University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Faculty Publications from the Harold W. Manter
Laboratory of Parasitology

Parasitology, Harold W. Manter Laboratory of

6-1999

Histogenesis in the Metacestode of *Echinococcus vogeli* and Mechanism of Pathogenesis in Polycystic Hydatid Disease

Robert L. Rausch

University of Washington, rausch@uw.edu

Antonio D'Alessandro

Tulane University

Follow this and additional works at: <https://digitalcommons.unl.edu/parasitologyfacpubs>

Part of the [Parasitology Commons](#)

Rausch, Robert L. and D'Alessandro, Antonio, "Histogenesis in the Metacestode of *Echinococcus vogeli* and Mechanism of Pathogenesis in Polycystic Hydatid Disease" (1999). *Faculty Publications from the Harold W. Manter Laboratory of Parasitology*. 400.
<https://digitalcommons.unl.edu/parasitologyfacpubs/400>

This Article is brought to you for free and open access by the Parasitology, Harold W. Manter Laboratory of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications from the Harold W. Manter Laboratory of Parasitology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

HISTOGENESIS IN THE METACESTODE OF *ECHINOCOCCUS VOGELI* AND MECHANISM OF PATHOGENESIS IN POLYCYSTIC HYDATID DISEASE

Robert L. Rausch and Antonio D'Alessandro*

Department of Comparative Medicine, School of Medicine, Box 357190, University of Washington, Seattle, Washington 98195-7190

ABSTRACT: Histogenesis of the metacestode of *Echinococcus vogeli* was traced mainly in rodents inoculated intraperitoneally with finely minced infective vesicles. The fragments aggregated in the peritoneal cavity and coalesced, forming structures (plaques) from which primary vesicles arose. From primordia in their germinal tissue, exogenous vesicles developed, enlarged, and migrated outward to the surface of the laminated membrane, where they remained attached and proliferated. Each unit of vesicles so formed retained discrete identity and, within 6–8 mo, acquired an adventitia; thereafter, exogenous multiplication ceased and endogenous proliferation supervened. Large numbers of daughter cysts arose in the germinal tissue lining chambers within the units; endogenous proliferation also finally ceased, and the daughter cysts produced brood capsules containing protoscoleces. Primordia of exogenous vesicles were not observed in the walls of daughter cysts. Production of protoscoleces involved 3 processes: they developed in typical brood capsules, singly in minute brood capsules, or directly from germinal tissue. Exogenous proliferation is not characteristic in the natural intermediate host of *E. vogeli*, the paca. Evidently in primates, the initial proliferation in the liver is followed by extension of the metacestode into the peritoneal cavity and eventual invasion of abdominal and thoracic organs. Exogenous proliferation by a process unique to *E. vogeli* accounts for the clinical course of polycystic hydatid disease.

Cestodes of the genus *Echinococcus* Rudolphi, 1801 in the larval stage (metacestode) have some capacity for invasive proliferation. That potential may not be manifest when development takes place in the natural intermediate host of the respective species, but such growth may be induced when mammals of other species, usually phylogenetically disparate from the natural host, become infected and also (less commonly) when oncospheres become established at anomalous anatomic loci.

The metacestodes of the 4 recognized species of *Echinococcus* consist of identical structural components (laminated membrane, lined by germinal tissue from which brood capsules and protoscoleces are derived) whose organization differs among the 4. In the natural intermediate hosts, the histogenesis in each species is such that it provides for production of extensive areas of germinal tissue, thereby ensuring maximal asexual reproduction. The extent to which development and reproductive processes are modified in atypical intermediate hosts is readily discernible by comparisons with conspecific metacestodes in the natural hosts, i.e., in those involved in cycles that are independent of anthropogenic influences. Under natural conditions, the predator–prey relationship between the intermediate and final host ensures the integrity of the coevolved assemblage. The intrusion of human populations in biocoenoses of which *Echinococcus* spp. and their natural hosts are components, and in the case of *Echinococcus granulosus*, the adaptation of 1 biotype to synanthropic hosts has led to significant levels of morbidity and mortality in people as a consequence of infection by the respective metacestodes in an atypical host.

The larval stage of the northern biotype of *E. granulosus* (Batsch, 1786) occurs typically in the lungs of deer (Cervidae). After formation of the primary lacuna, the developing vesicle soon acquires a translucent laminated membrane (by 3 mo, with diameter of 0.8–0.9 mm, in experimentally infected elk, *Alces alces* (L.); R. L. Rausch, unpubl. obs.), and thereafter enlarges concentrically, usually not attaining a diameter of more than 6–7 cm. When fully developed, the spherical to subspherical, flu-

id-filled cyst consists of an external laminated membrane lined by germinal tissue that produces a single layer of uniform, contiguous brood capsules in which protoscoleces arise. The larval stage of the biotype adapted to synanthropic hosts is pleomorphic, its form depending on degree of compatibility with the respective species of domestic ungulates. Simple (unilocular) and multichambered cysts are most common, but a multicystic form may develop in the liver of bovinds. Similar anomalous forms in people were formerly taken as evidence of occurrence of a morphological type transitional or intermediate between those of cystic and alveolar hydatid disease (Dew, 1931; Dévé, 1949). Indeed, the metacestode of *E. granulosus* may proliferate in bone, producing osseous hydatid disease (Dévé, 1948; Saidi, 1976), and great numbers of daughter cysts may be produced in the peritoneal cavity following rupture of an hepatic primary cyst.

