DEPARTMENT OF THE AIR FORCE
Detachment 3, Air Force Institute for Environment,
Safety, Occupational Risk Analysis (AFIERA) (AFMC)
APO AP 96368-5213

MEMORANDUM FOR 18 MDG/SGPM 20 June 2001

FROM: Detachment 3, AFIERA/CDM
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Unit 5213
APO AP 96368-5213

SUBJECT: Land snail infection rates for the human parasitic nematode, *Angiostrongylus cantonensis* (rat lung worm) with notes on snail and parasite biology and distribution on Kadena AB, Okinawa Japan. Consultative Letter, IERA-DO-BR-CL-2001-0049.

1. SURVEY PERSONNEL AND CONTACTS:

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2. PROBLEM: The death of a seven year old dependent on Kadena AB, Okinawa in July 2000, initially diagnosed with a parasitic disease known as eosinophilic meningoencephalitis or angiostrongyliasis prompted an investigation to determine the risk of human infection based on parasitism rates of the four most common species of land snails (intermediate hosts) found throughout residential and recreational areas on Kadena AB. Although the child's infection was later diagnosed as something other than angiostrongyliasis, the misinformation on how humans can become infected from snails and the importance of snails as agents of disease persists. This report details the biology of the causative agent of eosinophilic meningoencephaltis (*Angiostrongylus cantonensis* or rat lung worm) and provides information on the snail composition, parasite distribution and infection rates for snails throughout Kadena AB. Recommendations are provided to minimize the risk of becoming infected.

3. BACKGROUND:

a. Parasite Biology/Life Cycle. Angiostrongyliasis or eosinophilic meningoencephalitis is a human disease most commonly caused by a parasitic nematode (roundworm) *Angiostrongylus cantonensis* (rat lungworm). The indirect life cycle of the rat lungworm requires an intermediate host (usually a mollusk) for the development of various larval stages. The adult worms live in the pulmonary arteries and/or the right side of the heart of a rodent definitive host. Eggs laid in the pulmonary artery lodge themselves in the small lung capillaries where they develop into first stage larvae. The mobile first stage larvae migrate into the

alimentary tract and pass in the rodent's feces. Numerous mollusks, and most commonly land snails and slugs serve as intermediate hosts. The first-stage larvae gain access to the snail/slug either by direct tissue penetration or ingestion of infected rodent feces. These larvae molt twice in the mollusk muscle tissue where they become second and third-stage larvae at 7 to 9 and 12 - 16 days later respectively. Third stage larvae are considered the "infective" stage and remain encysted in snail muscle tissue for the life of the mollusk or until eaten by an accidental (human), paratenic (amphibian) or definitive host (rodent). Of note, various animals act as paratenic (transport) hosts: after ingesting infected snails, they carry the third stage larvae that can resume development when the paratenic host is ingested by a definitive host. Humans can acquire the infection by eating uncooked snails or slugs, contaminated vegetables, or infected paratenic animals (crabs, freshwater shrimps, frogs). Snail feces and slime trails are not normally infective, though dead mollusks killed with insecticides or other means can release infective larva into water or onto vegetables/vegetation. Upon consumption, infective larvae enter the circulatory system of the host, ultimately reaching the brain in one or two days. In humans, the development of the third stage larvae stalls in the brain, where they die and serve as the source of infection. Within the rodent brain tissue, the larvae migrate to the olfactory lobes and the cerebral hemisphere. During migration in the brain, the larva molts into the fourth and fifth stage young adults respectively. Fifth stage larvae generally move to the subarachnoid space eventually migrating to the pulmonary arteries and completing their life cycle.

b. Importance and Life Cycle of the Giant African Snail (*Achatina fulica*). Due to its longevity, wide food host range and migratory habits, the giant African Snail (GAS) has historically been the most important reservoir and heavily infected of the intermediate hosts that can infect humans. The large biotic potential of the African snail enables populations to build quickly. As hermaphrodites, each snail lays up to 1000 eggs per year with clutches ranging from 100-400 eggs. Egg-laying begins upon reaching 60 mm (about 1 year) in length. A large peak in egg production occurs in June and another small peak in October-December. Eggs produced in the latter generally do not survive the winter. Those that are laid in June hatch in a couple of days and quickly grow to 30-40 mm in length. Snails become inactive in late November and bury themselves in the soil. Activity resumes in April with snails growing to 50-70 mm by the next summer. Growth is slow hereafter with snails living 5-6 years to a maximum of 9 years. During the warmer parts of the year, aestivation occurs during drought or other unfavorable weather. The life cycle of the snail on Okinawa is shown in Figure 1.

