to chemotherapy, early diagnosis, improved surgery and medical care of patients may contribute to the success of treatment (12, 123).

Indications

There are several indications for chemotherapy, as follows:

- chemotherapy is indicated for a limited period of time after radical surgery. Since residual parasite tissue may remain undetected at radical surgery, post-operative chemotherapy for at least 2 years should be carried

out and patients should be monitored for a minimum of 10 years for possible recurrence

- long-term chemotherapy for several years is mandatory in inoperable AE patients, in cases following incomplete surgical resection of the parasite lesions and after liver transplantation
- pre-surgical chemotherapy is not indicated in cases of AE. However, in rare cases for whom surgery was contraindicated at the time of diagnosis of AE, surgery can be carried out after a prolonged course of chemotherapy.

Contraindications

In view of the severity of AE and the relative low toxicity of the drugs currently used (mebendazole or albendazole), there are only a few contraindications for chemotherapy. In some cases (pregnant women, etc.) certain precautions and limitations or modifications of drug administration are necessary (Annex 2.2.).

Choice of drugs

Two benzimidazoles (mebendazole and albendazole) are preferentially used for chemotherapy of AE (Annex 2.2.).

Mebendazole (MBZ) (Vermox® 500 mg, Janssen, Belgium) is given as 500-mg tablets in daily doses of 40 mg/kg-50 mg/kg bw in three divided doses postprandially. After an initial continuous treatment of 4 weeks, it is advisable to adjust the oral doses in order to obtain plasma drug levels of >250 nmol/l (= 74 ng/ml). The latter level was experimentally determined as effective in rodents (31). These data and results from human trials suggest that mebendazole plasma concentrations in excess of 80 ng/ml-100 ng/ml maintained for long periods may be necessary to achieve high efficacy (132). In special situations, the oral dosage may be higher than the above recommended dose, but a daily dose over 6 g per adult patient should not be given. The duration of treatment is at least 2 years after radical surgery or continuously for many years in inoperable cases, as well as for patients who have undergone incomplete resection or liver transplantation. For some patients, mebendazole has been administered for more than 17 years.

Albendazole (ABZ) (Eskazole®, Zentel®, SmithKline Beecham) is given as 400-mg tablet or as a 4% suspension at daily doses of 10 mg/kg-15 mg/kg bw (in two divided doses). In practice, a daily dose of 800 mg is given to adults, divided into two doses of 400 mg (53). The divided dose is supported by pharmacokinetic data (58). According to the original recommendation of the manufactuer, repeated cycles of 28 days treatment should be followed by a 'wash out' phase without chemotherapy of 14 days. However, recent data from the People's Republic of China (65) and Italy indicate that a continuous ABZ treatment of AE is at least equally or more effective and well tolerated. Sporadically ABZ was given in higher doses of 20 mg/kg/day for up to 4.5 years (65). The duration of necessary chemotherapy has not yet been determined but might well be life-long for most of the patients without complete resection of the AE lesions.

Praziquantel (PZQ) has been used for the treatment of human AE, but experimental data obtained from animal models indicate that its efficacy against the metacestode stage of *E. multilocularis* is far less pronounced than that of the benzimidazoles mentioned above, even when PZQ is given in very high doses (2, 31).

Benefits of benzimidazole treatment

This is a non-invasive treatment with a relatively low toxicity. However, in most patients benzimidazoles are only parasitostatic.

Risks

The main risks are neutropaenia, alopecia and liver dysfunction. Because of the potential embryotoxicity and teratogenicity (only observed in some laboratory animals), it should not be used in women of child-bearing age, unless contraceptive measures are taken, and during pregnancy especially the early stages (Annex 2.2.).

Medical requirements

Hospitalisation is not needed, but regular medical and laboratory checks for adverse reactions and efficacy are necessary. The costs of anthelmintics and repeated medical examinations are high.

Monitoring of patients

In the initial phase, monitoring of AE patients is similar to that in CE patients (Chapter 2.2.4.3.). Subsequently, haemogram and serum transaminases should be checked at intervals of 3 months. At intervals of 6 to 12 months, the patients should be examined in a clinical reference centre, where US and special imaging (for example CT) can be performed to monitor parasitic lesions and their response to chemotherapy. A long-term follow-up of more than 10 years is recommended.

2.3.4.3. Interventional procedures

With AE patients for whom surgery is contraindicated, a number of local complications may occur for which interventional procedures have to be considered (118, 129). Dilation and stent implantation in vessels and/or bile ducts, and endoscopic sclerosing of oesophageal varices are the main interventional procedures performed in AE. Drainage of necrotic liver lesions may be indicated if bacterial infection has occurred. In conjunction with chemotherapy, these procedures can be beneficial for patients.

Indications

Interventional procedures are indicated, when surgery is contraindicated because of disturbances of essential organ functions, i.e. hyperbilirubinemia due to cholestasis, vena cava or portal vein thrombosis, colliquative liver necrosis with risk of rupture into the abdomen, and/or severe bacterial infection or bleeding of oesophageal varices secondary to portal hypertension.

Contraindications

Interventional procedures have the potential risk to spread parasite material and – except the emergent and/or palliative ones – are not indicated when post-interventional chemotherapy is not possible.

Benefits

Interventional procedures together with chemotherapy as options for treatment can improve the life expectancy and quality of life of patients with AE.

2.3.4.4. Liver transplantation

In Europe, liver transplantation (LT) has been carried out in approximately 40 patients with inoperable AE and chronic liver failure (10). In a French series, 21 patients had received liver grafts between 1986 and 1991 for incurable AE (10). Among 15 patients who survived more than one year, ten were alive 6.5 to 11.5 years after transplantation (10). This study has shown that the risk of recurrence of parasite proliferation and metastasis formation after LT is relatively high (10).

Indications

Liver transplantation should only be considered in patients with very severe hilar extension, leading to uncontrolled biliary infections, symptomatic secondary biliary cirrhosis with ascites or severe variceal bleeding owing to portal hypertension (10). Such patients become more rare due to earlier diagnosis of the disease (10) so that the indication for liver transplantation is rather limited. It requires long-term and continuous post-operative chemotherapy (see above).

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Contraindications

Liver transplantation is not indicated in extensive AE that is not confined to the liver or for patients with contraindications for prolonged immunosuppressive treatment, and concomitant benzimidazole treatment.

Benefits

Liver transplantations can be a life-prolonging procedure for patients with severe liver dysfunction (10).

Risks

These include general surgical risks, specific risks of long-term immunosuppressive treatment, and induction of proliferation of metacestode remnants and metastases formation (particularly in the brain) under immunosuppression.

Medical requirements

Liver transplantation requires a highly specialised team and equipment with the competence to deal with the current post-transplantation problems as well as with the clinical problems of AE. Supportive medical care includes post-transplantation clinical observation, adaptation of immunosuppressive drugs, and diagnosis and management of complications of immunosuppressive treatment under continuous chemotherapy with benzimidazoles.

2.4. Other forms of echinococcosis

General aspects

Forms of human polycystic echinococcosis (PE) are caused by *E. vogeli* and *E. oligarthrus*, which are confined in their distribution to Latin American countries. Aspects of their biology are described in Chapter 1 (8, 20, 32). Up to 1999, at least 96 cases of human PE have been recorded in 11 countries of Central and South America (Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Venezuela, Suriname, Brazil, Uruguay, Argentina and Chile). Of the 96 cases, 37 were due to *E. vogeli*, three to *E. oligarthrus*, and in the other cases the *Echinococcus* species could not be determined. It appears that this number of cases is only 'the tip of the iceberg' (see also Chapter 4.3.) (8, 20).

2.4.1. Polycystic echinococcosis due to *Echinococcus vogeli*

The metacestode stage of *E. vogeli* is characterised by a polycystic structure and development in visceral organs. In 59 patients with PE, the liver was the most frequently affected organ. In 78% of the patients, the liver was infected alone or together with other organs (spleen, pancreas, stomach, omentum, mesenteries, lung, diaphragma, pericardium, intercostal muscle, etc.) (20). The second most frequently infected organ was the lung 14%, either singly or together with liver or other organs. Single site infections were observed in the liver and lung, but also in other organs (i.e. mesenteries and stomach) (20). Clinical and radiological presentation is very similar to infection with multiple cysts of *E. granulosus*, and differential diagnosis depends on isolation of protoscoleces and morphological hook characteristics (20). Immunodiagnosis using a purified antigen of *E. vogeli* allowed discrimination between cases of PE and CE, but differentiation between PE and AE was not always possible (46). Albendazole has been used for chemotherapy in six cases with success of treatment in four and improvement in two (20).

2.4.2. Polycystic echinococcosis due to *Echinococcus oligarthrus*

The causative agent is the metacestode of *E. oligarthrus*, which is polycystic in structure, and in naturally infected animals, it has been most commonly found in the musculature and the skin, but also in viscera. Only three human cases have been reported to date, two orbital in Venezuela and Suriname and one cardiac in Brazil with 2 cysts (1.5 cm diameter) (20). The diagnosis was based on morphology of protoscolex hooks.

2.5. Ethical aspects

In human echinococcosis ethical aspects have to be considered carefully in activities related to:

a) pure research (e.g. drug testing)

b) optimal and/or novel diagnostic or therapeutic approaches (e.g. diagnostic cyst puncture, therapeutic cyst puncture)

c) population-based studies (mass ultrasonographic or serological screening for CE or AE).

In all the situations, basic human rights have to be respected, according to the Helsinki declaration II (23), CIOMS documents (15, 117) and ethical review committees rules (24). The aim of all these documents is to minimise the risk that medical intervention may bring to the patients.