The invasive process characteristic of the larval stage of *Echinococcus multilocularis* Leuckart, 1863 in the human liver was concisely described by Elenevsky (1907: 452) long before the specific independence of the cestode had been established: “Ein bedeutender Theil der Sprösslinge des Parasiten stirbt ab. Die Überlebenden aber wuchern an der Peripherie durch Infiltration immer weiter.” Both in natural intermediate hosts and people, the oncosphere localizes in the liver, where exogenous proliferation takes place by means of thin extensions of germinal tissue peripherally (Rausch, 1954; Vogel, 1978). As very small vesicles are formed, beginning by 5 days postinfection in rodents, e.g., in the northern vole, *Microtus oeconomus* (Pallas), they acquire a thin covering of laminated membrane. When fully developed in the natural intermediate host, the larval cestode consists of a mass of small, interconnected chambers, each surrounded by laminated membrane and lined by germinal tissue and containing a few protoscoleces arising in brood capsules. Compatible with the short life expectancy of the intermediate host (less than a year in rodents of the family Arvicolidae), development is very rapid, resulting in the presence of infective protoscoleces as early as 60 days postinfection. In primates, the larval cestode is able to persist in a proliferative phase in the long-lived, incompatible host, gradually replacing hepatic tissue; the lesion consists of a matrix of collagen in which great numbers of vesicles are embedded, with invasive

Received 16 June 1998; revised 10 December 1998; accepted 10 December 1998.

* Department of Tropical Medicine, 1501 Canal Street, Tulane University, School of Public Health and Tropical Medicine, New Orleans, Louisiana 70112.

growth continuing peripherally. The metacystode is able also to extend from the liver to adjacent organs and to metastasize to distant sites (usually lungs or brain), in cases of long standing.

Echinococcus oligarthrus (Diesing, 1863), a cestode of felids, is the least known of the 4 species. Rodents serve as intermediate host. In experimentally infected agoutis, *Dasyprocta* sp., Sousa and Thatcher (1969) found subspherical, fluid-filled cysts in muscle and subcutaneous tissues, as well as in heart and lungs. The larval stage is distinguished in part by the formation of layers of brood capsules on the surface of germinal tissue. Exogenous proliferation has not been recorded. Only 3 human cases have been reported (D'Alessandro, 1997; Basset et al., 1998).

Rodents, typically the paca, *Cuniculus paca* L., serve as intermediate host also of *Echinococcus vogeli* Rausch et Bernstein, 1972. The characteristics of the metacystode in naturally infected pacas have been described (Rausch et al., 1981); herein, we trace the histogenesis of the larval stage in other rodents infected experimentally. A process of exogenous proliferation unique for the genus *Echinococcus* accounts for the mechanism of pathogenesis in human primates.

MATERIALS AND METHODS

After defining its cycle in eastern Colombia (Rausch et al., 1978), we maintained *E. vogeli* in rodents at the International Center for Medical Research, Cali, Colombia (1975–1984), at the University of Saskatchewan (1977–1978), and at the University of Washington (1978 to the present), and we also obtained experimentally the strobilar stage in dogs.

Three isolates of *E. vogeli* from naturally infected pacas were maintained in rodents by means of intraperitoneal (i.p.) inoculation of tissues of the metacystode. Mice, *Mus musculus* L. (CFW strain), and Mongolian gerbils, *Meriones unguiculatus* Milne-Edwards, were used initially, and gerbils only since 1984. Hystriognath rodents, neotropical species considered potential intermediate hosts, were also used: nutrias, *Myocastor coypus* (Molina), trapped in southern Washington State or laboratory reared; degus, *Octodon degus* (Molina), laboratory reared from stock originating in Chile; and a chinchilla, *Chinchilla lanigera* (Molina), of captive stock. Two laboratory-reared voles, *M. oeconomus*, and 2 muskrats, *Ondatra zibethicus* (L.), (Arvicolidae) were inoculated. After defining the prepatent period, infection of dogs was discontinued, and eggs were administered to 2 nutrias only. Material obtained at surgery or autopsy provided information on the course of polycystic hydatid disease in people.

The larval stage was studied from 281 gerbils and 88 mice, examined at intervals. In gerbils, on which this work was mainly based, durations of infection were as follows: less than 1 mo, 5 animals; 1–5 mo, 51; 6–10 mo, 51; 11–15 mo, 65; 16–20 mo, 30; 21–25 mo, 40; 26–30 mo, 28; and 31–37.4 mo, 11. If intended for inoculation, tissues of *E. vogeli* were placed in Hank's basic salt solution buffered with 10 mM TES (N-tris [hydroxymethyl] methyl-2-aminoethanesulfonic acid), containing 100 U of penicillin/ml and 100 mg of streptomycin/ml, and refrigerated from 24 to 48 hr, then minced in saline with small scissors, after which ~0.1 ml of fragmented tissues in 1–2 ml of Hank's solution was inoculated i.p. in the lower right abdominal quadrant by means of an 18-ga needle. For observations on histogenesis, immediately after removal from the host, larval cestodes were examined with dissecting and compound microscopes; some were stained intravitaly in neutral red (0.5% solution). Photographs were made routinely, from living specimens. Tissues from each rodent were preserved in 10% formalin solution.

Formalin-fixed vesicles or their components were processed by the paraffin-embedding method, sectioned at 5–15 μ m, and stained in hematoxylin-eosin, Gomori's trichrome, and by the periodic acid-Schiff (PAS) method. Additional stains were applied for specific purposes, e.g., to study mineralized vesicles. To avoid distortion of laminated membrane, representative tissues were fixed in 2% glutaraldehyde, embed-

ded in glycol methacrylate, sectioned at 2–5 μ m with a 9-mm glass knife on a Porter-Blum JB4 microtome, and stained in PAS-hematoxylin or azure III-methylene blue. Rostellar hooks stated below to be developed fully had maximal lengths falling within the range observed in material from naturally infected pacas: large hooks, 38.2–45.6 μ m; small hooks, 30.4–36.9 μ m. Hooks were measured under oil immersion (1,000 $\times$).

RESULTS

Development of the larval cestode in gerbils

Organization of inoculated tissue fragments: With reference to phases in the i.p. development of the metacystode, 2 terms are applied herein: "plaque" designates the initial aggregation of tissue fragments that attached to serosal surfaces; masses of vesicles arising from single plaques and persisting individually in the peritoneal cavity are designated units. The inoculum usually contained all components of the metacystode. Fragments of germinal tissue and laminated membrane ranged in size from very small to the largest that would pass through an 18-ga needle. When injected, fragments were dispersed posteriorly in the peritoneal cavity but, apparently mediated by movement of abdominal organs, were transported anteriorly, where they aggregated, forming flattened, dense masses, variable in size, that usually adhered to surfaces of the liver, stomach, and body wall.

