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What is Lyme Arthritis - also called

Lyme Disease, or Chronic  
Lyme Arthritis?

Lyme Arthritis is an epidemic,  
immune-mediated inflammatory  
disorder - usually begins with  
a characteristic skin lesion, ref. to  
as erythema chronicum migrans,  
- that may be followed weeks to  
months later by neurologic or  
cardiac abnormalities, migratory  
polyarthritis, intermittent attacks  
of oligoarticular arthritis, or chronic  
arthritis in the knees.

The endemic areas for this  
disorder include the northeastern  
coast of the U.S., particularly  
southern Connecticut, southern Rhode  
Island, Cape Cod, southern New  
York and Long Island, New Jersey,  
Delaware and Maryland. It has  
also been reported in Northern Wisconsin  
in northern California and Guyana.

Lyme Arthritis or Lyme disease got its  
name from the small community of  
Lyme in eastern Connecticut where

Lyme arthritis is thought to be caused by an infectious agent transmitted by I. ricinus.

By a characteristic expanding skin lesion, ERM, patients may often be identified 1-3 weeks after exposure and before the onset of arthritis.

When the skin lesion is present, most patients have circulating immune complexes. At that time, those with high serum IgM levels, erythrocytina containing IgM, and low IgG levels are the ones at risk of developing arthritis within months; those with high IgG usually do not.

- the above occur at m

- the name - Erythema chronicum  
migrans.

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ECM appears about 1-3 weeks after tick bite as an erythematous macule or papule. The borders of this lesion then expand to form a red ring as great as 20-30 cm in diameter, with central clearing. Occasionally secondary rings may form within the original one (erythema multiforme), sometimes expansion of the ring may not be accompanied by central clearing. The lesion which often itches, prickles, or burns may be accompanied by fever, headache, vomiting, fatigue, and regional adenopathy.

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It is of interest to note that the relatively voluminous European literature on ECM does not report or refer to arthritis - there is one possible exception - a report describing pain in a patient, unlike a few weeks after appearance of the ECM.

It also should be noted that the first case of ECM in the United States was reported in 1970, from the town of Redford in north central Wisconsin - so the

Even

A second passage to a third person was ~~not~~ successful and produced the typical ECM imp - two weeks after inoculation!

Of particular interest to us - as well as to the studies currently done at CDC in Atlanta - is a 1948 paper by Lenhoff (Lenhoff C, 1948 - spirochetes in acrologically obscure diseases, Acta Derm Venereol. Hockh. 28; 295-324)

Lenhoff reported on the presence of spirochetes in the ECM lesion. However, subsequent studies by others using darkfield and phase contrast microscopy failed to confirm Lenhoff's claim.

> In 1962, the French microbiologist Degas, Teuvaine and Abouete reviewed the clinical histories of 7 ECM cases that occurred in France between 1958 and 1961. They submitted the sera of their patients to Paul Girod (L'Inst. Pasteur, Paris) who examined them for antibodies to acetaldehyde by his famous slide agglutination test. Girod's results showed antibodies in titers up to 1:320 against R. proazaphie and up to 1:160 against R. moorei and R. canoni in early

disease has been recognized before the outbreaks in Connecticut.

What are the factors that suggest that Lyme arthritis and ECM are tick-borne?

- (1) - the geographic distribution of ECM and of Lyme arthritis coincide with the distribution of ixodid ticks, namely
- I. ricinus in European countries
  - I. dammini along the northeastern coast and in Wisconsin and
  - I. pacificus in California and Oregon,

In many instances patients remembered having been bitten by ticks and in some instances patients possessed the ticks for identification. Thus history of tick-bites prior to onset of the disorders are frequent -

- (2) In a study carried out along the Connecticut River in Connecticut, it was found that the incidence of Lyme disease during 1977 was 2.8 cases per 1,000 residents on the east side of the river - whereas on the west side the incidence was only 0.1 cases per 1,000 residents. An accidental survey revealed that the

population of I. damminis was much greater on the east compared to the west side of the river.

(3) The occurrence of cases in the summer and early fall, in the US at least, coincided with activity seasons of I. damminis.

(4) The incubation period of 1 to 3 weeks is relatively constant in both ECM & Lyme arthritis -

slide of ticks

There are regions such as in Scandinavia that report ECM cases but have no ticks; in those ~~cases~~<sup>areas</sup> a mosquito vector has been suspected.

Alan Barlowe



The causative agent of ECM and Lyme arthritis are still unknown. Some investigators postulated a reaction to the tick bite - however the interval from the tick to the lesion - up to 8 months seems too long for an allergic reaction to an irritant antigen. The most widely held view is that an infectious agent is involved: in fact German investigators succeeded to transmit the lesion among themselves by inoculating tissue from the edge of the lesion (Binder F., Deegfmer, R., Horstein G., 1958, Klin. Wochenschr. 33: 727-728)

Let us now briefly discuss the biology of this mammalian tick vector and life to observations made by Carey, Hinnsley and Mann, Jan of the Department of Epidemiology and Public Health, Yale University School of Medicine at New Haven Conn. J Med Entomol, vol 17: 89-99, 1980 who studied this tick within the Lyme arthritis area of southern Connecticut.

