

STUDIES ON THE CANINE DEMODICOSIS
III. EXAMINATION ON THE ORAL-INTERNAL INFECTION,
INTRA-UTERINE INFECTION AND INFECTION
THROUGH RESPIRATORY TRACT

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(Received for Publication, September 12, 1962)

Introduction

It is generally believed that infection of demodectic mange occurs by direct skin contact, however nowadays a question of other infective routes has been discussed since Cánepa *et al.* (1941-1945) first recorded the presence of mites in the inner organs of the dogs suffering from demodectic mange. The investigation of the skin and inner organs in the clinical normal dogs and diseased dogs performed by the authors (1957, 1962), had not shown the findings which proved the oral infection reported by Cánepa *et al.* In this paper, the possibility of the other infective route is discussed from the results of basic tests such as the artificial transmission.

Methods and Results

1. *Investigation of the oral infection*

a) Survival and mortality of mites in the contents of stomach and intestines of the diseased dogs.

Methods: Contents (2g) of each digestive tract from tongue to rectum were taken from three diseased dogs and were treated in a similar method as fecal sedimentation. Three diseased dogs anew for examination of mites in the feces were added. Fecal examination was carried out five times on each dog. Survival and mortality were estimated by movement of the mites when collected mites were

gradually heated till 40°C.

Results: (Table 1) It was found that most of the mites transmitted orally from the skin died in the stomach.

b) Influence of the juices pressed from the content of each digestive tract and the artificial gastric and intestinal juices on the mites.

Methods: The juices pressed from the stomach and intestine contents of a healthy dog (6 months old), equivalent solution of 3% pepsin and 3% HCl solution for artificial gastric juice, and a solution which contained 2g of pancreatin and 5ml of dog's bile in 100ml of the water for artificial intestinal juice, which was adjusted at pH. 7.0 with addition of NaHCO₃ were used. And saliva obtained by injection of atropine sulphate was used. The material scraped from the patient's skin was added to a small quantity of physiologic sodium chloride solution. One drop of this suspension contained about 30 mites. Each 1ml of the above mentioned juice was put into 3 small test tubes apiece and 3 drops of the suspension of mites were added to 1 tube apiece and then was kept at 38°C. Plain water and 2% K₂Cr₂O₇ solution were used as controls.

Survival time and rate of the mites in the tube were counted once daily.

Result: (Table 2) Most of the mites in the artificial gastric juice and the half in the other juice died on the 1st day. The third day, no surviving mite was found but in the saliva. Higher survival rate of the mite was found

Table 1. Number of surviving mites in the contents of each digestive tract of the affected dogs

Parts	Number of surviving mite					
	No. of animal					
	201	206	210	213	220	225
Tongue	0/4	1/2	4/6			
Pharynx	1/2	0/2	3/3			
Esophagus	3/4	0/4	1/2			
Stomach	2/28	13/60	11/79			
Duodenum	0/14	0/2	0/20			
Jejunum	0/24	0/43	0/26			
Ileum	0/6	0/2	0/3			
Caceum	0/2	0/18	0/6			
Feces (Total mites of 5 times)	0/788	2/900	0/1268	0/18115	0/1054	1/1364

Numerator : Surviving mites. Denominator : Dying mites.

Table 2. Influence of juices pressed from contents of each digestive tract on the mites

	pH before experiment	Survival rate of mites			PH after experiment
		Period of observation			
		1 day	2 days	3 days	
Saliva	8.4	31.6%	11.2%	1.2%	8.6
Pressed juice of stomach	4.0	30.1	1.6	0	4.2
Pressed juice of duodenum	6.6	30.6	3.5	0	6.8
Artificial gastric juice	1.0	4.6	0	0	1.0
Artificial intestinal juice	7.0	22.7	0	0	7.4
Water	6.4	36.7	25.6	18.7	6.4
2% K ₂ Cr ₂ O ₇		57.4	26.6	14.8	

in the controls. Although the mites receive some influence in the digestive tract, the mites are able to survive on the parts higher up than the duodenum, even a few days. According to the results, it seems that if oral infection might be possible, invasive ports were on these upper parts.

c) Transmission of the mites to the digestive mucosa.