Plaques had formed by day 5 postinoculation (PI), when a maximal size of 12 $\times$ 3 mm was recorded. At 35 days, 3 from 1 animal measured 4 $\times$ 2 mm, 11 $\times$ 3 mm, and 12 $\times$ 3 mm. After 16–20 days PI, small, flattened vesicles began to arise from the plaques (Fig. 1). Soon after initial vesicles appeared, a process of exogenous multiplication began (Fig. 2). The plaques usually persisted well after proliferation was advanced, remaining attached for at least 110 days PI to 1 surface of the mass of vesicles thus produced.

Exogenous proliferation: Vesicles arising from plaques attained diameters of up to 2–3 mm by 20–35 days PI, when the process of exogenous proliferation began. Primordia of secondary vesicles first were apparent (in sections) as accumulations of minute, PAS-positive granules in germinal tissue from nutrias (Rausch et al., 1981: Fig. 3). In gerbils, such primordia could be recognized when they had attained a diameter of 15–18 μ m in the walls of intact primary vesicles, appearing as circular, clear areas in germinal tissue. At about 50 μ m in diameter, a distinct membrane, circular in optical section, was present, closely surrounded by granules ~12–14 μ m in greater diameter that were involved in the formation of laminated membrane. At a diameter of 60–80 μ m, the early-stage vesicles had become enclosed by laminated membrane, 4.8–12 μ m in thickness (Fig. 3).

As they increased in size, the exogenous vesicles gradually passed from the germinal tissue into the laminated membrane, moving outward to its external surface, first appearing as small, surfacial convexities. Such movement was apparently passive, effected in part by intravesicular pressure. They emerged completely, but their laminated membrane remained connected with that of the vesicle from which they arose. Each emergent vesicle began anew to produce exogenous vesicles, initially forming an irregular mass (Fig. 4).

In series graded by time length, or age, the vesicles observed to arise from a single plaque persisted as a discrete unit for the life of the host. That the number of units in a gerbil did not

FIGURES 1–7. Formation and differentiation of tissues in *Echinococcus vogeli* in gerbils. **1.** Plaque (bottom left) with flattened primary vesicles (arrows), prior to onset of exogenous proliferation. **2.** Mass of exogenous vesicles, with portion of plaque (arrow) attached, 30 days postinoculation. **3.** Early-stage exogenous vesicles arising from germinal tissue of an older vesicle. Translucent laminated membrane (arrows) surrounds the internal, newly formed germinal tissue. **4.** A unit, free in the body cavity, undergoing exogenous proliferation, each vesicle enveloped by laminated membrane (arrows), 40 days PI. **5.** Unit of vesicles within adventitia, 22 mo PI. A portion of the peduncle by which the unit was attached to the serosal surface is present. **6.** Internal surface of germinal tissue in which endogenous daughter cysts are embedded or attached, 25.4 mo PI. Some of the innermost (older) vesicles exhibit beginning formation of brood capsules (arrows). **7.** Germinal tissue (background) with brood capsules (arrows), each enclosing a single, fully developed protoscolex. The very small, circular bodies are calcareous corpuscles. Scale bar in Figures 1, 2, and 4, 1.5 mm; Figure 3, 250 μ m; Figure 5, 20 mm; Figure 6, 2 mm; Figure 7, 250 μ m.

increase over time by means of proliferation by detached vesicles was evident from findings in animals autopsied at intervals PI. In an unselected series of 35 gerbils, infected for periods of 2–35 mo, the number of units ranged from 1 to 7, for a total of 93 ($\bar{x}$ = 2.6; mode = 1). In 18 of those gerbils, infected for 2–12 mo, numbers of units ranged from 1 to 6 ($\bar{x}$ = 2.6; mode = 1); in the remaining 17, infected for 20–35 mo, the range

was similarly 1–7 ($\bar{x}$ = 2.4; mode = 1). The units were usually free in the peritoneal cavity, but some remained attached to a serosal surface by a thin peduncle, apparently at the locus of the original plaque.

Initially, the vesicles arising from a single plaque formed an irregular mass, which within 6–8 mo PI began to acquire an adventitia of connective tissue. After about 8 mo PI, the en-

FIGURES 8–10. Formation and differentiation of tissues in *Echinococcus vogeli* in gerbils. **8.** Fully developed protoscoleces that have arisen directly from germinal tissue, to which each body (arrows) remains attached. **9.** Vesicular transformation of a protoscolex; the vesicle at the posterior end is enclosed by a laminated membrane (short arrow), and the anterior portion of the body and scolex are persisting (long arrow). **10.** Daughter cyst with protoscoleces developing from its external surface; others (arrows) have arisen within the lumen. Scale bar in Figure 8, 400 μ m; Figure 9, 250 μ m; Figure 10, 1 mm.

closing capsule caused the units to assume a spherical to ellipsoidal form, with smooth external surface (Fig. 5), their shape evidently a result of continuing proliferation by the enclosed vesicles. Except for increasing size, the units did not change thereafter in appearance externally. Internally, reorganization of the tissues occurred, apparently attributable to fusion of vesicle walls. In each unit, an intact layer of laminated membrane became contiguous with the adventitia, and large cavities formed within the mass of formerly discrete vesicles. After about 10 mo, perhaps as a response to increased internal pressure from the confining adventitia, exogenous proliferation ceased and a process of endogenous proliferation supervened.

Endogenous proliferation: With beginning endogenous formation of daughter cysts, primordia of exogenous vesicles were found only in tissue adjacent to the adventitia, and they did not undergo development. The chambers within the units became filled with daughter cysts arising directly from germinal tissue, their origin evident by the large numbers of very small, spherical vesicles embedded therein and many of greater size emerging from that tissue but still attached (Fig. 6). When actively producing daughter cysts, the germinal tissue was as much as 4 mm in thickness, and embedded vesicles were so numerous as to impart to it a reticular appearance. Transformation of protoscoleces to vesicles occurred to some extent, but that process did not result in a significant production of new vesicles.