Risks of medical intervention in echinococcosis may result from the following situations (117):

a) not respecting indications and contraindications in high risk groups of patients (e.g. with young or advanced age, with coagulation defects before liver biopsy)

b) using inadequate instruments (e.g. blunted or nonsterile biopsy needles, imaging equipment of poor quality)

c) carrying the intervention in ways that do not minimise the direct risks (e.g. haemorrhage or anaphylactic shock on liver puncture) or complications (infections, if non-sterile equipment is used, or secondary echinococcosis, if anthelmintic cover is neglected)

d) performing interventions by inexperienced or careless operators (e.g. by not referring patients to a competent centre)

e) making a false interpretation (e.g. results of the serological tests, imaging technologies or biopsy specimens).

General rules related to research

All research activities in echinococcosis aspects necessarily must follow some general rules: any planned research in echinococcosis should avoid a repetition of a previously well done study, have serious justification, with well defined objectives and appropriate study design. The study has to be performed in properly selected and representative population with a careful justification of the necessity of using special vulnerable groups of participants (small children, pregnant or nursing women). The selection of study methods should in optimal way address the objectives, consider necessary sample size and statistical power estimates as well as assure quality control. The personnel involved in the study should be well trained in the use of instruments and procedures, and there should be clearly described emergency procedures. The data have to be properly collected, avoiding bias, respecting confidence rules and defining the way and extent of notifying patients about the study findings. Final data analysis should concentrate on original discoveries, follow basic rules of statistical methodology and respect limitations of the study. Data management, including storing and protecting of the data and their final disposition, has to be decided before the study is undertaken. Other important conditions are: analysis of risks for patients involved and methods to minimise those risks; defining of the study benefits for patients and introduction of an informed consent procedure. This should ensure patient's rights not to participate in the study, or to withdraw his/her agreement to participate any time during the study as well as to regulate any financial aspects of participation or compensation in case of any harm related to the study (15, 23).

Some ethical problems related to the particular situations in echinococcosis research, individual clinical care and population interventions are presented, as follows:

- **Drug testing study**

Drug testing studies are to be best designed by the comparison of an investigational drug versus already available drugs in two randomly selected comparable groups. The consent form is required for each participating patient. The study has to be stopped as soon as the investigational drug is found to be of lower efficacy or too harmful; in that case the group assigned to an investigational drug should be offered a full conventional treatment without any delay (22). In the multicentre study, a uniform protocol has to be

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prepared and followed unless it is in conflict with the individual patient's interest according to the best judgement of the researcher.

- **Modification or extension of the standard medical care**

Much progress in the diagnosis and treatment of human echinococcosis originates from the observations of the results of *ad hoc* modified or extended care of the clinical patients, e.g. pharmacokinetic studies, evaluation of the efficacy of chemotherapeutic treatment by imaging techniques, dosing of anthelmintics. In some of these studies, a consent form from the patient may be needed, but in all such studies it is essential that the patient's interest and the benefits for the future patients due to the improved knowledge and experience would outweigh any risk or inconvenience of the modified procedure to the patient.

- **Novel diagnostic or therapy procedures**

It is very important that the initial sporadic clinical observations or experiences that may suggest a novel or improved procedures are described in detail and with maximal objectivity. However, the study aiming at introduction of the novel diagnostic procedures, such as diagnostic biopsy of the liver cysts or the novel therapeutic interventions such as PAIR or liver transplantation should be reserved for selected reference centres before enough experience is gained about their efficacy and safety elsewhere (35).

- **Selection of the optimal diagnostic procedures and treatment methods**

The variety of possible diagnostic procedures and treatment methods available frequently poses a question of the best choice. The choice has to respect the patient's interests, the availability of the diagnostic facilities and drugs as well as the cost of interventions. Unnecessary diagnostic procedures such as diagnostic biopsies, additional radiological or imaging examinations should be avoided. On the other hand, patients should be referred to specialised centres whenever practically possible. The information about the optimal treatment of CE and AE patients is widely available and regularly updated (86, 129).

- **Population-based study**

The population-based study should fully respect the human rights and ethical requirements. First of all, the study should not be undertaken in case the results will be of no benefit for individual participants found to be infected and/or any further use for public health services improvement. Before the population-based study is undertaken, it has to be accepted by the local public health authority and the population at large. When it is impracticable to elicit adequately informed consent from every individual involved in the study an acceptable procedure is to delegate the power of consent to local independent representative body. However, a rule has to be accepted that any individual person involved may refuse his/her participation in the study at any time. The study should be carefully designed and a decision made regarding the way in which any individual participant found to be infected will be further diagnosed, treated and cared. The population-based study have to be performed very carefully as small inadequacies in methodology of the study, in examination of the individual patient and interpretation of the results may lead to the false general conclusions. The documentation of such a study should be as complete as possible, in order of gaining the highest credibility. For example, in mass screening for liver CE, the results should mention – in addition to the age, sex, locality and the number of people examined – the number of persons with any liver space occupying lesion, the number of the persons with lesions suspected for liver CE and the number of patients with confirmed CE by other techniques including surgery and the types of *E. granulosus* cysts (Table 2.5.) or AE lesions (Table 2.13.).

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Annex 2.1.

Determination of performance characteristics for immunodiagnostic assays

F. Grimm

Detailed information on principles of validation of diagnostic assays for infectious diseases can be obtained from a recent review (3). Some basic aspects are described here.

Selection of the cut-off point (positive/negative threshold)

To achieve estimates of the diagnostic sensitivity and specificity (see below) of an assay, for example of an ELISA for serum antibody detection, the results first must be allocated to positive (antibodies detected) and negative (no antibodies detected) categories. The threshold or cut-off point between these categories may be selected by visual inspection of the frequency distributions of test results of groups of infected and uninfected reference individuals (3). However, visual inspection is not precise. Therefore, the cut-off is usually determined by calculating the mean of testing results (optical densities in the ELISA) + 2 or + 3 standard deviations (SD) for groups of individuals that are not infected with a specific agent, for example *Echinococcus*. All testing results above the cut-off point are regarded as positive.

The selection of negative reference groups is crucial. The cut-off point can be based on testing results of individuals that are free of parasites or that are free of a specific agent, i.e. *Echinococcus*, but may harbour other parasites which do not interact with the assay (for example with protozoan parasites in case of an *Echinococcus* assay). Since geographic and ethnic variation is known to occur in antibody response, it might be necessary to determine the cut-off point for each population under evaluation. In cases where information on the parasitological status of the population under study is not available, cluster analysis may provide a powerful statistical tool for the determination of a threshold value (2).

Calculation of diagnostic sensitivity and specificity

- **Definitions**

Diagnostic sensitivity (DS) is defined as the proportion of known infected individuals that test positive in an assay. Infected individuals that test negative are considered as false negatives. Analytical sensitivity defines the smallest amount of the analyte – for example antigen – which is detectable (3). Diagnostic specificity (DSP) is defined as the proportion of uninfected reference individuals that test negative in the assay. Uninfected reference individuals that test positive are regarded as false positives (3). With regard to parasitic infections two types of diagnostic specificities may distinguished:

DSP1: proportion of uninfected reference individuals that test negative in a population of individuals free of parasites;

DSP2: proportion of uninfected reference individuals that test negative in a population of individuals that are not infected with a specific parasite (for example *Echinococcus*), but harbour other parasites or infective agents.

After the cut-off point is established, the testing results of sera can be classified as true positives (TP) and true negatives (TN) if they are in agreement with those of the gold standard (3). The gold standard in human patients with echinococcosis is the diagnosis of the infection by imaging or by other methods of direct parasite identification. Alternatively, they are classified as false positive (FP) or false negative (FN).

Diagnostic sensitivity and DSP are calculated as follows and expressed as percentages:

Diagnostic sensitivity percentage: $DS = \frac{TP}{TP + FN} \times 100$

Diagnostic specificity percentage: $DSP = \frac{TN}{TN + FP} \times 100$.

Hypothetical example

Among 100 individuals 15 had confirmed echinococcosis with the following serological testing results:

Test result	Reference individuals	
	With confirmed echinococcosis	Without echinococcosis
Positive	13 = TP	3 = FP
Negative	2 = FN	83 = TN
Diagnostic sensitivity: DS = 13/13 + 2 × 100 = 86.6%		
Diagnostic specificity: DSP = 83/83 + 3 × 100 = 96.5%		

Calculation of predictive values

- **Definitions and general aspects**

The predictive value (PV) can be expressed as positive (PV+) or negative (PV–) value. The PV+ is an indicator of the probability that individuals with positive test results do have the disease, whereas the PV– expresses the probability that individuals with negative testing results do not have the disease. With other words, the PVs are indicators of the probability of the correctness of the diagnosis. The PVs are determined by the prevalence of a disease (P), and both the diagnostic sensitivity (DS) and diagnostic specificity (DSP) of the test. It is important to understand that the PVs are not inherent assay characteristics. Especially positive PVs are strongly dependent on the prevalence of an infection/disease in the population under study, and on the DSP of the assay used.

- **Formulas for calculating predictive values**

$$\text{Positive predictive value in percentage} = \frac{P \times DS}{P \times DS + (100 - P) \times (100 - DSP)} \times 100$$

$$\text{Negative predictive value in percentage} = \frac{DSP \times (100 - P)}{DSP \times (100 - P) + (100 - DS) \times P} \times 100$$

P : prevalence of the disease

DS : diagnostic sensitivity

DSP : diagnostic specificity

- **Example for calculating predictive values**

The expected prevalence of echinococcosis in a population is 2%, the available ELISA for a serological survey has a diagnostic sensitivity of 70% and a diagnostic specificity of 90% (1).