Methods: Sixteen healthy dogs, from 2 to 12 months old in which mites were negative, a piece (0.5 cm²) of one side of the muzzle skin, one of the so-called predilection site, was

used. For materials of transmission, some pieces of skin about 2 cm² were removed from the painted area of diseased dog and a liquid paraffin was dropped on these surface, and the surface of these skins was then scratched by a scalpel. After laparo-enterotomy, the above scraped material was applied to the mucous membrane of the operated tracts of each dog, and the removed skin was stuck and sutured on the same part. Such an operation was made at pylorus, fundus gland region, duodenum, jejunum, ileum and colon. The dogs died or were slaughtered at 20 hours to 60

days. Examination was carried out to search the mite in the operated tracts, mesenteric, portal, colic, internal and external iliac, mediastinal, pulmonary, renal, mandibular, supra-pharyngeal, antero-cervical, prescapular, axillary, inguinal, popliteal lymphatic glands and the skin (1 cm² apiece) connecting with the above nodes, namely, the eye lids, temporal region, the sides of the muzzle, lower jaw, fore breast, armpit, fore legs, abdomen, hind legs and lumbar. For detecting mites from materials in operated tubes, each material was divided into two equal pieces and one half was dissolved in 10% NaOH solution and the other half sectioned by paraffin method and stained with hematoxylin and eosin.

Result: (Table 3) In 3 cases out of 16, the mites were found on the inoculated parts of digestive tract by the dissolution method and the total mites were very few. There were never any found by the section method. However it was found on a microscopic prepara-

tion that some parts of sutured skin adhered to the serous membrane of the operated part; it appears that the mites which were found in digestive tube tissue have been encapsulated with adherent substance of wound lesion and veiled. Examination of the lymphatic glands and the skin was negative in all cases.

d) Oral transmission test.

Methods: Five puppies (Nos. 61, 146, 147, 148, 151) of 5-days-old, a litter of mange-free mothers, were used. Once a day during a week, forced administration of skin pieces, which contained about 50 thousand mites, liquid paraffin was dropped on the surface and incisions were made by a scalpel to make easy the movement of mites, was made to No. 61. Other puppies were given scratched materials containing about 5 thousand mites once a day during 20 days. After administration No. 61 was slaughtered after 3 days, No. 146 after 34 days, No. 147 after 2 months, No. 148 after 4 months, and No. 151 after 6 months,

Table 3. Showing the result of transmission of the mites to the digestive mucosa by direct inoculation

Dog's number	Age (Month)	Part of inoculation	Days after inoculation	Number of mites in the inoculated tissue			
				Methods of examined		Lymph nodes	Skin
				Dissolving in NaOH	Sectioning of tissue		
152	4	Pylorus	3 days	—	—	—	—
153	4	"	5	—	—	—	—
177	4	"	6	A. 1	—	—	—
182	4	"	7	—	—	—	—
183	4	"	7	—	—	—	—
184	6	Fundus	8	—	—	—	—
185	2	"	11	—	—	—	—
186	12	"	36	—	—	—	—
187	4	Duodenum	3	—	—	—	—
188	4	Jejunum	4	A. 2	—	—	—
189	12	"	5	—	—	—	—
190	12	"	6	—	—	—	—
192	4	Ileum	3	—	—	—	—
193	12	"	4	—	—	—	—
194	12	"	60	—	—	—	—
196	12	Colon	20 hours.	L. 2 A. 3	—	—	—

A: Adults. L: Larvae.

and examination of the lymphatic glands and the skin were carried out by the method mentioned (C). Especially, all organs and tissues of No. 61 were examined by the dissolving method. (Namely, the digestive tract, lung, liver, kidney, spleen, and blood of the heart).