The GAS was introduced onto Okinawa from Taiwan as a medicine for asthma from 1935 to 1940 and from other South Pacific islands as a source of food during the Second World War. The first rodent and human infections were not recorded until 1964 and 1969 respectively. Although the snail was consumed as food during and after the last World War in the Ryukyus Islands, this habit is no longer practiced. Indeed, efforts to control the snail are in affect in many areas due to their importance as a serious agricultural and quarantine pests. Human ingestion of the parasite will not always result in the disease. Although skin tests conducted on Okinawan's in 1976 showed 22.6 percent testing positive for exposure to *A. cantonensis*, only 11 patients were positively confirmed for the disease up until 1975. Many of these cases were from ingesting live slugs or frog livers for medicinal purposes. For the last 30 years, there has been less than 1 case per year on Okinawa. There are no known deaths due to this disease in Japan. The only known

cases of angiostrongyliasis in Americans was reported in October 1976 when 15 Marines consumed both raw and cooked giant African snails during survival training in the Northern Training Area. Only the three Marines eating raw snails became ill three weeks later. Those eating cooked snails did not become ill. Symptoms included pruritis, lower extremity weakness, muscle pain, flaccid paralysis, stiff neck, eosinophilia and eosinophilic pleocystosis. Six of 55 (11%) giant African snails sampled from the NTA following the Marine cases were found to contain parasites.

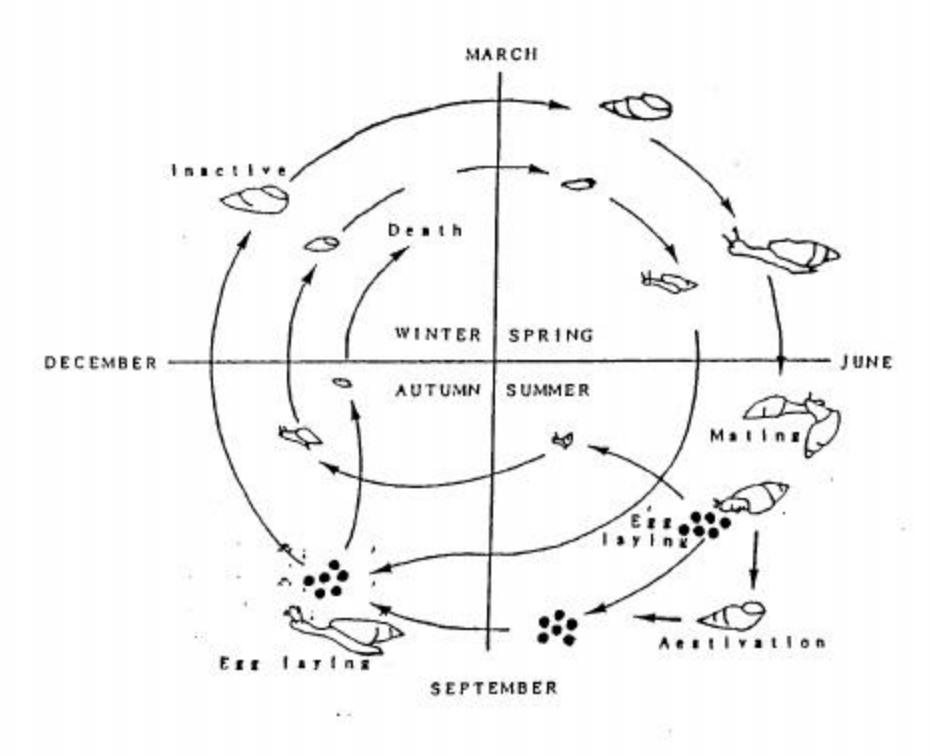


Figure 1. Life cycle of giant African Snail (*Achatina fulica*) on Okinawa.