Positive predictive value

$$PV+ \frac{2\times 70}{2\times 70+(100-2)\times(100-90)}\times 100=\frac{140}{140+980}\times 100=\mathbf{12.5\%}$$

Negative predictive value

$$PV- \frac{90\times(100-2)}{90\times(100-2)+(100-70)\times 2}\times 100=\frac{90\times 98}{90\times 98+30\times 2}\times 100=\mathbf{99.3\%}$$

The probability for a correct negative result is high, but low for a correct positive result.

- **Examples of predictive values (PV) for different prevalences (P) and diagnostic sensitivities (DS) and specificities (DSP)**

DSP	DS	PV+ P: 10%	PV+ P: 2%	PV+ P: 0.5%	PV– P: 10%	PV– P: 2%	PV– P: 0.5%
90%	70%	43.8	12.5	3.4	96.4	99.3	99.8
90%	97%	51.9	16.5	4.6	99.6	99.9	>99.9
99%	70%	88.6	58.8	26.0	96.7	99.4	99.8
99%	97%	91.5	66.4	32.8	99.7	99.9	>99.9

These examples show that for any prevalence of a disease, the PV+ depends predominantly on DSP, whereas PV– is more dependend on DS. It is to be underlined that in areas with a low prevalence of a disease assays with high DSP's are of crucial importance for reliable seroepidemiological studies.

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Annex 2.2.

Characteristics of benzimidazoles

Source: WHO (1996) (3)

Mebendazole (MBZ) (Vermox 500 mg®, Janssen) is poorly absorbed (<10%) after oral administration. The rate of absorption is increased (up to 8-fold) if the drug is taken during a meal, especially one with a high fat content. After oral administration of standard doses, serum drug levels are highly variable among individuals and are not correlated with the doses given. In blood plasma, >90% of the drug is protein-bound. Based on data from animal experiments, the serum drug concentrations required for effective chemotherapy are estimated to be >250 nmol/l (= 74 ng/l). However, several studies have shown that such serum levels may

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not be attained by more than 30% of the patients and that lower (as yet undetermined) levels may be sufficient for of long-term therapy. Mebendazole is rapidly metabolised in the liver and excreted via urine and bile. The elimination half-life times are short (2.5 h-5.0 h) and may be increased in patients with cholestasis and other disturbances of liver function. Serum mebendazole concentrations 4 h after the morning dose have a high degree of predictability for the 24 h average serum concentrations, and the 4-h value has therefore been proposed for monitoring serum drug levels.

Albendazole (ABZ) (Eskazole®, Zentel®, SmithKline Beecham), has similar pharmacokinetic properties to mebendazole with low absorption rates and high interindividual variability of serum drug levels that may lie in the range 200 nmol/l-6,000 nmol/l; average values are 1,000 nmol/l-2,000 nmol/l (albendazole sulfoxide). Serum drug levels are higher in patients with cholestasis and other liver dysfunctions, and intestinal absorption rates are increased by fatty food. The mean half-life elimination time in 14 persons was 8.5 h (SD, 6.0). The effective serum drug levels are not well defined; based on data from animal experiments, they are estimated to be around 650 nmol/l-3,000 nmol/l.

Drug efficacy

Mebendazole and the main metabolite of ABZ – albendazole sulfoxide have anti-parasitic properties.

Animal experiments have shown that long-term treatment with various benzimidazole derivatives (for example: albendazole, fenbendazole and mebendazole) has the following effects against *E. multilocularis* metacestodes: inhibition of metacestode proliferation, resulting in reduction of parasite masses; destruction of protoscoleces and partial destruction of the germinal layer of the metacestode; prevention or suppression of metastasis formation; calcifications; and prolongation of host animal survival.

Long-term animal studies have shown that *E. multilocularis* metacestodes are usually not killed by drug treatment, but that their proliferation is inhibited. The effect of the drugs in animals is therefore not parasitocidal, but parasitostatic. On the other hand, *E. granulosus* cyst may be killed by a long-term benzimidazole treatment.

Adverse reactions

Mebendazole and ABZ are generally well tolerated and adverse reactions are relatively mild. Examples of such reactions from two larger series are presented below.

- Adverse reactions in 70 patients with alveolar echinococcosis under long-term chemotherapy (mean duration: 6.5 years. Number of patients treated: MBZ: 61, ABZ: 4, MBZ/ABZ: 5) were: elevation of transaminases (27%); proteinuria (21%); loss of hair (18%); gastrointestinal disturbances (16%); neurological symptoms (e.g. vertigo) (11%) and leukopaenia (6%) (1).

- Adverse reactions associated with albendazole treatment of 780 patients with CE (the duration of treatment is generally shorter than for alveolar echinococcosis) were elevation of transaminases: (14.7%); abdominal pain: (5.7%); loss of hair: (2.8%); headache: (2.1%); abnormal liver biopsy: (1.7%); vertigo/dizziness: (1.3%); nausea: (1.3%); fever: (1.2%); reversible leucopaenia: (1.2%); abdominal distension: (0.6%); urticaria: (0.5%); jaundice: (0.5%); thrombocytopaenia: (0.3%); allergic shock: (0.3%); bone marrow toxicity: (0.1%); and cyst pain: (0.1%) (R.J. Horton, personal communication, 1997).

In a recent publication, Horton (2) listed 817 adverse events in 3,282 patients with echinococcosis, who had been treated with albendazole. The majority of adverse reactions referred to the liver and the gastrointestinal tract. During 12 years, there was not a single fatal case in patients with echinococcosis related to chemotherapy with ABZ. Two thirds of the patients experienced one or more side effects, but they were mostly of minor importance and reversible. Only in rare instances (3.8%) was a permanent discontinuation of chemotherapy indicated. Allergic reactions may also occur.

Precautions

Patients with CE or AE under chemotherapy should be carefully monitored (Chapters 2.2.4.3. and 2.3.4.2.). Monitoring of serum drug levels is suggested to avoid severe toxic reactions.

Pregnancy and nursing

Under certain conditions, MBZ and ABZ may induce embryotoxic or teratogenic effects in some animals. Although such effects have not been observed in humans, it is recommended that use of these drugs be avoided for pregnant women, or the drugs to be used only in urgent cases in the second or third trimester after a careful benefit/risk analysis. For women of child-bearing age, contraceptive measures are indicated during treatment. Experience with MBZ or ABZ treatment during breast feeding does not appear to put the infant at risk of side effects.

Liver disturbances

For patients with cholestasis or hepatocellular disturbances, the drug doses may have to be reduced. Such patients require frequent monitoring of liver function parameters and of serum drug levels, especially those with chronic cholestasis.

Diabetes

Mebendazole may reduce the insulin requirement; therefore, the serum glucose blood levels of diabetics must be carefully monitored.

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Chapter 7

Prevention of echinococcosis in humans and safety precautions

J. Eckert, B. Gottstein, D. Heath and F.-J. Liu

Summary

In view of the high potential pathogenicity of the Echinococcus *infection to humans safety precautions in laboratories and for field workers are of special importance. Heat remains the most reliable method for killing of* Echinococcus *eggs. They may also be inactivated by deep-freezing, but only at temperatures of –70°C to –80°C and minimum exposure times of 96 h and 48 h, respectively. The high cold resistance of the eggs of* E. multilocularis *is well documented. On the other hand, it is still unclear whether strains* E. granulosus *may differ in various regions with regard to cold resistance of their eggs. Chemical disinfection is difficult as most of the commercial disinfectants are ineffective against* Echinococcus *eggs. Of some value is sodium hypochlorite solution. Recommendations are given for disinfection of materials and objects contaminated with* Echinococcus *eggs, for decontamination of living-rooms and cars, and for inactivation of metacestode material. Furthermore, guidelines for precautions during treatment of dogs and cats infected with* E. multilocularis *and for prevention of cystic and alveolar echinococcosis in humans are presented.*

7.1. Safety precautions and disinfection

Safety precautions formed an important section in the 'Guidelines for Surveillance, Prevention and Control of Echinococcosis/Hydatidosis' published in 1984 (31) and are again emphasised in this publication.

7.1.1. Awareness of the problem

Persons at special risk and dangerous material

All personnel handling dogs, foxes and other carnivores known or suspected to be final hosts of *Echinococcus* species in endemic areas should be aware of the health risk both to themselves and to the general public (15, 31). This applies with special force to personnel involved in diagnostic work (necropsies of foxes, dogs, etc., faecal examination of carnivores) or in echinococcosis surveys and control programmes. In areas with endemic echinococcosis, they should be encouraged to regard all definitive hosts as potentially infected. Furthermore, they should always treat any faeces or other materials possibly contaminated with *Echinococcus* eggs under strict safety precautions. Safety precautions are also important in laboratory work and to some extend in clinical investigations (see below).

7.1.2. Sources and routes of infection

Primary echinococcosis in humans (Chapter 2) usually results from the ingestion of *Echinococcus* eggs. However, there is also evidence that the hatching and activation of embryos can occur in extra-intestinal sites (29). This raises the possibility that infection may result from the inhalation of eggs with subsequent development in the lungs. Experimental studies with sheep support this possibility (2). However, this has never been substantiated for natural infections. On the other hand, it may well be that eggs are inhaled, then swallowed and transported to the intestinal tract. Furthermore, secondary echinococcosis may possibly follow contamination of the conjunctiva with protoscoleces, but such cases have never been described.

