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Hard Tick as a Reservoir and Potential Vector of Tuberculosis

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BOOPHILUS annulatus and Hyalomma dromedarii reared on experimentally infected guinea pigs and rabbits were capable of transmitting M. tuberculosis and M. bovis to another free hosts. Transovarial and transstadial passage of infection was also detected.

M. tuberculosis survived in starved ticks of both species for 15 weeks indoor (at 20° ± 3) and for 7 weeks outdoor (at 28° ± 2). Under the same environmental conditions, M. bovis remained viable for 10 and 5 weeks, respectively.

The presence of arthropods and arthropod-borne diseases in practically all parts of the world is well known, although variety and intensity of both vary according to environmental conditions. Ticks are of special importance in transmitting serious virus and protozoan diseases peculiar to the tropics and subtropics. Williamson and Payne (1959) mentioned fourteen arthropod-borne diseases, of which nine are mostly transmitted by ticks.

Among bacterial diseases, various species of hard ticks were responsible for transmission of anthrax (Williamson and Payne, 1959) brucellosis (Pritulin, 1954 and Mowafy,1974), salmonellosis (Buxton, 1958 and Mowafy, 1974), tularaemiasis (Karpov and Popov, 1944 and Allerd *et al.*, 1956) pasteurellosis (Macadam, 1962) and listeriosis (Petrov, 1966). Otherwise knowledges about the role of ticks in transmitting tuberculosis is still shorter andthis work was planned, in an attempt, to explain this role.

Material and Methods

1. *Experimentalanimal*

Three groups of mature guinea pigs and rabbits were used. Each group contained 3 animals.

2. *Ticks used* :

Adult stages of Boophilus annulatus (Say, 1821) and Hyalomma dromedarii (Koch, 1844) were used. The ticks were distributed separately in plastic tubes (4 cm in diameter x 10 cm length), each contained 20. The ticks were maintained under controlled environment of 28° and 80% R.H. for 3 days to be starved fully before being used for parasitism.

Aliquot speciments of the used ticks were tested bacteriologically to ensure its freedom from the tested organisms before being used.

3. *Test organsims*:

4- weeks old culture of virulent strains of:

i- *Mycobacterium tuberculosis.*

ii- Myoobacterium bovis.

Heavy suspension of each organism, 10 g moist culture per ml, in steralized saline solution was prepared for use.

4. *Culture media*

i- Modified Lowenstein-Jensen medium with glycerol.

ti- Stonebrink medium.

Experimentation

A. Transmission of infection from one animal to another

(1) Experimental infection of anmials

A group of 3 guinea pigs was infected with human tubercle bacilli. Each animal was injected intraperitoneally with 1 lm of bacterial suspension. Also each of a rabbit group was injected intravenously with 0.20 ml of tubercle suspension of bovine type.

(2) Rearing of ticks on infected animals

20 starved ticks of both species were used for each animal of the two infected groups. The ticks were taken carefully and distributed into two rimmed alluminium capsules with minute pores for ventilation, covered with gauze and tied with rubber band on a shaved skin area of the test animal (Woke, 1951). At the distal end of the capsule, tioks were in contanct with the skin of the host to suck what they need of the blood during the period of their adjustement.

(3) Detection the transmission of infection to ticks through blood meal

Trials were applied for isolation of the test organisms from ticks on animals at intervals of , 5 and 7 days post-parasitism. One fully engorged tick of each tick species on each infected animal was taken and triturated in a sterile morter with sterile glass sand and 2 ml of 4% sodium hydroxide solution, and placed in the incubator for 30 min at 37° . The supernatent fluid was transferred to a clean tube and centrifuged at 3000 r.p.m. for 30 min. The deposit was neutralized with 8% H Cl using phenol red as indicator, and evenly distributed onto two slopes of Modified Lowenstein-Jensen medium with glycerol for isolation of human tubercle bacilli or Stonebrink medium for culturing bovine type. The cultured slopes were incubated at 37° for 8 weeks, during which periodical examination were made daily for the first 7-10 days and weekly after that. Suspected growth was assured to be the respective test microbe according to Buchanan and Gibbons (1975).