After about 20 mo PI, when the cavities within units were filled with daughter cysts, endogenous proliferation ceased. At that phase, the germinal tissue was thin. Brood capsules with fully developed protoscoleces were present in all daughter cysts, indicating that none of the latter was of recent origin. The fluid-filled daughter cysts maintained their spherical form and contributed significantly to weight of the units. At 15.8 mo PI, a unit 60 $\times$ 55 mm weighed 52.8 g; a second, 54 $\times$ 45 mm, weighed 40.4 g.

Production of endogenous daughter cysts, often numbering

more than 1,000, caused individual units to increase in size, to an observed maximal diameter of 80 mm. A unit measuring 35 $\times$ 33 mm at 24.7 mo PI contained 394 daughter cysts >3 mm in diameter and 1,291 ranging from 0.5 to 3 mm. Each was enclosed by laminated membrane 50–70 μ m in thickness, and almost all contained brood capsules, with numbers increasing in proportion to size of vesicle. The smallest had only 1–3 brood capsules. That the rostellar hooks of protoscoleces had attained full development indicated a considerable age for even the smallest daughter cysts.

Brood capsules and protoscoleces: Processes involved in development of structures within individual units varied, reflecting the high degree of histogenetic potential of germinal tissue. Protoscoleces were produced in 3 ways: they arose (1) in considerable numbers in brood capsules on the surface of germinal tissue within large chambers or in brood capsules in daughter cysts; (2) in very small brood capsules wherein they persisted singly (Fig. 7); or (3) directly from germinal tissue (Fig. 8). The large brood capsules contained many protoscoleces and were identical with those observed in vesicles in the natural intermediate host. The tegument of protoscoleces was 1–2 μ m in thickness and PAS positive; brood capsules were often lined by a similar membrane, continuous with that of the enclosed protoscoleces. Often, the lining membrane could not be discerned, and the wall of the brood capsule apparently consisted only of germinal tissue. When only 1 protoscolex was present, a distinct space separated it from the wall of its brood capsule (Fig. 7); in such cases, capsule walls were readily discernible with use of the dissecting microscope at 25 or 50 $\times$. Such brood capsules (containing a single protoscolex) frequently formed loose aggregations on the surface of germinal tissue. Protoscoleces that arose directly from germinal tissue were attached by thin filaments. Like those developing in brood capsules, they first appeared as solid, conical buds, but their scoleces were often partly or entirely evaginated, imparting to them a more

elongate form. They also attained full development, as determined from the rostellar hooks. In a gerbil examined 93 days PI, only such single protoscoleces were present; in another, at 3.9 mo, in a mass of vesicles 25 mm in greater diameter, brood capsules contained numerous protoscoleces. All protoscoleces were transparent, and contained many calcareous corpuscles.

A transformation of protoscoleces to vesicles occurred in some units. That process first was observed at 5.8 mo PI, in a vesicle holding many protoscoleces within brood capsules as well as some that had arisen directly from germinal tissue. In protoscoleces undergoing such change, the scoleces usually had evaginated, and the entire structure was much elongated. Spherical vesicles formed within the body (Fig. 9). When the new vesicles attained a diameter of about 225–340 μm , they had become enclosed by laminated membrane. The appended scoleces degenerated, with loss of their rostellar hooks. Such transformation was observed in units enclosed by an adventitia. Frequently, at longer intervals PI, small daughter cysts had protoscoleces projecting from their external surface, with few or none within the lumen (Fig. 10).

Pathogenicity: Inoculated gerbils remained clinically normal for many months, except for some degree of distension of the abdomen. In a few, the liver contained 1–2 small vesicles that probably had arisen at loci of plaques on the hepatic capsule, but exogenous proliferation of vesicles did not occur in the liver of gerbils. One animal died 14 mo PI following spontaneous rupture of an hepatic vessel. Ascites was a rare finding. A small amount of fluid was present intraperitoneally in a gerbil examined after 25.3 mo, but it also had nodules of a spontaneous tumor on the abdominal walls and posterior surface of the diaphragm. In 3 animals, vesicles that became attached anteriorly proliferated exogenously and penetrated the diaphragm, producing additional vesicles in the pleural cavity; in 1, a lung had been invaded. In another, a unit had ruptured, releasing daughter cysts into the peritoneal cavity.

In a few gerbils, portions of some units exhibited degenerative changes, usually within a single chamber. Such cavities became filled with a white, amorphous substance in which daughter cysts were embedded. In a typical case, at 20.5 mo PI, the daughter cysts, 0.5–8 mm in diameter, were mostly intact but lacked calcareous corpuscles; the fully developed protoscoleces were transparent and without internal structures other than the rostellar hooks, still in normal position. Sections of affected vesicles were stained in Alcian blue–Kernechtrot, PAS–Mayer’s hematoxylin, and by the von Kóssa method for calcium. That method gave positive results, and we concluded that the condition represented a process of dystrophic calcification involving a calcium phosphate complex. From roentgenograms, the deposited material was found to be radio-opaque.

When fragmented tissues of metacestodes of *E. vogeli* and *E. multilocularis* were inoculated simultaneously into a single host, the vesicles resulting formed a single mass, but those of the 2 species remained discrete. The metacestodes of both developed normally.

Development of the larval cestode in laboratory mice

As compared with gerbils, in mice of the CFW strain the larval cestode was more pathogenic. Clinical signs of infection appeared relatively early, at which time the animals were au-

topsied. The maximal duration of infection in the mice was 26.1 mo; only 5 other animals were kept as long as 20.5–25.7 mo.

Early-phase development of the larval cestode was dissimilar to that in gerbils. Formation of well organized plaques was not observed. At 16 days PI, small masses of vesicles were present; they did not remain as discrete units, but many became detached and multiplied independently. As early as 2.6 mo PI, single masses of exogenous vesicles ranged up to 20 mm in diameter, and brood capsules had formed. During the interval 7.8–9 mo, large, irregular masses of vesicles ranged from 23×18 mm to 40×25 mm. Part of 1 mass had become enclosed by an adventitia at 9 mo, but most vesicles proliferated without restriction. At 11.3 mo, daughter cysts covered by thick laminated membrane were present. Larger vesicles contained many singly arising protoscoleces, often in aggregations and associated with typical brood capsules.