Infection of humans with *Echinococcus* eggs may result from:

a) Handling infected definitive hosts, egg-containing faeces or egg-contaminated plants or soil followed by direct hand to mouth transfer. It has been shown that eggs of *Echinococcus* adhere to the coat of

dogs (25), particularly to the hairs around the anus, on the thighs, muzzles and on the paws (23). The same applies to dogs infected with *Taenia* species (5) and to foxes infected with *E. multilocularis* (unpublished findings).

b) Ingestion of vegetables, salads, uncooked fruits and other plants which have become contaminated directly with *Echinococcus* eggs. Foodstuffs or surfaces may possibly be secondarily contaminated with *Echinococcus* eggs via agents such as wind, birds, beetles and flies (see Chapter 5.1. for experimental evidence).

c) Drinking of water contaminated with *Echinococcus* eggs by faeces of infected carnivores is a potential route of infection. Recent studies in the People's Republic of China (Sichuan) have shown that people drinking water from small ditches which are accessible to animals have a higher risk to acquire CE than others consuming well-water (F.-J. Liu, personal communication, 1998) (Chapter 5.2.).

d) Inhalation of eggs in dust cannot be excluded as an infection route (15), but is apparently unimportant.

Reliable data on the actual importance of the various potential routes of infection are not available so far.

7.1.3. Resistance of *Echinococcus* eggs

Resistance to temperatures

Echinococcus eggs are highly resistant, and may remain infective for about one year in a suitable, moist environment at lower temperatures. For example, eggs of *E. multilocularis* remained viable for about 16 months at +4°C in water (30). It can be assumed that, because eggs survive at low temperatures, large numbers will accumulate during the cold season in sites where definitive hosts defecate, for example in yards where livestock live with their guard dogs. By the end of the winter, such environments must be loaded with *Echinococcus* eggs. On the other hand, desiccation and high temperatures are the two most important factors reducing the longevity of the eggs (7, 15, 16, 20, 30) (Table 7.1.).

Echinococcus eggs are killed by boiling water or dry heat. Eggs of *E. granulosus* are killed within 5 min at +60°C to +80°C and instantaneously at 100°C (Table 7.1.). *Taenia* eggs are killed by exposure to these temperatures (7), and this is also very likely for eggs of *E. multilocularis*. It has to be stressed, however, that the length of time for which contaminated materials should be heated will vary. For example, heat penetrates dog faeces slowly and such material should be boiled for at least 5 min to ensure killing of all eggs (15). Most sewage treatment processes (for example sedimentation) do not totally eliminate taeniid eggs (7). Based on experiments with *Ascaris* eggs, it can be assumed that the eggs of *Taenia* and *Echinococcus* are killed in sewage sludge and compost after exposure for at least 30 min to temperatures of +65°C or higher, generated by heating or fermentation processes (7).

On the other hand, eggs of both *E. granulosus* and *E. multilocularis* are highly resistant to freezing temperatures (Table 7.1.). Therefore, the temperatures of a household deep-freezer of –18°C to –20°C are insufficient for inactivating the eggs within a reasonable time.

However, very low temperatures of –70°C to –80°C are able to kill eggs of *E. granulosus* and *E. multilocularis* within 96 h or 48 h, respectively (Table 7.1.). The effective temperatures have to reach all parts of the contaminated material. For example, carcasses of foxes have to be frozen at –80°C for at least 4 days (routinely 7 days) in order to achieve thorough deep-freezing. The high cold resistance of the eggs of *E. multilocularis* is well documented. On the other hand, it is still unclear whether strains *E. granulosus* may differ in various regions with regard to cold resistance of their eggs.

Resistance to desiccation

The eggs of *Echinococcus* are sensitive to desiccation. At a relative humidity of 25% eggs of *E. granulosus* were killed within 4 days and at 0% within 1 day (21). Eggs of *E. multilocularis* lost infectivity to rodents after exposure at +25°C and a relative humidity (RH) of 27% for 2 days, at +43°C and 15% RH for 2 h, and at +45°C and 85%-95% RH for 3 h (30).

Table 7.1.
Resistance of *Echinococcus* eggs to heat and low temperatures

***Echinococcus* species**	**Temperature (°C)**	**Survived (+) or killed (–) after periods indicated**	**References**
E. granulosus	+45 to +55	5 min: +	Colli and Williams (3)
	+60 to +80	5 min: –	Colli and Williams (3)
	+100	1 min: –	Meymerian and Schwabe (24)
	–30	24 h: +	Colli and Williams (3)
	–50	24 h: +	Colli and Williams (3)
	–70	24 h: –	Colli and Williams (3)
E. multilocularis	–18	240 days: +	Veit *et al.* (30)
	–27	54 days: +	Schiller (28)
	–30	24 h: +	Colli and Williams (3)
	–50	24 h: +	Colli and Williams (3)
	–70	96 h: –	Blunt *et al.* (1)
	–80 to –83	48 h: –	Frank (14), Eckert *et al.* (12) Veit *et al.* (30)
	–196	20 h: –	Veit *et al.* (30)

Resistance to chemicals

Echinococcus and *Taenia* eggs are highly resistant to numerous chemicals (22). For example, eggs of *T. pisiformis* survived for 3 weeks in 10% formalin, eggs of *E. granulosus* retained viability in ethanol (50%, 70%, 95%) after 5 min to 60 min exposure (19, 24, 26), but only a few survived in glutaraldehyde (5% and 10%) (27). Most of the commercial disinfectants with activity against viruses and bacteria are ineffective against *Echinococcus* eggs (see below).

7.1.4. Ovicides and disinfection

Heat

Heat remains the most reliable and effective method for killing the eggs of *Echinococcus* and can be applied in various forms for disinfection (Table 7.2).

Deep-freezing

Eggs of *E. multilocularis* or *E. granulosus* in carcasses or intestines of final hosts (foxes, dogs, etc.) infected with the parasite or in contaminated faecal material can be inactivated by deep-freezing at –70°C to –80°C for at least 4 or 2 days, respectively (Table 7.1.).

Irradiation

The infectivity of *E. granulosus* eggs after irradiation with doses of 10, 20 and 30 krad (= 100, 200 and 300 Gray) was diminished, but not lost (32). *Echinococcus multilocularis* eggs irradiated with a dose of 40 krad were apparently infective to rodents (as demonstrated by antibody detection), but metacestodes did not develop (30). For inactivation of taeniid eggs higher irradiation doses than 40 krad are apparently required. Indeed, after infection of rodents with eggs of *Taenia taeniaeformis* irradiated at 60 krad metacestodes did not develop (6).

Table 7.2.
Examples for disinfection of materials and objects contaminated with *Echinococcus* eggs, or of viable metacestode material

Type of material or object	Method of disinfection	Further usability of materials/ objects
Contamination with *Echinococcus* eggs		
Faecal samples	Boiling, 5 min	Examination for eggs and pro-glottids
	Steam sterilisation (autoclave)	Disposal
	Incineration	Disposal
	Deep-freezing at –80°C, 2 days	Examination for eggs and proglottids, coproantigen detection (CA), PCR
Whole carcasses or intestines of foxes, dogs, etc.	Steam sterilisation (autoclave)	Disposal
	Incineration	Disposal
	Deep-freezing at –80°C, at least 4 days	Examination for cestodes and other parasites, CA, PCR
Metal trays	Steam sterilisation (autoclave)	Re-utilisation
	NaOCl[a] solution (3.75%) for at least 1 h	Re-utilisation
Metal tables and other work surfaces	NaOCl[a] solution (3.75%) for at least 1 h	Re-utilisation
Metal instruments	Steam sterilisation (autoclave)	Re-utilisation
	NaOCl[a] solution (3.75%) for 5 min	Re-utilisation
Concrete floors[b]	Boiling water or hot water/steam mixtures	Re-utilisation
	NaOCl[a] solution (3.75% or higher) for at least 2 h-3 h	Re-utilisation
Clothing and other laundry	Steam sterilisation (autoclave)	Re-utilisation
	Washing in a washing-machine at +60°C, 1 h	Re-utilisation
Plastic sheets and disposable protective clothing	Steam sterilisation (autoclave)	Disposal
	Incineration	Disposal
Foodstuffs (vegetables, fruits, etc.) and water potentially contaminated	Heating, >60°C, at least 30 min	Consumption
***Echinococcus* protoscoleces or other viable metacestode material**		
Echinococcus metacestode material	Steam sterilisation (autoclave)	Disposal
	Incineration	Disposal
	4% formalin	Disposal or histological examination
	40% ethanol	Disposal or PCR, other examinations
	Deep-freezing, –20°C or –70°C, for at least 1-2 days	Antigen preparation, PCR, other examinations

a) sodium hypochlorite
b) alternatively the floor (or parts of it) of rooms in which potentially infective *Echinococcus* material is handled can be covered with plastic sheets which are disposed after use (see above)
Methods marked with are of variable efficacy
CA : coproantigen
PCR : polymerase chain reaction for detection of DNA

Chemical disinfection

Sodium hypochlorite solution (NaOCl) at a minimum concentration of 3.75% in water disrupts the embryophores of *Echinococcus* eggs and damages the majority of the oncospheres within a few minutes (4) (Table 7.2.). However, the effect of this disinfectant is variable and depends on the actual chlorine concentration, on temperature and the depth of penetration; it does not penetrate easily into organic materials. This may have been the reason that exposure of *E. multilocularis* eggs to a household disinfectant containing NaOCl with 'under 5% free chlorine' did not kill all eggs after 5 min (30). One should be aware that the concentration of active chlorine may decrease rapidly in a solution by evaporation. Therefore, high quality and fresh NaOCl solutions should be used. NAOCl solution is quite aggressive and has to be handled with care.

NaOCl solutions of about 1.3% to 4% are commercially available as bleaches or antifungal substances for use in households. It has been recommended (4) to use NaOCl solutions of 3.75% to wipe down work surfaces, soak instruments (3 min-5 min), plastic trays, glassware, etc. (time unlimited), both in the laboratory and in the field. In some laboratories, it is common practice to use NaOCl solutions at higher concentrations for longer exposure times for disinfecting work surfaces, floors, trays, plastic material, etc. (Table 7.2.).