c. Historical Background and Additional Host Information. Surveys conducted in the 1970's by the Okinawan Institute of Public Health found a number of definitive (rodents), intermediate (mollusks) and paratenic (amphibians, crustaceans) hosts. Roof (*Rattus rattus*) and Norway (*R. norvegicus*) rats generally have the highest infection rates of up to 18% and 37% respectively. Other animals such as *Mus caroli* (mouse), *Suncus murinus* (musk shrew) or *Herpestes edwardsii* (mongoose) are rarely, if ever infected. Although infected rodents and snails tend to be focal, other studies done in the 1970's found several land snail species serving as intermediate hosts including *Achatina fulica* (giant African snail, Figure 2), *Acusta despecta* (Figure 3), *Cyclophorus spp*. (Figure 4), *Bradybaena similaris, Satsuma mercatoria* (Figure 5), *Limax spp*. (slug, Figure 6), *Deroceras varians, Vaginulus plebeius* (slug), *Laevicaulis alte* (slug) and *Philomycus bilineatus* (slug). Historically, *A. fulica* has been the most heavily infected land snail species due to their long lives, diverse diet and migratory behavior. Once snails become infected, they remain infective for life. In the 1970's surveys found up to 57% of the GAS's collected from the middle part of the island were infected with fewer infected in the northern and

southern parts of the island. Likewise, *B. circulus*, *A. despecta* and *S. mercatoria* have been collected and had infection rates of 1, 1.5 and 35% respectively. *Laevicauslis alte* and *D. varians* are the most heavily infected species of slug. On Kadena AB, *A. fulica*, *Cyclophorus spp.*, *B. circulus*, *B. similaris*, *A. despecta* and *S. mercatoria* are the most common snails found on the walls, landscape plants, lawn and areas surrounding base housing. Slugs tend to be less common but can be locally abundant.

4. SAMPLING AND PARASITE DETECTION: Foraging and resting snails were collected from housing areas around Kadena AB including North Terrace, Sebille Manor, O'Donnell Gardens and Jennings. Pooled snail samples were tested for parasites using a pepsin-digest procedure. The pepsin artificial stomach juice solution was made using 5 grams of pepsin and 7 ml of concentrated HCL in 1 liter of distilled water. Muscle tissue was snipped from snails and placed into a food processor or chopped finely with scissors. Pooled snail samples separated by species varied in size but usually were as follows: *A. fulica*, n = 5; *A. despecta*, n = 10, *Cyclophorus spp.*, n = 10; *S. mercatoria*, n = 5. Snails/slugs were macerated for 10-15 seconds and transferred into a labeled jar containing the stomach juice solution. Samples were placed on a shaker overnight at room temperature. The next morning, digested samples were transferred to a gauze-lined, 3" plastic funnel with a piece of 5" rubber tubing attached to the bottom (apparatus called a Baermann funnel). A binding clip was used to pinch the rubber tubing thus preventing liquid from escaping. The digested snail samples were covered with ocular saline and allowed to sit for 4-6 hours. Sediment accumulating in the bottom of the rubber tubing was transferred into a test tube and centrifuged at 1000 rpm for 10 minutes. The test tube sediment was pipetted onto a microscope slide, covered with a cover slip and examined with a compound microscope at 10-30X for third stage infective larvae. Slides were recorded as positive or negative. Larvae, if present, were generally alive, active and about 800-1000 um in length.

Fig. 2. Non-native giant African snail (6-9 cm), historically the most heavily parasitized species on Okinawa.

Fig. 3. *Acusta (Fruticicola) despecta*, a common medium to small (1.0-2.5 cm) species found through out housing area on Kadena AB.

Fig. 4. *Cyclophorus spp*., a common medium to small (1-2 cm) species found in base housing on Kadena AB.

Fig. 5. *Satsuma spp*., (2.0-3.5 cm) a common medium to large snail species found in the woods near housing areas on Kadena AB.