(4) Rearing of infected ticks on healthy animals

Infected ticks from (2) were taken and starved for 3 days. Starved ticks were distributed in another aluminium capsules, each contained 10 ticks of both species, and reared again on another two groups of the respective test animals for seven sucessive days. Animal died or killed after six weeks were carefully autopside and suspected lesions were confirmed bacteriologically.

(B) Longivity of tuberole bacilli in starved ticks

From each infected tick species with any of the test organisms, two batches (each contained 20) were distributed asceptically in separate plastic tubes, 10 ticks each, covered with gauze and tied with rubber band, and placed with terminal end immersed in moistened sand. The prepared batches were arranged as any of the test microbe in each tick species was kept at room temperature ($20^0 \pm 3$) and open atmosphere ($28^0 \pm 2$).

Trials for detection of viable tubercle bacilli in ticks were applied at weekly intervals, using the same procedures mentioned before in A-3.

C- *Detection of tubercle bacilli through various tick generations*

From each infected tick species 5 fully engorged females were taken in plastic tubes and kept under controlled enviromnent of 28^0 and 80% R.H.for oviposition and larvae formation.

(1) Isolation of the test microbes from eggs

About 0.5 g of newly deposited eggs from each infected tick species was triturated well with sterile glass sand and 2 ml of 4% sodium hydroxide solution. The obtained suspension was treated as described before in A-3.

(2) Detection of tubercle bacilli in larval stage was carried out : by:

i. Trials for isolation of each test organism from 20 larvae of each tick species. The larvae were triturated well with sterile glass sand and 2 ml of 4% sodium hydroxide solution. The prepared suspension was treated as mentioned before in A-3.

ii- Rearing of newly hatched larvae of each tick species on another healthy groups of the respective test animal. Each animal was infested by 20 larvae of both tick species for 7 days. After engorgement they were collected and kept similar plastic tubes for molt.

Dead or killed animals were autopside and suspected lesions were bacteriologically inspected for presence of the test tubercle bacilli.

(3) Detection of tubercle bacilli in molted nymphs

Of the two tick species was carried out by isolation technique; as mentioned before in A-3.

Results

The obtained results are tabulated in 1-3.

Discussion

This study was planned to clarify the extraordinary mode of transmission of tuberculosis among animals, *i.e* vector of transmission such as ticks. Among these, Boophilus annulatus and Hyalomma dromedarii were described to be the most prevalent species infesting livestock at Sharkia governorate (Mowafy, 1974).

The obtained findings in Table 1 showed no difference in the role played by the two tick species. These vectors taken the infection of human and bovine tubercle bacilli after 3 days of blood meal on experimentally infected hosts, also they were capable of transmitting the infection to new hosts during seven days of attachment.

The test organisms were recovered from lungs, kidneys and livers of, previously infested, guinea pigs and rabbits killed at 6 weeks post-infestation.

Similar findings were obtained by Postoyan and Agabalov (1971) who mentioned that presence of Mycobacterium bovis in Ornthodorus Lahornesis fed on infected guinea pigs and rabbits was established by biological examination of ticks.

Although tubercle bacilli are essentially pathogenic, they could be detected viable in starved ticks for various periods (Table 2). M. tuberculosis remained viable for 15 weeks in ticks kept at room temperature ($20^0 \pm 3$), and for 7 weeks in those maintained outdoor ($28^0 \pm 2$). On other aspect M. bovis showed shorter periods, that it survived in ticks for 10 weeks indoor and 5 weeks outdoor.

From the hygienic point of view, infected ticks, other than infected animal, may constitute a dangerous reservoir of tubercle bacilli. A fact which should not be neglected during combating of tuberculosis, that animals should be free from ectoparasite-infestation.

Table 3 indicated that infected female ticks of both species could be able to transmit tubercle bacilli through eggs and larvae. The two test organisms successfully recovered from eggs and larvae of the used ticks. Moreover, active larvae of each tick species carried the infection to the specific hosts during seven days of attachment. The animals were severely affected, that all guinea pigs died after 15 and all rabbits showed severe emaciation, in comparison with those infected with infested adults ((Table 1). Furthermore, M. tuberculosis and M. bovis were recovered from molted nymphs of both tick species

TABLE 1. Transmission of tuberculosis infection through tick infestation.