In a recent study (30), the efficacy of 10 commercial disinfectants, containing phenol derivatives, aldehydes, ethanol phosphoric acid and other substances, was tested against *E. multilocularis* eggs. None of these disinfectants used in the recommended concentrations and application times killed the eggs as shown by *in vitro* activation of eggs and, in addition, peroral inoculation to rodents.

7.1.5. Decontamination of the environment

After purgation of dogs with arecoline, after drug treatment (Chapter 3) or maintenance of infected carnivores in confined areas, such as kennels, large numbers of infective *Echinococcus* eggs may contaminate the environment. Therefore, purgation or treatment should – whenever possible – be carried out in rooms or confined sites with a concrete floor, which can easily be cleaned and disinfected (Table 7.2.). Alternatively, sites of purgation/treatment may be covered with a plastic sheet, which can be incinerated. If soil has been contaminated with *Echinococcus* eggs, the surface layer (approximately 1 cm-2 cm) should be removed, and the ground thoroughly burned with a fire-lamp or a small flame-thrower. It should be considered that, although high temperatures are generated by these devices, decontamination may not be complete because of rapid decrease of temperature after contact of the flame with soil, especially moist soil (Chapter 7.1.11.).

7.1.6. Decontamination of living-rooms and cars

If dogs or cats with intestinal *Echinococcus* infection had access to living-rooms or cars, the question for an adequate method of disinfection may arise. There is no satisfactory solution, but thorough cleaning using a vacuum-cleaner, the focal application of dry heat (hair-drier, electrical heater, etc.) at sites preferably used by the animals, and heat-treatment of laundry may help to reduce the infection risk. During summer, cars can warm up to temperatures detrimental to *Echinococcus* eggs by exposing them for several hours to direct sunshine.

7.1.7. Inactivation of metacestode material

Protoscoleces of *E. granulosus* and *E. multilocularis* and germinal cells of metacestode cysts can be inactivated by heat, deep-freezing and some chemicals, such as ethanol (40% or higher concentration) or formalin (4%) (Table 7.2.). Deep-freezing (at –20°C or lower) normally kills protoscoleces of *E. granulosus* and *E. multilocularis* and also germinal cells. It should be noted, however, that cryopreservation of *E. multilocularis* tissue is possible if cryoprotectants and certain protocols for deep-freezing are used (11).

7.1.8. Precautions in laboratories

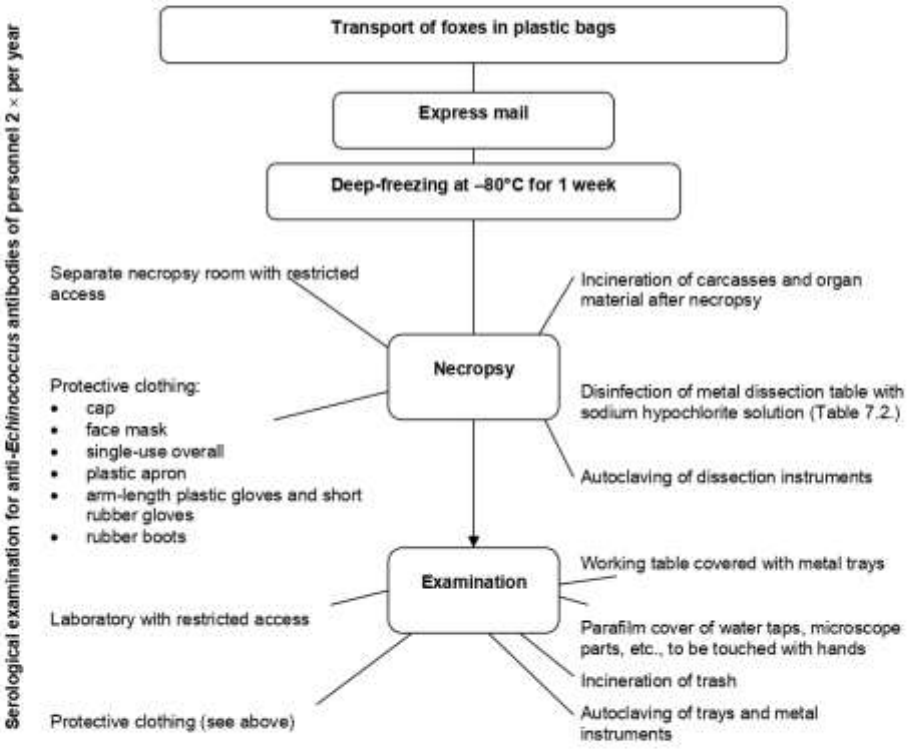
For work with *Echinococcus* infected definitive hosts, their intestines, faecal or other materials possibly containing infective *Echinococcus* eggs special laboratories or necropsy rooms should be used. In some countries, a biohazard safety level BL-3 is required. Such rooms should be marked as biohazard areas, they should be fully equipped with appropriate tables, wash-basin, containers, instruments, etc., and ideally with a sterile bench system; they should be adjacent to a changing room. Protective clothing, including overalls,

masks, caps, gloves and boots should be put on before entering the laboratory/necropsy room. Facilities should be available for decontamination of protective clothing; it should never be sent to a laundry without first being sterilised.

Infective material may be examined over sinks, in which an immersion heater can be placed to enable material to be boiled in water before it is passed into a sewerage system (15). In situations where the intestines of definitive hosts have to be examined, this should be done on metal trays or on disposable plastic foils. Following examination, the tray and all instruments should be sterilised, ideally by steam sterilisation in an autoclave. Plastic sheets, carcasses or organ material can be autoclaved or incinerated (Table 7.2.).

Faecal samples which are used for detection of coproantigen or DNA can be decontaminated prior to examination by deep-freezing at –80°C for at least 2 days.

Personnel involved in the examination of larval material from intermediate hosts should wear safety glasses. This will eliminate the possibility of protoscoleces being squirted into the eyes of the operator, with the risk of conjunctival echinococcosis. Remnants of metacestode material and infected intermediate hosts should preferably be heat sterilised or incinerated (Table 7.2.). For necropsy of foxes (and other final hosts) possibly infected with *E. multilocularis*, detailed safety precautions have been worked out (10, 12, 13) (Fig. 7.1.).



Source: Institute of Parasitology, University of Zurich/Switzerland and WHO Collaborating Centre for Parasitic Zoonoses

Fig. 7.1.
Safety precautions for parasitological examination of foxes or other definitive hosts infected with *Echinococcus multilocularis* (10)

7.1.9. Precautions in animal maintenance

Definitive hosts

If definitive hosts, experimentally infected with *Echinococcus* species, have to be maintained for research purposes special precautions are necessary. For some studies, for example drug testing, it may be sufficient to work only with prepatent infections and to finalise the experiment before excretion of infective eggs begins. For ethical reasons, maintenance of definitive hosts with patent infections should only be carried out in special isolation units under conditions in which transmission of *Echinococcus* eggs to humans is excluded. In addition, all persons working in such a unit should regularly receive screening for anti-*Echinococcus* antibodies (Chapter 7.2.).

Intermediate hosts

Maintenance of rodents infected with *Echinococcus* metacestodes by injection or surgical transplantation of metacestode material, such as protoscoleces, tissue homogenate or tissue fragments, does not require special safety precautions, but persons handling metacestode material should wear safety glasses, protective clothing and gloves. Care must be taken to inactivate metacestode material after the experiment (Chapter 7.1.7.).

After oral infection of rodents with *Echinococcus* eggs, there is a possibility of egg passage through the gastrointestinal tract and egg excretion for some days. Therefore, such animals should be maintained in the same cage for 3 to 4 days in a clean bench system preventing spreading of eggs to the environment. Thereafter, the animals should be transferred to a new cage and can be maintained under normal conditions. Isolation and handling of infective *Echinococcus* eggs requires strict biohazard safety precautions (Chapter 7.1.8.).

7.1.10. Precautions during handling of human patients with echinococcosis

Biological samples containing living protoscoleces and/or metacestode tissue of *Echinococcus* species could be infective to humans if accidentally injected to a person. Therefore, precautions are necessary, especially with regard to correct handling and disposal of needles, scalpel blades and glass ware. Spillage of such material to the face, for example during opening of a cyst, has to be avoided because of the hypothetical risk of a conjunctival infection with protoscoleces. Echinococcosis cannot be transmitted by serum samples of human patients or natural intermediate hosts.

7.1.11. Precautions for field workers

Ideally, personnel engaged in echinococcosis surveys should, at all times, wear appropriate protective clothing, i.e. impervious boots, gloves, coat or apron, and a face mask if necessary (for example during handling of faecal samples of *Echinococcus* infected carnivores). Regular screening (at least once per year) of the personnel for *Echinococcus* antibodies and the implementation of strict hygienic measures (for example thorough washing of hands after work with soap and water) are strongly recommended.

In situations in which faecal samples are being collected from potentially infected dogs following arecoline treatment, animals should be confined to a specific area. Subsequently, the ground from which faeces are collected should be thoroughly decontaminated by burning (Chapter 7.1.5.). Faeces should either be rendered safe in the field by being boiled, or by being packed in secure leak-proof containers for transport and later decontamination.