Fig. 6. *Limax spp*., a slug occasional found in some housing areas around Kadena AB (1-4 cm).

6. RESULTS:

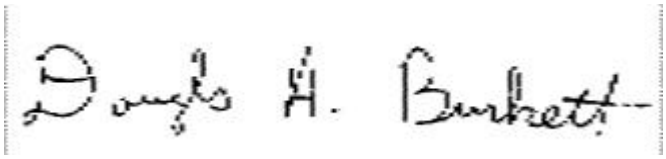
A total of 396 snails/slugs were collected in six housing areas throughout the housing areas Kadena AB. Results of the parasite rate for the four most common snail species and slug are organized by Kadena housing area and shown in Table 1. All snail/slug species evaluated tested positive for parasites in one or more of the housing areas. Overall, the common hard shelled snail, *Cyclophorus spp*. (Figure 4) were the most heavily infected species with 73% of the pools testing positive for infective rat lungworm larvae. The other species were parasitized in decreasing order including *A. despecta* (17%), *A. fulica* (17%), *S. mercatoria* (14%). Infective larvae were also found in the slug, *Limax spp*., though sample sizes were too small to estimate parasite rates. Overall, rat lung worm infection rates were highest in snails collected from O'Donnell Gardens and Sebile Manor housing areas where 45 and 53% of the pools tested positive for infective larvae respectively. In the fall of 2000, several hundred snails representing the four common species were collected in the neighborhood near where the girl died (Stearly Hts) and analyzed for parasites by the Okinawa Prefectural Government, Department of Microbiology. Similar to our findings, OPG found 2 of 20 pools (10% of the *B. circulus* pools (n=10) to contain parasites. None of the other species including the GAS's were found to contain infective larvae. *Bradybaenia spp*. snails were small during the Spring 2001 collection period and consequently not sampled or processed.

7. DISCUSSION AND RECOMMENDATIONS. Although infected snails are common, the risk of American military members and dependents stationed/living on Kadena (or other military installations on Okinawa) contracting angiostrongyliasis is very low. A thorough literature review shows < 1 reported infection per year in Japan and even those cases usually occur under unusual circumstances. There has never been a reported death from this disease in Japan. Most cases are of known etiology and usually result from ingesting raw snails and slugs for folk medicine, rough handling/mutilation of snails or accidental ingestion of living or dead snails and slugs on infested food. As the infective rat lungworm larvae are encapsulated in the mollusks muscle tissue that requires digestive juices for their release, casual contact with snails or their waste/slime trail has not been conclusively demonstrated as an important factor in disease transmission. Purposeful or accidental ingestion of raw infected living or freshly killed land or fresh water mollusks or other hosts must occur in order for humans to become infected. Free-living third stage infective larvae released from dead mollusks have been known to penetrate tissues and infect other hosts. However, this mode of parasitism has not been documented for humans. Even if humans were accidentally infected through exposure to free living larvae, or ingested a host containing few parasites, humans would only rarely become ill. Disease symptoms are most likely to occur following consumption of hosts containing large numbers of infective larvae.

A moderate to high percentage of some snail species captured throughout Kadena AB contained infective larvae. The snail infection rate is surprising because of the apparent low numbers of definitive hosts (commensal rodents) captured by CE pest management and observed throughout the base. The musk shrew (*Suncus murinus*) is the most common animal seen/captured in base housing. This animal is not a rodent and not known to be a carrier of rat lungworm. The larvae recovered from snail digestions in this investigation were presumed to be *A. cantonensis* based on size, shape and release from muscle tissue following the pepsin-hydrochloric acid digestion procedure. To a greater or lesser extent, infective rat lungworm larvae were found in all mollusk species tested. In general, the most heavily infected snail was *Cyclophorus spp*. that was consistently one of the most abundant snails in and around the various housing areas. Fortunately, the giant African snail (*A. fulica*) that historically been the most heavily parasitized species was not found to be heavily infected on Kadena AB.