In mice, vesicles frequently were attached to the liver or to other serosal surfaces (but never to the extent observed in individuals of the same strain inoculated with the metacestode of *E. multilocularis*). Perforation of the diaphragm was recorded once, at 9 mo, when 2 vesicles free in the pleural cavity were proliferating exogenously. Ascites was a frequent finding in mice, first observed at 3.5 mo PI. In 2, after 11.7 mo, abundant vesicles in the peritoneal cavity were associated with 20 ml and 35 ml of hemorrhagic fluid, respectively; the fluid formed a solid clot overnight.

Development of the larval cestode in hystricognath rodents

Two nutrias were examined at 48 days and 90 days PI, after receiving gravid segments of *E. vogeli* per os (p.o.), from a dog autopsied 129 days PI. Eight nutrias inoculated i.p. were examined from 6 to 23.3 mo PI.

In a subadult animal (4.1 kg) 48 days after receiving 10 gravid segments, many nodules, ~5 mm in diameter, were visible at the hepatic surface. As seen in sections, they contained spherical vesicles up to 1.2 mm in diameter. Laminated membrane and germinal tissue were well developed. Aggregations of minute PAS-positive granules were present in the germinal tissue, evidently corresponding to loci of early-stage primordia of exogenous vesicles, as observed also in gerbils. In a subadult (3 kg) at 90 days, vesicles in the liver were ~5 mm in diameter. The tissue response in the 2 animals was described elsewhere in detail (Rausch et al., 1981: cf. Fig. 3).

Findings were similar in 2 nutrias examined 7.1 mo PI. Masses of i.p. vesicles were 8.8–55 mm in greater diameter; in 1, daughter cysts ≈ 100 μm in diameter were present in the germinal tissue. Most vesicles contained numerous brood capsules, with fully developed protoscoleces (large hooks, 40–42 μm in length; small hooks, 32–34 μm). Additional vesicles were present in the liver. In an animal examined after 16.4 mo, the liver was massively invaded, its weight (1.35 kg) making up 20.9% of the total body weight (6.45 kg). Vesicles, mostly 10–20 mm in diameter, had replaced much of the hepatic parenchyma. Trabeculae were prominent in the larger vesicles, and large cavities contained many brood capsules with fully developed protoscoleces (large hooks, 40–44 μm , small hooks, 32–34 μm). The serosa exhibited many surfacial convexities, as a result of exogenous proliferation beneath the capsule. Masses of vesicles

FIGURE 11. Polycystic hydatid disease. From a patient in Colombia, exogenous vesicles of *Echinococcus vogeli* (peritoneal cavity).

ranging from ~30 to 40 mm in diameter were attached to the mesenteries; small vesicles covered the inner surface of the omentum, and some in the lungs were proliferating. The peritoneal cavity contained ~400 ml of hemorrhagic ascitic fluid. Another nutria (8.55 kg), after 23.3 mo, also had significant invasion of the liver (weight 450 g), with the greatest involvement around the hilus; the posterior portion of the spleen had been invaded. Numerous vesicles were attached to the peritoneum of the body wall, and some were proliferating within the inguinal canals. The lungs were not affected.

Two adult degus were examined 8 mo PI. A smooth-surfaced vesicle 25 × 23 mm, 4.5 g, was present in 1. Brood capsules with fully developed protoscoleces were numerous in vesicles of exogenous origin. Small vesicles sometimes contained protoscoleces not only internally, but others projected from the external surface (as observed also in gerbils). The second degu contained 1 vesicle 8 × 8 mm that was opaque as a result of dystrophic calcification. Daughter cysts, 1–2 mm in diameter, with well developed laminated membrane were numerous; they lacked calcareous corpuscles and in some the germinal tissue had become detached. The protoscoleces, like those in similar vesicles in gerbils, had no calcareous corpuscles.

In the single chinchilla, at 2.6 mo PI, a 12 × 10-mm vesicle was attached to the mesentery. Germinal tissue was well developed. Only 2 small vesicles, without brood capsules or protoscoleces, were present internally.

Development of the larval cestode in arvicoline rodents

At 4.7 mo PI, an i.p. mass 20 × 13 mm was obtained from a muskrat. Exogenous proliferation had occurred, and numerous brood capsules contained mature protoscoleces (large hooks, 41–44 µm; small hooks, 32–34 µm). A second muskrat was negative after the same interval. In a northern vole at 5.4 mo PI, a single

vesicle 10 × 7 mm was attached at the posterior end of the spleen. Exogenous proliferation had occurred, and internal trabeculae had formed; germinal tissue lacked calcareous corpuscles, and neither brood capsules nor protoscoleces were present. Findings were negative in a second vole at 30 mo PI.

Development of the larval cestode in primates

Evidently, following initial development in the liver, the metacestode of *E. vogeli* undergoes extensive proliferation within the peritoneal cavity of primates.

A patient with a history of intra-abdominal masses for at least 15 yr was found to have involvement of the liver, spleen, omentum, mesentery of the colon, ovaries, uterus, and urinary bladder. Two kilograms of vesicles each 5–6 cm in diameter were removed at palliative surgery in 1984; most were viable but some exhibited degenerative changes. At the time of the patient's death in 1990, the duration of infection was estimated to have been ~60 yr, as she had lived under urban conditions after leaving place of birth in rural Colombia at an age of about 7 yr (D'Alessandro, 1997). Vesicles in situ in the liver, obtained postmortem, ranged from 19 × 15 mm to 33 × 25 mm and were enclosed by thick, vascular adventitias. The germinal tissue was thin, but brood capsules as well as singly arising protoscoleces were numerous; rostellar hooks were fully developed. Vesicles in the peritoneal cavity were actively proliferating (Fig. 11). Findings were similar in numerous cases of polycystic hydatid disease from South America.