Animals necropsied in the field should be disposed according to the rules of the respective country (steam sterilisation, incineration, etc.). Intestines of potential definitive hosts should be ligated before removal from the carcass in order to prevent the dissemination of infective material. For the preservation of such material, fixative can first be injected into the litigated gut and the gut then immersed in fixative. It has to be stressed, however, that the normal fixatives (e.g. 4%-10% formalin or others) are not ovicidal (18, 25). Intestines fixed in formalin are not suitable for satisfactory recovery of *Echinococcus* species. A better method than formalin injection is deep-freezing of the intestines at –80°C for at least four days which kills *Echinococcus* eggs (see above).

7.1.12. Precautions during treatment of dogs (cats) infected with *Echinococcus multilocularis*

In view of the high pathogenicity of *E. multilocularis* to humans, special safety precautions should be observed if dogs (or cats), infected with *E. multilocularis*, have to be treated by application of an anthelmintic (8, 9) (Chapter 3).

a) Animals should only be treated under supervision of a veterinarian by informed and trained personnel.

b) Treatment should be performed under biohazard precautions in a veterinary clinic or under conditions where faecal material excreted after treatment can be collected and disinfected by heat or can be incinerated. Disinfection of kennels (for example by heat >80°C), the ground, equipment, etc. possibly contaminated with *E. multilocularis* should be feasible (Table 7.2.).

c) After treatment the animals should be shampooed and bathed in warm water in order to remove *Echinococcus* eggs adhering to the coat.

d) The result of treatment should be checked by repeated examination of faecal samples for taeniid eggs and for *Echinococcus*-specific coproantigen and/or DNA (Chapter 3).

e) Persons who had contact to a definitive host infected with *E. multilocularis*, should receive serological screening for serum antibodies using a highly specific and sensitive test (for example Em2 plus-ELISA, [17]) beginning about 4 weeks after suspected exposure and 6, 12 and 24 months later (Chapter 2).

f) These measures have to be adequately adapted to the situation of the individual case by the supervising veterinarian.

7.1.13. Precautions during purgation or treatment of dogs infected with *Echinococcus granulosus*

Several precautions have been described under Chapters 7.1.4. and 7.1.8. and in WHO Guidelines (31).

7.2. Prevention of cystic and alveolar echinococcosis in humans

- Prevention of CE

Control measures against the *E. granulosus* infection in dog populations are the basis for prevention of CE in humans. Details are described in Chapters 6.1. Some of the measures recommended in the prevention of AE (see below) are also applicable in prophylaxis against CE.

- Prevention of AE

Effective control of *E. multilocularis* in the sylvatic and the synathropic cycles is especially difficult (Chapter 6.2.). Therefore, some measures are recommended aiming at the reduction of the infection risk and of AE morbidity/mortality in humans. These measures refer to individuals or populations. For both groups education is an essential part of prevention (Chapter 6.1.3.).

Measures for individuals

The Swiss National Centre for Echinococcosis in Zurich has recommended the following measures for individuals to reduce the risk of AE (9):

a) In endemic areas where *E. multilocularis* is known to occur in foxes, wild berries, mushrooms, other plants or fruits from locations accessible to contamination with foxes' droppings should be thoroughly washed or better boiled before consumption. Deep-freezing at –18°C to –20°C does not kill eggs of *E. multilocularis* (they can only be killed at –70°C to –80°C) (Table 7.2.).

b) Foxes or other final hosts potentially infected with *E. multilocularis* should be handled with great care, always using disposable plastic gloves.

c) Special recommendations have been worked out for laboratory workers concerned with examinations of foxes for *E. multilocularis* (10, 12, 13) (Chapter 7.1.8., and Fig. 7.1.). In endemic areas similar measures may be applied to all laboratories in which necropsies of foxes are carried out, for example for rabies.

d) After agricultural or gardening work leading to contact with potentially egg-contaminated soil, hands should be thoroughly washed with soap and warm water (Chapter 7.1.11.).

e) Persons who have had single contact with infected final hosts or egg-contaminated materials (for example fox faeces), should receive serological screening for specific antibodies against *E. multilocularis* antigens at the following intervals after the suspected contact: 4 weeks, 6, 12 and 24 months. Highly sensitive and specific tests have to be employed for this purpose (Chapter 2). In unclear or doubtful cases US examination of the liver should be performed.

f) Individuals with repeated infection risk (for example fox hunters, laboratory personnel, etc.) should be serologically examined once or twice per year.

Measures for populations

In Japan and some other endemic areas, population-screening by serology and US examination of human populations has been successfully used for early detection of cases. This can reduce morbidity and mortality considerably (Chapter 6.2.5.).

7.3. Education

Education is an essential part of prevention and control of echinococcosis (Chapter 6.1.3.).

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ANEXO IV

96246

ORDENANZA Nro. 246/96.-

VISTO:
El Expte. Municipal Nro. 5996/96, caratulado: "elevaci¢n Proyecto de Ordenanza sobre Residuos Patol¢gicos"; y

CONSIDERANDO:
Que los residuos patol¢gicos generados en una poblaci¢n de la magnitud alcanzada por la Ciudad de General Pico, deben ser manejados en forma tal que no se constituyan en un riesgo para todas las personas que directa o indirectamente se relacionen con ellos, pudiendo adem s contaminar el suelo, el agua y la atm¢sfera, comprometiendo la calidad de vida de las generaciones futuras;

Que como consecuencia de lo expresado es necesario dotar al Municipio de un instrumento apto para la recolecci¢n, transporte, tratamiento y destino final de los residuos aludidos en forma independiente de los residuos domiciliarios;

POR ELLO:
EL HONORABLE CONCEJO DELIBERANTE DE GENERAL PICO
SANCIONA CON FUERA DE ORDENANZA:

Art¡culo 1ro.: Quedan comprometidos en las disposiciones de esta Ordenanza, todos los establecimientos sanitarios p£blicos y privados tales como: hospitales, cl¡nicas, sanatorios, maternidades, unidades de di lisis, salas de primeros auxilios, geri tricos, laboratorios de an lisis cl¡nicos, laboratorios de investigaciones biol¢gicas, consultorios m,dicos, odont¢logicos y veterinarios, gabinetes de enfermer¡as, servicios de emergencias m,dicas, farmacias y en general todo centro de atenci¢n de la salud humana y animal que manipulen y/o generen basuras clasificadas como residuos patol¢gicos.-

Art¡culo 2do.: Enti,ndase por Residuo Patol¢gico a todo elemento s¢lido, semis¢lido, l¡quido o gaseoso, que representa caracter¡sticas de toxicidad o actividad qu¡mica, f¡sica o biol¢gica, que puede afectar perjudicialmente en forma directa o indirecta, mediata o inmediata la salud humana, animal o vegetal y/o causar contaminaci¢n del suelo, agua o la atm¢sfera.-

.. 1 ..

.. 2 ..

Art¡culo 3ro.: Ser n considerados Residuos Patol¢gicos a los efectos de la presente Ordenanza:

a) Residuos provenientes de cultivos de laboratorios biol¢gicos y bioqu¡micos;

b) Residuos de sangre y sus derivados;

c) Residuos org nicos provenientes de quir¢fanos, morgues, salas de necropsias y laboratorios de an lisis cl¡nicos, de investigaci¢n qu¡mica, f¡sica o biom,dica, veterinaria y/o biol¢gica y de productos medicinales;

Página 1

d) algodones, gasas, vendas usadas, ampollas, jeringas, objetos cortantes o punzantes, materiales descartables, elementos impregnados con sangre u otras sustancias putrescibles, que no se esterilizan;

e) restos de animales productos de la investigaci¢n m,dica;

f) agentes quimioter picos de medicina humana o veterinaria en desuso, vencidos y/o sus residuos;

g) cualquier otro desecho que por distintos motivos pueda encuadrarse en lo dispuesto en el Art¡culo 2do. de la presente Ordenanza;

Art¡culo 4to.: Queda prohibida la entrega de los residuos individualizados en el art¡culo anterior, a firmas o personas no autorizadas expresamente por la Municipalidad a la recolecci¢n especial de los mismos.-

Art¡culo 5to.: Los establecimientos que reciban las prestaciones del servicio de recolecci¢n, transporte y tratamiento de Residuos Patol¢gicos deber n abonar mensualmente la tasa correspondiente.-

Art¡culo 6to.: Los establecimientos comprendidos en el Art¡culo 1ro. deber n contar con recipientes retornables y/o descartables en cantidades y tipos adecuados a sus necesidades.-

Art¡culo 7mo.: Los recipientes deber n contener una leyenda que identifique en forma clara que son Residuos Patol¢gicos y al generador.-

.. 3 ..

Art¡culo 8vo.: Los recipientes contar n en su interior con un envase de polietileno de 100-120 micrones como m¡nimo de espesor o de cualquier material de similares caracter¡sticas, que a¡sle totalmente el material acumulado de las paredes del recipiente.-

Art¡culo 9no.: Los recipientes se colocar n en lugares que no est,n en contacto o cercan¡a con reas de preparaci¢n de material, comida, esterilizaci¢n, etc.-

DEL PERSONAL

Art¡culo 10mo: Todo personal que de cualquier manera se relacione con la recolecci¢n, transporte y disposici¢n final de Residuos Patol¢gicos eliminados por los establecimientos pfblicos y privados se¤alados en el Art¡culo 1ro., recibir conocimiento y adiestramiento en el servicio, para el manejo de los mismos.- Asimismo se establecer un programa de inmunizaci¢n preventiva para proteger la salud de dicho personal.-

DEL TRATAMIENTO Y DISPOSICION FINAL DE LOS RESIDUOS PATOLOGICOS

Art¡culo 11ro.: La Municipalidad solo podr autorizar sistemas o m,todos de tratamiento y disposici¢n final, cuya tecnolog¡a garantice la muerte de todo agente que contenga y la completa destrucci¢n de dichos residuos.-

Art¡culo 12do.: Todo generador podr tratar sus propios residuos patog,nicos en unidades de tratamiento que funcionen dentro del establecimiento u optar por la contrataci¢n de un centro de tratamiento.-

Art¡culo 13ro.: Todos aquellos generadores que traten sus propios residuos deber n contar con la autorizaci¢n correspondiente de la Direcci¢n de Prevenci¢n y Protecci¢n a la Comunidad dependiente de la Municipalidad, debiendo agregarse a la solicitud presentada la memoria descriptiva del sistema de tratamiento a adoptar y una vez habilitado el mismo, se efectuar peri¢dicamente el control para verificar la eficacia del procedimiento a trav,s de sus organismos competentes.-

.. 3 ..