Result: Careful examination for the mites was negative in all cases.

2. Investigation of the intra-uterine infection

a) Demonstration of the mite in the fetus and the non-suckling puppy of the affected dam.

Method: Six fetuses of the affected dam which died 40 days after pregnancy and 8 puppies in a litter were used. Four puppies of these had been isolated from the dam. The other 4 puppies were used as control, which were suckled directly for 2 days. One puppy of their litter, suckled for 16 hours, was examined also. All kind of tissues and organs of these animals were examined.

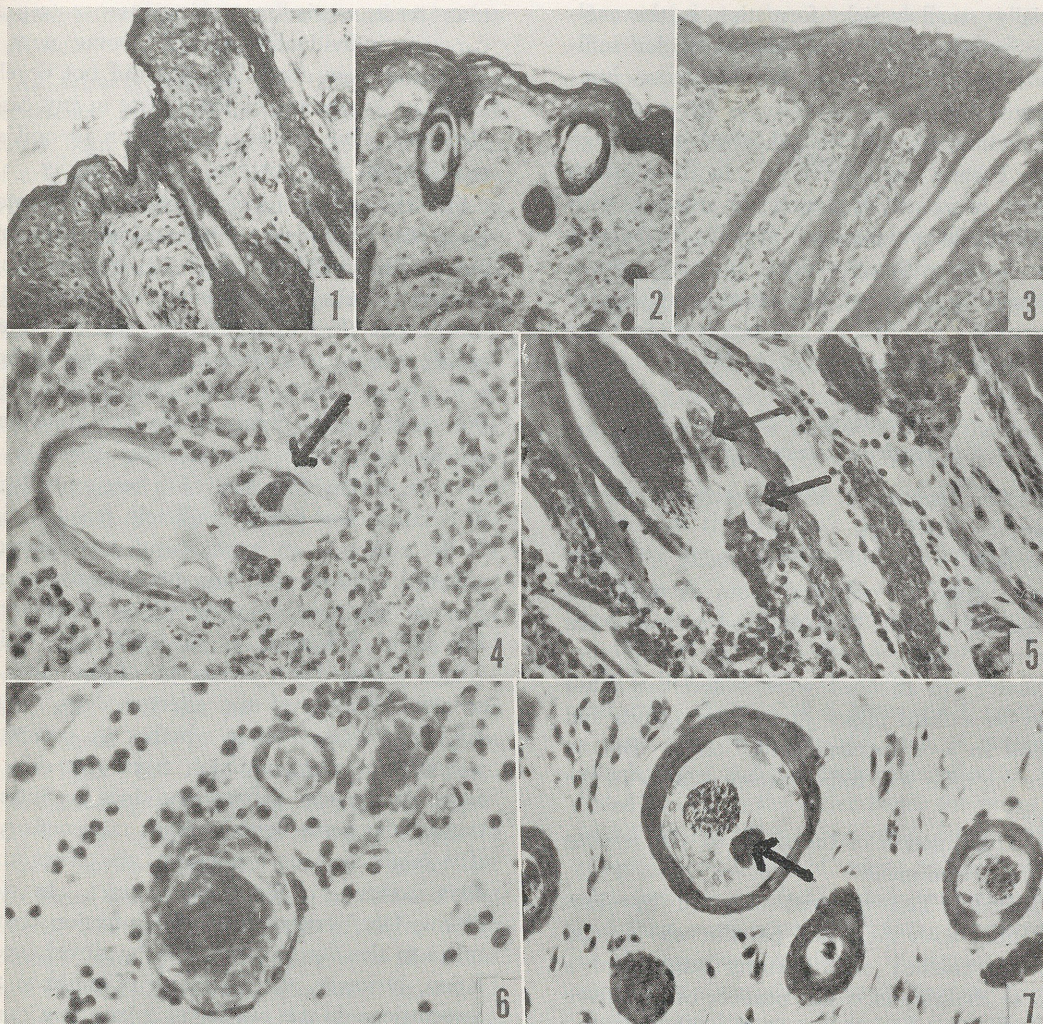
Result: Examination on the fetus and the non-suckling puppy failed to reveal the mite