DISCUSSION

In pacas, the larval stage of *E. vogeli* has been found almost exclusively in the liver; in a series of 61, naturally infected, examined by us in Colombia, the liver only was site of locali-

FIGURE 12. Blanchard's (1889) concept of exogenous proliferation in the metacestode of *Echinococcus granulosus*; an illustration based on Leuckart's written description of such a process. Blanchard's labeling (letters o–t) indicates the developmental sequence of vesicles arising in the laminated membrane.

zation in all but 2, in which vesicles also were attached to the hepatic ligament and mesentery, respectively. In most, only 1 or a few vesicles were present in the liver, although clusters of up to 59 were observed (Rausch et al., 1981). When numerous, the vesicles were usually contiguous, possibly indicating local proliferation; however, they were of similar size, and neither extensive invasion of the liver nor spread to the peritoneal cavity was observed. Pacas are relatively long-lived rodents, attaining an age of at least 12 yr (Collett, 1981), but we saw no indication in the oldest individuals of our series that intrahepatic invasion had progressed over their lifetimes. The massive invasion of the liver in 1 experimentally infected nutria (see above) we conclude to be a manifestation of occurrence of the larval cestode in an atypical host. The bush dog, the only known natural final host of *E. vogeli*, and the nutria are not sympatric (Redford and Eisenberg, 1992: map 11.112; D'Alessandro, 1997: Fig. 8); the nutria therefore is not to be considered a component of the coevolved assemblage of the cestode and its hosts.

Our work described herein provides evidence that the course of development of the metacestode of *E. vogeli* in experimental animals is variable with respect to production of protoscoleces, but that a distinctive characteristic is exogenous proliferation

occurring through a process that has not been observed in other species of *Echinococcus*. Such proliferation accounts for the invasiveness and pathogenicity of the larval cestode in other than natural hosts, especially primates; polycystic hydatid disease, like alveolar hydatid disease, is the consequence of the high degree of incompatibility existing between the larval cestode and primates as intermediate hosts. A further distinction, in experimental animals, appears to be the formation of plaques resulting from aggregation of inoculated tissue fragments, transported antegrad in the peritoneal cavity. Similar movement of inocula consisting of finely minced tissues of a transmissible mesothelioma resulted in development of tumors on the dorsal surface of the stomach or at other anterior loci (Rausch et al., 1987).

Exogenous proliferation, with respect to *E. granulosus*, has been the subject of much discussion since at least the middle of the last century. Figures intending to illustrate the origin of exogenous vesicles have been based mainly on Leuckart's (1879–1886: 777) description of the process, and versions of Blanchard's (1889) modification (Fig. 12 herein) of Leuckart have been published repeatedly. Leuckart considered that exogenous vesicles arose from aggregations of granular material that became enclosed in the laminated membrane, initially with-

in its innermost layers. In reference to that concept, Dévé (1949) considered that folding or other disruption of laminated membrane might result in the incorporation of germinal tissue from which exogenous vesicles could develop. Such a process, if it occurs, must be rare, and no information exists to indicate that it might lead to invasion of tissues around the primary cyst.

Another concept of proliferation in *E. granulosus*, the formation of exogenous daughter cysts, was that of Fairley and Wright-Smith (1929), based on findings in domestic ungulates, mainly bovines, wherein vesicles arose when herniation of germinal tissue and laminated membrane permitted the escape of germinal tissue or daughter cysts into the space between the external surface of the laminated membrane and the surrounding adventitia. Such vesicles increased in size and number (process involved not explained), exerting greater pressure on the membrane, causing collapse, whereupon the lumen within the adventitia became filled by daughter cysts. Fairley and Wright-Smith (1929: 322) stated that in a late stage of the development, the adventitia was disrupted and that "... a more universal extension of this process may lead in certain cases to a condition of complete loculation and honeycombing of the tissues characteristic of multicystic bovine echinococcosis..." However, we have no evidence to support the concept that the multicystic formation sometimes found in the liver of bovines is associated with a primary cyst of *E. granulosus*, i.e., one from which it might have been derived.

Whereas the larval stage of the synanthropic biotype of *E. granulosus* may develop in a wide range of organs in people, an atypical growth occurs in bones. Osseous hydatid disease is considered a consequence of dispersal of oncospheres via the systemic circulation, reaching the most vascularized regions of bones, the epiphyses, followed by proliferative growth in the cancellous interior. Constrained in development by the character of the tissue, proliferation takes place within Haversian canals and among trabeculae. Pressure effects lead to resorption of the cancellous matrix, with formation of cavities in which typical cysts develop. With the destruction of cortical bone, the metacestode may pass through the periosteum and invade the surrounding soft tissue, where again cysts of typical structure develop. The growth process is not well understood, in part because of confusion of osseous hydatid disease caused by *E. granulosus* with alveolar hydatid disease, which occurs very rarely in bones (Posselt, 1932). A case was described recently by Fujioka (1996).

Traumatic rupture of hepatic cysts of *E. granulosus* usually releases generative tissues into the peritoneal cavity, with the consequence that great numbers of daughter cysts may be produced, resulting in extreme abdominal distension. Consension has prevailed that daughter cysts arise by means of transformation of protoscoleces or brood capsules. According to Dew (1928), masses of cysts may develop from contiguous vesicles or brood capsules. Intraperitoneal cysts are typical in structure, and no reports have indicated the involvement of exogenous proliferation as occurs in the metacestode of *E. vogeli* (cf. Dew, 1928; Dévé, 1948; Saidi, 1976).