.. 4 ..

Art¡culo 14to.: Todo incinerador para residuos patol¢gicos deber poseer como m¡nimo las siguientes caracter¡sticas:

a) Ser del tipo de c maras m£ltiples, debiendo poseer:

1- C mara de combusti¢n primaria, con piso macizo, cuya funci¢n ser el secado e incineraci¢n de los residuos;

2- C mara de combusti¢n secundaria, donde se complete la combusti¢n de los vol tiles y gases generados en la c mara primaria y dise¤ada para asegurar un tiempo de residencia de los gases de 2 segundos.-

3- La llama del quemador ubicado en la c mara de combusti¢n primaria, deber incidir directamente sobre los residuos a quemar.-

4- Deber poseer un quemador auxiliar en la c mara secundaria que permita lograr una combusti¢n completa de los vol tiles.

b) R,gimen del Horno: En la c mara primaria deber alcanzar como m¡nimo una temperatura de 830 §C y en la secundaria de 1200 § C.-

c) Todos los hornos deber n poseer:

1- Dispositivo para regular el tiraje.-

2- Instrumentos para verificar las temperaturas de las distintas c maras de combusti¢n.-

3- Orificios para "toma de muestras" en la chimenea.-

4- La evacuaci¢n de los humos debe practicarse por medio de chimenea con salida a los cuatro vientos y cuya altura deber superar la topograf¡a de las construcciones m s pr¢ximas.-

ESTADISTICAS

Art¡culo 15to: Los establecimientos sanitarios comprendidos en

el Art¡culo 1ro. llevar n un registro de los residuos patol¢gicos producidos por rea y por semana, su destino final, as¡ como la adecuaci¢n a las presentes normas.-

.. 4 ..

.. 5 ..

Art¡culo 16to: La Direcci¢n de Prevenci¢n y Protecci¢n a la Comunidad a trav,s del Dpto. de Saneamiento Ambiental de la Municipalidad ser la autoridad de aplicaci¢n de la presente Ordenanza.-

Art¡culo 17mo: En caso de incumplimiento o irregularidades a lo dispuesto en la presente Ordenanza por parte de los establecimientos generadores de Residuos Patol¢gicos estos se har n pasibles de:

a) Apercibimiento: Dentro de las 24 Hs. de verificada la anormalidad, deber n regularizar la situaci¢n. Dicha regularizaci¢n deber ser comunicada fehacientemente al organismo de contralor.

b) En caso de persistir dicha/s irregularidad/es se proceder a la clausura preventiva del mismo.-

c) Las acciones u omisiones a la presente Ordenanza dar n lugar a las siguientes multas:

1- a la primera infracci¢n: $ 250.

2- a la segunda infracci¢n se duplica el monto de la primera infracci¢n.

3- a las sucesivas infracciones el monto se triplicar por el valor de la primera infracci¢n y se proceder a la clausura temporaria del establecimiento de 5 a 15 d¡as.

d) Clausura definitiva con prescindencia de la responsabilidad civil o penal que le cupiera a los infractores.-

Art¡culo 18vo: Der¢ganse las Ordenanzas Nro. 36/92 y 37/92.-

Art¡culo 19no: Comun¡quese, reg¡strese en la Carpeta de Ordenanzas del H.C.D. y pase al D.E. Municipal para sus dem s efectos.-

DADA en el Recinto de Sesiones del H.C.D., a los 30 d¡as del mes de Diciembre de 1996.-

REGLAMENTACION DE RESIDUOS PATOLOGICOS

Art¡culo 1ro.: El objeto de la presente reglamentaci¢n es asegurar la generaci¢n, recolecci¢n, transporte, tratamiento y disposici¢n final ambientalmente de los residuos patol¢gicos a fin de evitar perjuicios a la salud de la poblaci¢n y promover la preservaci¢n del medio ambiente.-

Proh¡bese en la jurisdicci¢n que compete a la Municipalidad de la Ciudad de General Pico la disposici¢n de los residuos aludidos sin previo tratamiento que garantice la preservaci¢n

ambiental y en especial la salud de la poblaci¢n.-

Art¡culo 2do.: Ser n considerados residuos patol¢gicos a los efectos de la presente reglamentaci¢n los enumerados en el Art¡culo 3ro. de la Ordenanza Nro. /96.-

OBLIGACIONES DE LOS ESTABLECIMIENTOS GENERADORES

Art¡culo 3ro.: a) Todo generador deber inscribirse en el Registro Municipal de Establecimientos Generadores de Residuos Patol¢gicos en un plazo de 30 d¡as a partir de la publicaci¢n de la presente, presentar debidamente firmada una Declaraci¢n Jurada (ver Anexo 1). En la misma quedar debidamente explicitada la frecuencia de recolecci¢n, d¡as y horarios de entrega en virtud de las necesidades de acuerdo al tipo de establecimiento aludido.

b) Todos los generadores que traten sus propios residuos, deber n solicitar la aprobaci¢n del destino y/o sistema de tratamiento de los mismos, transporte y disposici¢n final cuando correspondiera, ante las autoridades competentes.

c) Todos los generadores de residuos deber n descargar en planillas de control los residuos generados y su destino final.

d) Todos los establecimientos deber n separar adecuadamente los residuos patol¢gicos de los no patol¢gicos.

e) Se proh¡be la mezcla de residuos patol¢gicos y no patol¢gicos o la inclusi¢n de residuos patol¢gicos en la basura domiciliaria.

f) A los efectos de dar cumplimiento a lo estipulado en los incisos a, b, c, d, e y f de este Art¡culo, deber n realizarse los tr mites pertinentes ante la Direcci¢n de Prevenci¢n y Protecci¢n a la Comunidad, Dpto. de Saneamiento Ambiental.-

DERECHOS DE LOS ESTABLECIMIENTOS GENERADORES DE RESIDUOS PATOLOGICOS

Art¡culo 4to.: a) A solicitar a la Municipalidad la provisi¢n del servicio de recolecci¢n y eliminaci¢n de los residuos patol¢gicos por ellos generados.

b) A la provisi¢n por parte del prestador del servicio de los recipientes higienizados en cantidad, tiempo y forma para almacenamiento de residuos patol¢gicos.

c) A denunciar ante la Direcci¢n de Prevenci¢n y Protecci¢n a la Comunidad, Dpto. de Saneamiento Ambiental, las anormalidades o irregularidades que se registren en la prestaci¢n del servicio, dentro y/o fuera del establecimiento.-

d) A capacitar su personal en los cursos que dicta la autoridad pertinente y requerir de la misma el certificado correspondiente.

e) A exigir la comunicaci¢n fehaciente de cualquier modificaci¢n que se introduzca la sistema de gesti¢n de Residuos Patol¢gicos.

CONDICIONES DE MANIPULACION DE LOS RESIDUOS EN EL GENERADOR

Art¡culo 5to.: La disposici¢n transitoria de los Residuos

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Patol¢gicos dentro del establecimiento generador se efectuar £nicamente en bolsas de polietileno que tendr n un espesor m¡nimo de 120 micrones, tama¤o que posibilite el ingreso en hornos incineradores u otros dispositivos de tratamiento de Residuos Patol¢gicos, impermeables, opacas y resistentes.-

El cierre de las mismas se efectuar mediante el uso de un precinto resistente y combustible, el cual una vez apretado no permitir su apertura. Coloc ndose en cada bolsa la tarjeta de control, seg£n modelo que se detalla en el Anexo 2 de la presente reglamentaci¢n.-

Art¡culo 6to.: Las bolsas que contengan Residuos Patol¢gicos se colocar n en recipientes tronco c¢nicos (tipo balde) livianos, de superficies lisas en su interior, lavables y resistentes a la abrasi¢n y a golpes, con tapa de cierre herm,tico y asas para posibilitar su traslado, de hasta 50 lts. de capacidad. Los mismos deber n contar con una leyenda clara que identifique su contenido y el establecimiento al que pertenecen.-

Art¡culo 7mo.: Los residuos constituidos por elementos desechables cortantes o punzantes (agujas, hojas de bistur¡es, etc.) deber n ser colocados en recipientes herm,ticos resistentes a golpes y perforaciones antes de su introducci¢n a las bolsas.-

Art¡culo 8vo.: Aquellos Residuos Patol¢gicos de alto contenido l¡quido, ser n colocados en sus bolsas respectivas a las que previamente se les haya agregado material absorbente que impida su derrame.-

ALMACENAMIENTO

Art¡culo 9no.: a) Ser responsabilidad del usuario el almacenamiento provisorio de los Residuos Patol¢gicos, del lugar que destine para tal fin, de que se hallen en condiciones, en horario y forma hasta el retiro por parte del personal de recolecci¢n, seg£n lo establezca el organismo de contralor.-

b) La circulaci¢n dentro del establecimiento de la basura patol¢gica generada debe estar a cargo de personal especializado (de mantenimiento o quien se designa para tal fin). Solo se permitir el movimiento interno de basura patol¢gica desde el lugar de generaci¢n al de almacenamiento transitorio.

c) El acarreo de las bolsas (cuando sea necesario) hasta el dep¢sito de residuos del establecimiento se har con carros reglamentarios o bien con los contenedores pl sticos adecuados.-

d) En los establecimientos donde exista m s de un sitio donde se generan residuos, una vez retiradas las bolsas de las reas generadoras ser n colocadas en los contenedores que se encuentran en el rea de dep¢sito, los que a su vez deber n tener colocada la bolsa reglamentaria.-

Una vez lleno el contenedor se cerrar la bolsa con los precintos y se proceder al cierre del contenedor. El mismo quedar en dep¢sito hasta que sea recogido por el personal autorizado por Municipalidad.-

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DE LA RECOLECCION

Art¡culo 10mo: El servicio de recolecci¢n ser prestado todos los d¡as, excepto los Domingos y feriados nacionales, provinciales y municipales.