A base-wide snail control campaign is not necessary, though common sense is in order to avoid becoming infected. Wash locally grown produce with running water (not soaking) and cook potentially infested vegetables such as cabbage. The Okinawan's rarely eat uncooked vegetables. In other studies, infected snails/slugs (living or dead) have been found hiding under leaves of various leafy vegetables As children are most likely to become infected, advice youngsters not to crush or play with snails or to place dirty or potentially contaminated items in their mouths, particularly while playing outdoors. Snails found in drainage ditches seem to be particularly heavily infected. If snails must be removed from around base housing, pick up snails using rubber gloves, tongs, or an inverted plastic bag. Place snails in plastic bag and throw in trash. Snails are most numerous and easy to collect at dawn especially following a rainy or humid night. The important thing is to keep fingers out of your mouth, and get into the habit of washing hands after being outdoors.

8. Contact me at DSN: 634-2639, or e-mail douglas.burkett@kadena.af.mil if there are any questions concerning this report.



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Kadena Location	Snail Species	Sample Dates	Pool Size	# of Snails/ Pools Tested	Infective Larvae (%+) (Pooled Samples)
North Terrace	*Achatina fulica*	05/03/01	1	24	0/24 (0.0%)
North Terrace	*Achatina fulica*	05/28/01	7	1	0/1 (0%)
North Terrace	*Acusta despecta*	05/03/01	6	1	0/1 (0.0%)
North Terrace	*Satsuma mercatoria*	05/03/01	4	1	0/1 (0.0%)
North Terrace	*Cyclophorus spp.*	05/03/01	10	1	0/1 (0%)
	Total Snails			**51**	**0/28 (0%)**
Sebile Manor (Mallard Rd)	*Achatina fulica*	5/29/01	5	8	4/8 (50%)[1]
Sebile Manor (Mallard Rd)	*Cyclophorus spp.*	5/29/01	10	3	3/3 (100%)
Sebile Manor (Mallard Rd)	*Acusta despecta*	5/29/01	5	4	1/4 (25%)
	Total Snails			**90**	**8/15 (53%)**
Stearly Hts	*Achatina fulica*	6/4/01	5	3	0/3 (0%)
Stearly Hts	*Cyclophorus spp.*	6/4/01	10	3	2/3 (66%)[1]
Stearly Hts	*Acusta despecta*	6/4/01	1	1	0/1 (0%)
Stearly Hts	*Satsuma mercatoria*	6/4/01	5	3	0/3 (0%)
	Total Snails			**61**	**2/10 (20%)**
O'Donnel Gardens	*Achatina fulica*	5/7/01	5	4	0/4 (0%)
O'Donnel Gardens	*Cyclophorus spp.*	5/7/01	20	3	3/3 (100%)
O'Donnel Gardens	*Acusta despecta*	5/7/01	10	3	1/3 (33%)
O'Donnel Gardens	*Satsuma mercatoria*	5/7/01	5	1	1/1 (100%)
	Total Snails			**115**	**5/11 (45%)**
Jennings (Near Merik Park)	*Achatina fulica*	5/24/01	2	1	0/1 (0.0%)
Jennings	*Achatina fulica*	6/8/01	3	1	0/1 (0%)
Jennings (Near Merik Park)	*Cyclophorus spp.*	5/24/01	10	3	2/3 (66%)
Jennings	*Cyclophorus spp.*	6/8/01	6	2	1/2 (50%)
Jennings	*Acusta despecta*	6/8/01	5	3	0/3 (0%)
Jennings (Near Merik Park)	*Satsuma mercatoria*	5/24/01	4	1	0/1 (0%)
Jennings	*Satsuma mercatoria*	6/8/01	3	1	0/1 (0%)
Jennings	*Limax spp.* (Slug)	6/8/01	5	2	2/2 (100%)[1]
	Total Snails			**79**	**5/14 (36%)**
Total				396	20/78 **(26%)**

Table 1. Infection rates of common land snails and slugs with the 3rd stage infective larvae of the human parasitic nematode, rat lung worm (*Angiostrongylus cantonensis*), found in various housing areas on Kadena AB, Okinawa Japan.

[1]Few infective larvae recovered

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