I. dammini like the other members of the Ixodes ricinus complex - has 3 developmental stages; - the larvae, the nymphs, and the adult. Larval and nymphal ticks require a blood meal - to reach the nymphal and adult stage, respectively. A blood meal is also required by the male female tick before copulation of eggs occur. Male ticks do not ingest blood.

In 1978, according to Carey and associates larvae I. dammini were most abundant in the late summer and adults in the spring and fall.

I. dammini was found to parasitize a large variety of mammals - with the principal small host for larvae

and nymphs being - the white-footed mouse (*Peromyscus leucopus*) and the Eastern chipmunk (*Tamias striatus*). The principal host for the adult was the white-tailed deer. The immature stages of *T. dammisi* infest also ear, dog, and - very important - man!

White-tailed deer - have been reported to be important hosts for all stages of *T. dammisi* (Pomeroy et al. 1979, J. Wildl. Entomol. 15: 537-540)



unsuccessful. Also it should be stated that they have tested hundreds of patients' sera for antibodies to tick-borne viral - other arboviral and bacterial agents with uniformly negative results.

In 1980, Bob Philip and I tested a large series of Dr. Heu's sera by indirect immunofluorescence and microagglutination, respectively, against all available tick-borne antigens. The data were interpreted as being against a tick-borne etiology of Lyme Disease.

Similarly, sera collected by Dr. Claus Winkelmack from ECN patients in Sweden had no significant titres when tested against the European members of the spotted fever group.

2nd September, I unexpectedly received a shipment of adult I. dammanni from my colleague, Dr. Joyce Benach from the New York Department of Health at Hony Brook. In the past few years Joyce and I have collaborated on RITF on Jay Island. He, more recently, has been interested in the role of I. dammanni on a vector of Babesia microti, the

earrings of - human Gallinosa  
in that area. Attached to the  
tick - was a pencil-written note  
saying - Collected from an area where  
several cases of Lyme arthritis - have  
occurred. More ticks to follow if  
you want to look at them - if not -  
Don - Jay.

Yes, we were interested in ~~the~~ <sup>these</sup> ticks,  
particularly since previous examination  
of I. clammii from that area yielded  
specimens infected with spotted fever  
group rickettsiae. Attempt for isolation  
- however, - had failed.

Thus the 2133 and 2399 were  
rejected - to the hemolymph test. Although  
- they were negative for rickettsiae, - two  
ferrets contained in these hemolymph  
masses (clumps) of a microplasma.

✓✓✓✓✓✓ slides -

Although I am not a-helminthologist  
a review of the literature on zoonotic  
placental infections of North America,  
suggests - that the "beast" in question  
is a Dirofilaria.

Our slides have been forwarded for  
identification - to the Department of  
Tropical Medicine and Public Health  
at the Tulane University.

until we receive final identification

~~in the meantime~~ let us realize that  
Dirofilaria is a nematode found in  
camions - throughout the world Mosqui-  
toes and fleas are incriminated as the  
usual vector.

In the U.S., identifications re Dirofilaria  
as heartworms in dogs and as sub-  
cutaneous infection in raccoons.

About 50 human cases have so far  
been reported - most of them in  
enough - from New York, Long Island,  
Vermont, Massachusetts, Cape Cod,  
Florida and Wisconsin - i.e. within  
the distributional area of I. dammani

Could it be that the tick rather than  
mosquitoes or fleas is the vector to  
man?

After discovering the microplasmae, we decided to take a close look at the remaining live ticks. Each tick was dissected for the preparation and examination of tissues from tissues including midgut, salivary glands, Malpighian tubules, central ganglion and genital systems.

Our first attention was directed towards a characterization of the tick's symbiote.

Symbiote as you must know are associated with every species of tick and occur in every tick specimen where they are usually limited to certain tissues such as the ovary, malpighian tubules and certain parts of the midgut. Their physiologic function and purpose are as yet unknown - but to the best of my knowledge they have ~~not~~ never been linked to disease of a tick's natural or accidental hosts. Because of their absence from the tissues of the salivary glands, their transmission by tick can be ruled out.

The other rickettsia-like symbiote occur either free in the cytoplasm or

as colonies in membrane-bound vacuoles.

✓✓✓

The symbiotes of I. dammiti - as illustrated in the next slides - are readily recognized as highly pleomorphic ciliated cyanoforms varying from coccoid to threadlike. They occur free in the cytoplasm where they produce massive infestations especially in the ovarian tissues.

✓✓

In their fine structure they greatly differ from the symbiotes of Dama-centa, for instance, in that

- a) they are not membrane-bound
- b) there is no differentiation of their cytoplasm, and
- c) they do not exhibit a multilamellar cell wall.