En caso de que dichos d¡as sean sucesivos, se podr disponer de la ejecuci¢n del servicio.-

Art¡culo 11ro: La frecuencia de recolecci¢n prestada a los establecimientos inscriptos que no traten sus propios residuos patol¢gicos, resultar de lo manifestado en la Declaraci¢n Jurada, la cual, para generadores habituales no ser nunca menor a una frecuencia semanal.-

Art¡culo 12do: A la llegada del m¢vil de recolecci¢n de residuos, se proceder a controlar que las bolsas se encuentren precintadas, se completar la tarjeta identificatoria (ver Anexo 2) y ser n retiradas previa confecci¢n de un recibo con la cantidad de recipientes entregados (ver Anexo 3).-

Art¡culo 13ro: El establecimiento recibir del recolector los recipientes previamente higienizados en igual n£mero a los que fueran retirados en la recogida anterior.-

DE LOS VEHICULOS DE TRANSPORTE

Art¡culo 14to: - Ser de uso exclusivo para el transporte de residuos patol¢gicos.-

- Las unidades ser n de color blanco en su totalidad y se identificar en ambos laterales y parte posterior el tipo de servicio al que est afectado y deber n estar provistos de una baliza luminosa de color amarillo.-

- La cabina ser independiente del resto de la unidad y la c£pula para carga deber estar construida de materiales inoxidables y en forma tal que evite el escape de l¡quidos, en caso de eventuales derrames facilidad de limpieza y desinfecci¢n.-

- Poseer un sistema que permita el alojamiento de los contenedores evitando su desplazamiento.-

- La parte de carga de la unidad deber estar permanentemente cerrada y con llave durante el transporte de los residuos.-

- Contar con pala, escoba y bolsas de repuesto de 100 micrones y desinfectante para su uso en caso de derrames eventuales.-

DE LA HIGIENIZACION DE LOS VEHICULOS

Art¡culo 15to: - La caja del veh¡culo ser lavada y desinfectadas mediante la utilizaci¢n de antis¡pticos de reconocida eficacia, una vez finalizado el traslado o despu¢s de cualquier contacto con residuos patol¢gicos.-

- Para la higienizaci¢n de los veh¡culos y de los contenedores, se deber disponer de un local de uso exclusivo con:

a) Piso, z¢calo sanitario y paredes y techo lisos,

impermeables de f cil limpieza.

b) Piso con inclinaci¢n hacia un vertedero o desagote, conectado a la red cloacal, previa c mara de decantaci¢n.

c) Provisi¢n de agua, manguera, cepillo y dem s elementos de limpieza.

d) Elementos de protecci¢n personal para los operadores, los que ser n suministrados diariamente en condiciones higi,nicas y en perfecto estado de uso.

Art¡culo 16to: En caso de que los residuos sean esparcidos durante la operaci¢n de transporte y/o recolecci¢n, ser responsabilidad del prestador del servicio el retiro de los mismos como as¡ tambi,n la desinfecci¢n del rea de volcado.-

TODO PERSONAL AFECTADO A LA RECOLECCION, TRANSPORTE Y DISPOSICION FINAL DE LOS RESIDUOS PATOLOGICOS DEBERA:

Art¡culo 17mo: Recibir por cuenta de su empleador:

a) Capacitaci¢n sobre:

1) Riesgos y precauciones a tener en cuenta en el traslado y manipulaci¢n de Residuos Patol¢gicos.

2) Primeros auxilios en caso de accidentes.

3) Uso de elementos de protecci¢n personal.

4) Prevenci¢n y lucha contra incendios.

b) Atenci¢n m,dica mediante un servicio asistencia a cargo del empleador, en la forma de ex menes m,dicos preocupacionales y peri¢dicos.

c) Deber n contar con seguros contra accidentes de trabajo y respecto de responsabilidad civil por los da¤os que puedan ocasionarse a terceros.

DEL TRATAMIENTO Y LA DISPOSICION FINAL DE LOS RESIDUOS PATOLOGICOS

Art¡culo 18vo: El lugar de procesamiento (incineraci¢n) de residuos patol¢gicos deber cumplir los siguientes requisitos:

a) Ser de acceso restringido a cualquier persona ajena a la actividad.

b) Energ¡a el,ctrica para alumbrado, equipamiento y limpieza.

c) Abastecimiento de agua y gas.

d) Protecci¢n contra incendios.

e) Balanza apropiada para determinar el peso de los residuos recibidos para su incineraci¢n.

f) Instalaciones higi,nico-sanitarias adecuadas para el personal afectado al servicio.

g) Oficina de recepci¢n y/o administraci¢n.

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h) Disponer de un dep¢sito de residuos con equipo de fr¡o (entre 8§ y 0§ C) contiguo al sitio de incineraci¢n, para el almacenamiento de los mismos en caso de no poder ser incinerados el d¡a en que fueron recepcionados, con capacidad estimada para disponer los residuos sin tratar por un plazo de 4 o 5 d¡as, ante un eventual inconveniente en el servicio.

i) No se podr modificar de manera alguna en el almacenaje la estructura f¡sica de los residuos infecciosos generados.

Art¡culo 19no: Las caracter¡sticas del horno se regir n conforme lo establecido en el Art¡culo 13ro. de la Ordenanza Nro. /96.

Art¡culo 20mo: Los residuos una vez tratados deber n ser colocados en bolsas resistentes para su traslado y disposici¢n final y podr n recibir tratamiento similar al de los residuos domiciliarios.-

DE LAS SANCIONES

Art¡culo 21ro: En caso de incumplimiento o irregularidades a lo dispuesto en la presente Ordenanza por parte de los establecimientos generadores de Residuos Patol¢gicos estos se har n pasibles de:

a) Apercibimiento: Dentro de las 24 hs. de verificada la anormalidad, deber n regularizar la situaci¢n. Dicha regularizaci¢n deber ser comunicada fehacientemente al organismo de contralor.

b) En caso de persistir dicha/s irregularidad/es se proceder a la clausura preventiva del mismo.-

c) Las acciones u omisiones a la presente Ordenanza dar n lugar a las siguientes multas:

1- a la primera infracci¢n: $ 250.

2- a la segunda infracci¢n se duplica el monto de la primera infracci¢n.

3- a las sucesivas infracciones el monto se triplicar por el valor de la primera infracci¢n y se proceder a la clausura temporaria del establecimiento de 5 a 15 d¡as.

d) Clausura definitiva con prescindencia de la responsabilidad civil o penal que le cupiera a los infractores.-

Ref..GOBIERNO-RESIDUOS PATOL¢GICOS-DEROG. 36 Y 37/92
Obs..REGLAMENTANDO EL SERVICIO DE RECOLECCI¢N DE RESIDUOS PATOL¢GICOS

ANEXO V

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ORDENANZA Nro. 58/97.-

VISTO:
El Expte. Nro. 2191/97 del Departamento Ejecutivo Municipal, relacionado con lo establecido en el Art¡culo 5to. de la Reglamentaci¢n de Residuos Patol¢gicos, aprobada mediante Ordenanza Nro. 246/96; y

CONSIDERANDO:
Que se hace necesario la modificaci¢n del mencionado Art¡culo que trata sobre condiciones de manipulaci¢n de los residuos en el establecimiento generador;

POR ELLO:
El HONORABLE CONCEJO DELIBERANTE DE GENERAL PICO
SANCIONA CON FUERZA DE ORDENANZA:

Art¡culo 1ro.: Modif¡case el Art¡culo 5to. de la Reglamentaci¢n de Residuos Patol¢gicos, aprobada mediante Ordenanza Nro. 246/96 el que quedar redactado de la siguiente manera:

"Art¡culo 5to.: La disposici¢n transitoria de los Residuos Patol¢gicos dentro del establecimiento generador se efectuar £nicamente en bolsas de polietileno que tendr n un espesor m¡nimo de 100 micrones, impermeables y resistentes, de color rojo, llevar n en blanco impreso la leyenda "Residuos Patol¢gicos". Su tama¤o deber ser de 60 x 75 cm. (vac¡a) para permitir el ingreso al horno incinerador.-
El cierre de las mismas se efectuar mediante el uso de un precinto resistente y combustible, el cual una vez apretado no perimitir su apertura. Coloc ndose en cada bolsa la tarjeta de control, seg£n modelo que se detalla en el Anexo II de la presente reglamentaci¢n.-"

Art¡culo 2do.: Comun¡quese, reg¡strese en la Carpeta de Ordenanzas del Honorable Concejo Deliberante y pase al Departamento Ejecutivo Municipal para sus dem s efectos.-

DADA en el Recinto de Sesiones del Honorable Concejo Deliberante de General Pico, a los 05 d¡as del mes de Junio de 1997.-

Ref..GOBIERNO-RESIDUOS PATOL¢GICOS-MODIFICANDO 246/96
Obs..

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