Tuberole bacilli	Animal	Adult stage of tick species	Transmission of infection								
			From animals to ticks					From ticks to animals			
			Period posin-fection	Period of attac-ment	Early isolation of TB ticks after rearing			Pe iod of attach-ment	Patho-genecity	Isolation of TB from tubercul-ous lesion; in
					3D	5D	7D			
Human type	Guinea pigs	Boophilus analatus. Hyalomma dromedarii	24 hr	7 days	+ve	+ve	+ve	7 days	all animals survived for 6 weeks	Lungs liver Kidneys
Bovine type	Rabbits		24 hr	7 days	+ve	+ve	+ve	7 days	all animals survived for 6 weeks	Lungs Liver Kidneys

Accordingly, one may conclude that M. tuberculosis and M. bovis could be transmitted through various developmental life-cycle of B. annulatus and H. dromedarii, *i.e* transovarial and transstadial passage. Blagodarnyi *et al.* (1970) reported that Mycobacterium aviam could be transmitted by the bite of infected ticks as well as transovarian passage.

TABLE 2. Survival of tubercle bacilli in ticks under various environmental conditions.

Tubercle bacilli	Survival period in weeks	
	Tick used B. annulatus and H. dromedarii	
	Room + temp. (20° ± 3)	Open air (28° ± 2)
Human type	15	7
Bovine type	10	5

TABLE 3. Detection of tubercle bacilli through tick tgenerations.

Tubercle bacilli	Tick speci ·	Tick generations					
		Eggs	Larvae				Nymph
			Isolation	Parasitism			
				Period	Pathogenecity	Isolation of TB from tuberculous lesions in	
Human type	Boophilus annulatus Hyalomma dromedarii	+ ve isolation	+ ve	7 days	all guinea pigs died after 15 days	Lungs Kidneys liver	+ve isolation
Bovine type		+ ve isolation	+ ve	7 days	all rabbits survived for 8 weeks with severe emaciation	Kidneys Liver	+ ve isolation

+ Ve = Positive

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حشرة القراد مستودع وناقل اضطرارى لمرض السل

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ثبت الآن بالقطع مدى خطورة الطفيليات الخارجية فى نقل الأوبئة للحيوان خاصة فى المناطق الحارة والشبه حارة . وان كان دور هذه الحشرات فى نقل العديد من أمراض الحيوان مازال غامضا . الأمر الذى دفعنا لاجراء هـذه الدراسـة فى محاولة لاستبيان دور حشرة القراد فى نقـل وباء السـل بين الحيوانات .

وفى سبيل ذلك تم اجراء دراسة معملية حية استخدم فيها الآتى :
١ ـ ثلاث مجموعات من الخنازير الهندية والأرانب ، كل منها يتكون من ثلاث أفراد ناضجة .

٢ ـ نوعين هامين من حشرة القراد هما : بوفيلس أنيولاتس وهيالوماد روميداري وقد ثبت آنفا مدى انتشارهما على الحيوانات الزراعية فى محافظة الشرقية •

٣ ـ عترتين ضاريتين من جرثومة السل الآدمى والبقرى •

٤ ـ منابت بكتريولوجية لجرثومة السل •

وقد تبين من الدراسة الآتى :

أولا ـ ثبت امكانية نقل جرثومتا السل الآدمى والبقرى لحشرة القراد ، فى مدة ثلاث أيام من تطفلهما على الحيوانات المصابة ، وقد نقلت هذه الحشرات المرض لحيوانات أخرى عندما انتقلت بالاعالة عليها •

ثانيا ـ بقيت جرثومة السل الآدمى حية داخل حشرة القراد لمدة ١٥ أسبوع عند حفظها داخل المساكن ، ولمدة ٧ أسابيع عندما بقيت خارج المساكن • فى حين أن جرثومة السل البقرى ظلت لمدد أقل ١٠ ، ٥ أسابيع بالتتالى تحت الظروف الموضحة سابقا •

ثالثا ـ ثبت امكانية انتقال جرثومتا السل بين أطوار الحياة المختلفة لحشرة القراد وكذا خطورة كل طور فى نقل المرض للحيوانات عند تطفله عليها •

وعليه فقد بات واضحا دور الحشرات ولاسيما القراد فى نقل جرثومة السل الآدمى والبقرى بين الحيوانات • الأمر الذى لا يمكن اغفاله عند متابعة سلوك المرض أو عند مقاومته •