In our experimental animals, the origin and development of protoscoleces in brood capsules of *E. vogeli* did not differ in process from that in *E. granulosus*, as described by Goldschmidt (1900), Dew (1922), and others. In the second edition of his work, Leuckart (1879–1886: 766) stated that protosco-

leces in *Echinococcus* always arise within brood capsules, and that contrary impressions were based on observations of cysts undergoing degeneration. Dévé (1949: 49) further stated that "Contrairement à ce qu'on admettait autrefois, les scolex ne naissent jamais directement ni isolément de la membrane germinative. Ils se développent toujours à l'intérieur d'une capsule prolifère, à laquelle ils sont attachés par leur mince pédicule." Our finding that the protoscoleces of *E. vogeli* commonly arose individually from the germinal tissue may be a process unique to *E. vogeli*; as the phenomenon was observed also in wild pacas, in vesicles in the liver (Rausch et al., 1981), clearly it is not limited to larval cestodes developing in other than the natural intermediate host. We observed commonly that protoscoleces developed externally on small, clearly intact daughter cysts that often contained brood capsules or protoscoleces internally as well.

The process in *E. vogeli* by which protoscoleces formed vesicles also appeared to differ from that described in *E. granulosus*. According to Leuckart (1879–1886), Dévé (1949), and others, the protoscolex of *E. granulosus* vesiculates while the scolex is still invaginated; by that means, the entire structure acquires a covering of laminated membrane, within which the scolex degenerates, and the rostellar hooks persist against the internal surface of the vesicle. In *E. vogeli*, the scolex typically evaginates, and vesiculation took place within the body of the protoscolex; the vesicle acquired laminated membrane, and the appended scolex soon disintegrated, the rostellar hooks being detached externally. With respect to protoscoleces of *E. multilocularis* maintained in vitro, Yamashita et al. (1962) observed that either the scolex or the body might vesiculate.

Like that of *E. multilocularis*, the larval stage of *E. vogeli* produces similar lesions in nonhuman primates and people. Useful information about the course of polycystic hydatid disease was provided by findings in apes at the Los Angeles Zoo, for which the approximate date of infection was known (Howard and Gendron, 1980; O'Grady et al., 1982). Infant primates were housed next to the pen of a bush dog (captured in South America), into which they could reach. Most of the apes died of polycystic hydatid disease within an interval of ~10 yr, or if they died of other causes, they were found at autopsy to have advanced infections. Total deaths included 7 orangutans, 3 chimpanzees, 2 gibbons, 1 siamang, and 3 gorillas; 2 additional gorillas were severely affected. Two gorillas that died had pendulous abdomens caused by the great volume of vesicles present; their livers were extensively infiltrated, and in the peritoneal cavity, vesicles were attached to the mesenteries or intestinal serosa, to the surface of the diaphragm, or were free. Protoscoleces were abundant. Older hepatic vesicles had thick adventitias (Howard and Gendron, 1980). Four gorillas examined by ultrasound had exhibited rapid enlargement of the abdomen, lethargy, and anorexia; in 2 also were clinical signs of hepatic failure (O'Grady et al., 1982).

In people, as in nonhuman primates, the oncospheres of *E. vogeli* typically localize in the liver. Extensive hepatic infiltration is followed by proliferation of successive generations of exogenous vesicles in the peritoneal cavity, often with involvement of various organs, including the pancreas, spleen, diaphragm, omentum, stomach, pericardium, and lungs (D'Alessandro et al., 1979; Meneghelli, 1985; Meneghelli et al., 1986, 1992; Nozais et al., 1994; Ferreira et al., 1995). Older

vesicles acquire adventitious, but exogenous proliferation continues, probably for the life of the host. In chronic infections, vesicles may become calcified, as was observed in a case of at least 22 yr duration (Meneghelli, 1985).

We conclude that the larval stage of *E. vogeli* exhibits a range of species-specific processes, and that it appears to equal the pathogenicity of *E. multilocularis* in degree of morbidity caused in the human host. Untreated, polycystic hydatid disease can be expected to terminate fatally.

ACKNOWLEDGMENTS

Housing and care of animals were generously provided by the Tulane-COLCIENCIAS International Center for Medical Research at Cali, Colombia, by the Department of Veterinary Microbiology, University of Saskatchewan, and by the Department of Pathobiology and Department of Comparative Medicine, University of Washington. The investigation was supported in part by grants AI-10050 and AI-15172 in Colombia, and RO1 AI-15172 from the National Institute of Allergy and Infectious Diseases. For assistance in this investigation, we express our gratitude to Olivia Hincapié and Jaime Torres at Cali, and to Andra B. Erickson and Denny Liggitt at the University of Washington.