in any tissues. On the other hand, mites were found in the skin of the 5 suckling puppies, however, it was negative in the internal tissues and organs. It seems that skin infestation of the mites will occur directly from skin of affected dam to skin of puppy after birth. In all these cases were not found the lesion of a clinical form, except No. 233 which showed a slight lesion as redness in Indian bean natural size only on the inside of the thigh. The number of the mites in 1 cm² area of the skin was from 2 to 94 and that in the lesion was not larger than other parts, as was shown in Table 4. Detected parts of the mites in the skin were around the upper and lower lip, sides of the muzzle, around the eyes, temporal region, fore legs, abdomen, lumbar, thigh and around anus. The life stages of the mite in these cases were adults alone or adults and eggs. In most cases which invaded the follicles were found microscopically near the surface of the skin. Though there were found no visible lesions, in the skin, dilation of the small vessels throughout the cutis and occasionally swelling of the prickle

Table 4. Showing the number of mites present in 1 cm² of skin and presence of mites in the skin tissue of puppies suckled from the demodectic dam

Part of skin examined	Animal's number														
	178			179			180			181			233		
	Life stage of mites														
	A.	Y.	E.	A.	Y.	E.	A.	Y.	E.	A.	Y.	E.	A.	Y.	E.
Upper lip	22	1	6	46	1	1	79	10	4	+			89	5	3
Lower lip	11	0	4	17	2	0	+			2	0	0	+		
Muzzle	+			+			+		+	37	2	4	+	+	+
Around eye	0	0	0	4	1	0	0	0	0	0	0	0	8	0	0
Temporal	+			6	0	0	+			1	0	0	-		
Fore leg	9	0	0	19	2	7	15	1	2	9	0	2	+		
Abdomen	2	0	0				4	0	0	0	0	0	9	0	1
Lumbar	-			-			-			7	1	0	32	0	0
Thigh	7	0	0	-			3	0	1	2	0	0	7	0	0
Around anus	+			+		+	+			15	1	3	4	0	0
Back	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A: Adults, Y: Young adults, E: Eggs



EXPLANATION OF THE PLATES

1. Swelling of the prickle cells. $\times 100$.
2. Vesicle formation in the malpighian layer. $\times 100$.
3. Suppuration of epidermis. $\times 100$.
4. & 5. Puncture and cellular infiltration of the hair follicle, mainly histocytes, lymphocytes. After 2 days. $\times 400$. \rightarrow Mites.
6. Collection of histocytes and a few eosinocytes surrounding blood vessels (in the infiltration around). $\times 400$.
7. Mite in the hair follicle of the part without lesions. $\times 400$. \rightarrow Mite.

cells were microscopically detected (Photo. 1). Partially small vesicle formation in the malpighian layer, (Photo. 2) slight cellular infiltration mainly consisted of histiocytes, lymphocytes and a few neutrocytes in papilla or surrounding hair follicles and rare haemorrhage in the dermis were observed. In these tissues bacteria were rarely found by staining with methylene blue. Detected mites in the follicles were found without relation to these lesions, however, in some of the affected follicles in the area of these lesions was observed the rupture of hair follicle (Photo. 4, 5).

3. Investigation of the infection through respiratory tract

a) Demonstration of the mites in the trachea of the diseased dogs.

Method: Survival and mortality of mites in the material obtained from secretion of larynx and trachea of 4 diseased animals were examined.

Result: In all cases were found 2-6 adult mites on a third upper part of the trachea and 1 adult in a bronchus of one cases. But no surviving mite was found except 1 mite in the trachea.

b) Transmission of the mites to the trachea by direct instilment.