They resemble ciliates in their gross appearance and presence of a typical slime layer but differ from pathogenic ciliates by the absence of a microcapsular layer and the fact that the outer and inner leaflets of the cell wall are of the same thickness (2-3  $\mu$ m).

Presently we are in the process of  
isolating - the symbiotes on tissue  
cultures for immunology and  
immunochemical characterization.

The result of preliminary FA  
stainings suggest - that the T. dam-  
mini symbiotes - have no antigenic  
relationship to - the symbiotes of  
Dermacentor tick for instance  
or to the spotted fever or - typhus  
group tickborne.

✓

While examining - the tissue  
smears of a series of 5 T. dam-  
mini 7 encountered yet another  
agent in the midst of the  
second 7 of that particular  
series - here it is - FA Spirochete!

To make certain that these organisms  
were alive, we prepared fresh pre-  
parations of midgut from the  
remains of the same tick and  
examined them under darkfield.  
Although many spirochetes were  
inactive, few exhibited the  
typical movement of BORRELIA  
I shared this information with  
Jozef Benach who immediately

went into the field to collect  
and furnish additional ticks -  
and also with Alan Barbour  
who is collaborating with Dr.  
Hammer on the antigenic makeup  
of the relapsing fever agent,  
Borrelia burgdorferi.

As of today I have dissec-  
ted 122 *I. dammini* (2599 &  
9738) from the collection site  
in Long Island - 76 (1299 &  
6438) were positive for spiro-  
chetes!

✓ Interestingly - these organisms  
appear to be present only in the  
ticks' digestive tract, particu-  
larly in the midgut where they  
are associated & often in clumps  
- with the microvillar  
border of the gut epithelium.  
Spirochetes have never been observed  
in the hemolymph or in any  
other tissue of the ticks.

That we are dealing here with  
a *Borrelia* becomes apparent  
also from the following slides - that  
illustrate the fine structure of  
- this microorganism.

✓✓ The first two slides are longitudinal sections showing the flagellum which consists of 6 to 8 fibrils (in the average) and which forms an integral part of the outer membrane. This is better seen in cross sections that also illustrate the plasma membrane (7-10 nm) surrounding a mottled cytoplasm with randomly distributed ribosomes.

✓ The diameter of these organisms varies from 15 to 25  $\mu$ .

of particular interest - and as yet unsolved - is the "blebbing" phenomenon - detectable even by conventional light and darkfield microscopy (a bleb or aneurism along the spirochete - as seen on previous slide).

✓ Electron microscopy reveals that these blebs represent a bulging or aneurism of the outer cell membrane - forming a bleb that may contain numerous rather large granules. This phenomenon has been the subject



✓ of many previous investigations by spirochetalogists and has been interpreted as a "sporulation phase" in the developmental cycle of *Borrelia* that usually multiply by transverse fission.

However, there are other investigators such as Pillot & Ryter (Ann. Institut Pasteur, Oct. 107, 1964) who state "spherical forms are only a degeneration product of the helical elements; they do not constitute resistance forms nor elements of an obligatory cycle."

I am certain that we will address ourselves to this question in the near future, for Alan has been successful in isolating and maintaining in Kelly's medium the Spirochete from a pool of tick midgut.

I should add here that inoculation of infected mixed suspensions and of heavily positive cultures into suckling and 21-day-old RML white mice, also BALB/c

mice, meadow vole, prairie dog,  
and rabbit did not result in  
detectable opioche ~~ferment~~ or illness.

✓✓

Finally, there was another  
agent in many tick smears that  
I had concluded to be of protozoan  
nature -  
show slide -

Does anyone who has seen  
- head of red tick - recognize  
it?

Well, we had seen difficulty  
in isolating this as Vero cultures -  
where we recognized it as the  
developmental stages of a yeast-  
cupriating from the exoskeleton  
of the ticks.

Thus my friends, we are looking  
at at least 4 parasitic or microbial  
agents that are associated with  
1. clammy from a highly endemic  
area of Lyme disease.

Without the knowledge that Parvovirus  
is highly effective in treating FCV and

lyme Disease, one could speculate  
- that each of these agents could  
be involved in the etiology of  
the disorder. Penicillin sensi-  
tivity, however, make the uickethia-  
like symbiote and particularly  
the spirochetes prime targets for  
further investigation.

Such investigations were  
initiated a few days ago with  
sera collected by Tony Borach  
of Lyme Disease patients from  
Long Island.

I am delighted to report that  
indirect fluorescence microscopy  
on either cultured or tick-associated  
spirochetes gave very strong and  
specific staining reactions but  
was uniformly negative when  
applied to the uickethialike  
symbiote.

These findings - as far as the  
spirochetes are concerned at least -  
were confirmed also by Alan  
who applied the Western Blot  
technique ~~to~~ to show the  
relationship between the patients'  
antibodies and the spirochetal  
antigens.