LITERATURE CITED

- BASSET, D., C. GIROU, I. P. NOZAIS, F. D'HERMIES, C. HOANG, R. GORDON, AND A. D'ALESSANDRO. 1998. Neotropical echinococcosis in Suriname: *Echinococcus oligarthrus* in the orbit and *Echinococcus vogeli* in the abdomen. *American Journal of Tropical Medicine and Hygiene* **59**: 787–790.
- BLANCHARD, R. 1889. *Traité de zoologie médicale*, Vol. 1. Librairie J.-B. Baillière et Fils, Paris, France, 808 p.
- COLLETT, S. 1981. Population characteristics of *Agouti paca* (Rodentia) in Colombia. Publications of the Museum, Michigan State University, Biological Series **5**: 489–601.
- D'ALESSANDRO, A. 1997. Polycystic echinococcosis in tropical America: *Echinococcus vogeli* and *E. oligarthrus*. *Acta Tropica* **67**: 43–65.
- , R. L. RAUSCH, C. CUELLO, AND N. ARISTIZABAL. 1979. *Echinococcus vogeli* in man, with a review of polycystic hydatid disease in Colombia and neighboring countries. *American Journal of Tropical Medicine and Hygiene* **28**: 303–317.
- DÉVÉ, F. 1948. L'échinococcose osseuse. A. Monteverde & Cie, Montevideo, Uruguay, 236 p.
- . 1949. L'échinococcose primitive (maladie hydatique). Masson et Cie, Paris, France, 362 p.
- DEW, H. R. 1922. Observations on the mode of development of brood capsules and scolices in the encysted stage of *Taenia echinococcus*. *Medical Journal of Australia*, September 30, p. 381–384.
- . 1928. Hydatid disease. Its pathology, diagnosis and treatment. Australasian Medical Publishing Co., Ltd., Sydney, Australia, 429 p.
- . 1931. *Echinococcus alveolaris*, with report of an Australian case. *Australian and New Zealand Journal of Surgery* **1**: 115–141.
- ELENEVSKY, K. 1907. Zur pathologischen Anatomie des multiloculären *Echinococcus* bei Menschen. *Archiv für Klinische Chirurgie* **82**: 393–461 + 5 Tafeln.
- FAIRLEY, N. H., AND R. J. WRIGHT-SMITH. 1929. Hydatid infestation (*Echinococcus granulosus*) in sheep, oxen and pigs, with special reference to daughter cyst formation. *Journal of Pathology and Bacteriology* **32**: 309–335.
- FERRERA, M. S., S. DE A. NISHIOKA, A. ROCHA, AND A. D'ALESSANDRO. 1995. *Echinococcus vogeli* polycystic hydatid disease: Report of two Brazilian cases outside the Amazon region. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**: 286–287.
- FUJIOKA, F. 1996. Histologic investigation on distant metastases from alveolar echinococcosis in the liver. In *Alveolar echinococcosis. Strategy for eradication of alveolar echinococcosis of the liver*, J. Uchino and N. Sato (eds.). Fuji Shoin, Sapporo, Japan, p. 225–231.
- GOLDSCHMIDT, R. 1900. Zur Entwicklungsgeschichte der *Echinococcus*-köpfechen. *Zoologische Jahrbücher. Abteilung fuer Anatomie und Ontogenie der Thiere*. **13**: 467–493 + 1 Tafel.
- HOWARD, E. B., AND A. P. GENDRON. 1980. *Echinococcus vogeli* infection in higher primates at the Los Angeles Zoo. In *The comparative pathology of zoo animals*, R. J. Montall and G. Migaki (eds.). Symposium of the National Zoological Park, Smithsonian Institution Press, Washington, D.C., p. 379–382.
- LEUCKART, R. 1879–1886. Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten. Ein Hand- und Lehrbuch für Naturforscher und Aerzte. Zweite Auflage. C. F. Winter'sche Verlagshandlung, Leipzig und Heidelberg, Germany, 950 p.
- MENEGHELLI, U. G. 1985. Calcificações hepáticas múltiplas decorrentes de doença hidática policística. *Revista Goiana Medicina* **31**: 53–60.
- , M. L. P. BARBO, J. E. MAGRO, A. D. BELLUCCI, AND M. A. S. LLORACH VELLUDO. 1986. Polycystic hydatid disease (*Echinococcus vogeli*): Clinical and radiological manifestations and treatment with albendazole of a patient from the Brazilian Amazon region. *Arquivos Gastroenterológicos de São Paulo* **23**: 177–183.
- , A. L. C. MARTINELLI, M. A. S. LLORACH VELLUDO, A. D. BELLUCCI, J. E. MAGRO, AND M. L. P. BARBO. 1992. Polycystic hydatid disease (*Echinococcus vogeli*). Clinical, laboratory and morphological findings in nine Brazilian patients. *Journal of Hepatology* **14**: 203–210.
- NOZAIS, J.-P., J.-P. CHIGOT, C. SEIGNALET, P. ASTAGNEAU, C. HOANG, F. GAY, AND M. DANIS. 1994. À propos d'un cas d'échinococcose probablement dû à *Echinococcus vogeli* (Rausch et Bernstein, 1972) en provenance du Surinam. *Cahiers Santé* **4**: 335–339.
- O'GRADY, J. P., C. H. YEAGER, G. N. ESRA, AND W. THOMAS. 1982. Ultrasonic evaluation of echinococcosis in four lowland gorillas. *Journal of the American Veterinary Medical Association* **181**: 1348–1350.
- POSSELT, A. 1932. Die vielkammerige Blasenbandwurmgeschwulst ausserhalb der Leber. (Extrahepataler Alveolarechinococcus). *Ergebnisse der Allgemeinen Pathologie und Pathologischen Anatomie* **26**: 424–611.
- RAUSCH, R. L. 1954. Studies on the helminth fauna of Alaska. XX. The histogenesis of the alveolar larva of *Echinococcus* species. *Journal of Infectious Diseases* **94**: 178–186.
- , A. D'ALESSANDRO, AND V. R. RAUSCH. 1981. Characteristics of the larval *Echinococcus vogeli* Rausch and Bernstein, 1972 in the natural intermediate host, the paca, *Cuniculus paca* L. (Rodentia: Dasyproctidae). *American Journal of Tropical Medicine and Hygiene* **30**: 1043–1052.
- , W. E. GIDDENS, JR., AND J. G. BRIDGENS. 1987. Mesothelial tumors associated with larval taeniid cestodes maintained by intraperitoneal inoculation in rodents. *Canadian Journal of Zoology* **65**: 1755–1758.
- , V. R. RAUSCH, AND A. D'ALESSANDRO. 1978. Discrimination of the larval stages of *Echinococcus oligarthrus* (Diesing, 1863) and *E. vogeli* Rausch and Bernstein, 1972. *American Journal of Tropical Medicine and Hygiene* **27**: 1195–1202.
- REDFORD, K. H., AND J. F. EISENBERG. 1992. *Mammals of the Neotropics*, Vol. 2. The southern cone. University of Chicago Press, Chicago, Illinois, 430 p.
- SAIDI, F. 1976. Surgery of hydatid disease. W. B. Saunders Co., Ltd., London, U.K., 396 p.
- SOSA, O. E., AND V. E. THATCHER. 1969. Observations on the life-cycle of *Echinococcus oligarthrus* (Diesing, 1863) in the Republic of Panama. *Annals of Tropical Medicine and Parasitology* **63**: 165–175.
- VOGEL, H. 1978. Wie wächst der Alveolarechinokokkus? *Tropenmedizin und Parasitologie* **29**: 1–11.
- YAMASHITA, J., M. OHBAYASHI, T. SAKAMOTO, AND M. ORIHARA. 1962. Studies on echinococcosis XIII. Observations on the vesicular development of the scolex of *E. multilocularis* in vitro. *Japanese Journal of Veterinary Research* **10**: 85–96 + 2 plates.