Method: The material obtained by skin-scratching with the liquid paraffin was poured into the trachea of 3 healthy puppies of a litter of 15-days-old. The number of the given mites was about 200 to 500. The puppies were slaughtered at the end of a month.

Result: In any parts of the lung, liver, spleen, blood of heart, superficial and internal lymphatic glands and skin of the predilection, mites were not detected.

Discussion

On the evidence that internal invasion of mite is active and significant, Cánepa *et al.* (1945) reported that 10 healthy puppies of mange-free mothers received administration of the diseased skin pieces and the all test animals became mangy as early as two months after

administration. With one exception, these tests were carried out with litter-mates, apparently individually isolated; some pups of each litter were controlled and did not develop lesion or symptoms of the disease in any cases. However, the process of infection in oral administration was not based histologically.

On the other hand, reexaminations were made by Unsworth (1946) using 3 puppies and by Enigk (1948) using 12 puppies, however, the results obtained were negative. Enigk stated that the animals on the test of Cánepa *et al.* might have been infected with manges before test. Lucker *et al.* (1952) stated that the occurrence of infection via oral route cannot be accepted as proven, since there is no way in which the skin of a test animal can be demonstrated to be completely free of mites at the outset of an experiment, as Unsworth acknowledged in reporting his successful transfers of mites to skin by direct inoculation. But both of Cánepa *et al.* (1942) and Enigk (1948) reported the occurrence of the mites in mesenteric nodes of the affected dogs. Although it was not certain whether its occurrence was due to contamination from the skin or feces, or the true infestation, since its infestation on the tissue sections were not confirmed, if it might be true infestation, the mites must have invaded via the digestive tract. In view of this fact, writers made the infection test of mites to the digestive mucosa by direct inoculation, however, the migration of mites to the mesenteric node was not found. In a few cases, a very small number of mites was found on the inoculated part. But from the histological findings, it is difficult to consider that the mites may invade the digestive mucosa. According to the population and the proportion of mites in the skin and the lymph nodes of the clinical normal dogs and the diseased dogs suffering from demodectic mange which were described in our previous papers, and from the facts that the mites received the harmful influence in the digestive tract and that oral transmission test was negative and it cannot be accepted that infestation of the skin can occur via the oral internal route. Lucker *et*

al. stated also as follows — It is at present to assume tentatively that active inward migration can occur.

As primary reasons for this are reported occurrence of mites in the lymph nodes of newly born pups (Watson, 1948) and a report of a litter being born with demodectic mange (Kirk, 1949). Actually the finding of mites in the skin or internal tissues of a fetal puppy would constitute uncontrovertible proof of tissue penetration. — But Watson did not state exactly about the interval from the birth to the death of the pups and Kirk cited only a communication obtained from a client. In this connection, Enigk (1948), on failing to find mites in the skin and internal tissues of 18 still-born puppies, stated that intra-uterine infection such as reported by Kirk could not occur. The writers found infestation in the skin of the sucking group in a litter divided into two groups but never in the non-sucking group of fetus. If this infection occurred via the oral internal route, it was too early to reach the skin, and it is not just to consider that this fact occurred only in these puppies among the same litter by the intra-uterine infection. For discussing the channel of inward migration to the lymph nodes or other organs of clinical normal dogs, it is a noteworthy fact that there was found rupture in some of the affected follicles in the areas of slight tissue lesions. Namely, it seems that, once the mites have reached the dermis, they may be carried into the lymph nodes via blood or lymphatic vessels. On this occasion, though it could not define whether rupture of hair follicles was caused by the mites alone, or was due to a prerequisite formation of abscess, from the findings of non-infested follicles in some parts of these lesions, probably these lesions of surrounding hair follicles might occur prior the rupture of the follicles, and from the findings of the slight inflammation, the mites may destroy the hair follicles in such a tender skin parts. And also as the stray invader, the mites may be able to destroy as well blood or lymphatic vessels as the hair follicle with inflammation, however it may be accidentally, since

the rupture of the hair follicle with partial inflammation in the normal dog is very rare. Further precise experiments are required on the process of the mite migration.

Summary

In relation to the infection modes of *Demodex folliculorum canis*, examination of the oral internal infection route, the intra-uterine infection and of the infection through respiratory tract there is no finding to support those routes.

1. Namely, it was found that most of mites degluted down by licking infected skins in the stomach and most of the mites in the digestive juice died within 1 day. No mites did migrate in the mesentric lymph node of 16 dogs transmitted to the digestive mucosa by direct inoculation, it could not prove (histologically) the invasion of the mites in the digestive tissue inoculated (by the tissue section). Examination on the oral transmission of 5 puppies of 5 days old failed to reveal a mite in any tissues. Detection of mites of 6 fetus and 8 puppies in a litter born from affected dogs, 4 puppies of which were isolated from the affected dam, failed too. On the other hand, the mites were found in the skin alone of 4 other suckling puppies. Number of mites inhaled in the bronchus by a affected dog was very small and they were never surviving. Transmission to the trachea by direct instilment of mite contained materials using 3 puppies failed to reveal the mites in any organs and tissues at one month after inoculation.

2. Adults and eggs were found in the hair follicles of the puppy sucked from the affected dam for 16 hours after birth. The infected parts of skin were head, fore legs, abdomen, lumbar, thigh and around anus.

3. While no visible lesions were found in the skin, dilation of the small vessels, occasional swelling of the prickle cells, partial small vesicle formation in the malpighian layer, slight cellular infiltration of mainly histocytes, lymphocytes and a few neutrocytes in papillar or around hair follicles and rarely haemorrhage in the dermis were microscopically found.

Infestation of mites in the hair follicles were found without relation to this lesions, however some of the affected follicles in the area of these lesions had rupture of hair follicles.

4. The mites may be able to destroy hair follicles and lymphatic or blood vessels only in this condition, and as the stray invader the mite may migrate accidentally in the lymph nodes or other organs of the clinical normal dog.

Acknowledgements

We are grateful to Prof. H. Hosoya the laboratory of Veterinary Public Health of Nihon University for much advice and criticism.

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犬アカルス疹に関する研究

(3) 経口感染、胎盤感染ならびに気道感染の検討

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Demodex folliculorum canis の感染は経皮感染法によるものと一般に信じられている。しかしながら、健康犬および病犬の内部臓器とリンパ節に本虫が検出されるにおよび、その存在意義をめぐって、本虫の感染法の問題が再検討されつつある。すなわち Cánepa らにより経口感染説、Kirk らにより子宮感染説が提唱され、さらにこれ等臓器への侵入経路および侵入方法等が論議されているが、今日未だ意見の一致をみないようである。筆者らもこの問題について先報において論じて来たが、今回はさらに人工感染試験等の基礎的試験を行い、検討した結果、次の如き成績を得た。

1. 本虫の経口ならびに胎盤および経気道感染の可能性を支持し得る所見は認められなかった。

すなわち、経口的に摂取された虫体の大多数は胃液内で1日以内に死滅し、十二指腸においては生存虫体を検出できなかった。また経口的に虫を投与した5頭(生後5日)の仔犬および腸粘膜に含虫材料を直接接種した16頭の健康犬を検索したが、接種部における虫体の侵入像および皮膚その他の臓器における虫体の感染は全く認められなかった。

病犬の胎児6頭についても皮膚その他の臓器における虫体検索は陰性に終わった。

病犬より出産した1腹仔8頭を2群にわけ、1群4頭を分娩直後より母犬から隔離した結果、皮膚および臓器のいずれからも虫体を検出できなかった。反之、他の群4頭を2日間母犬に哺乳させたところ、全例の皮膚のみから虫体が検出された。

病犬の気管支に吸入される虫数は極めて少く、いずれも死虫であつた。

また3頭の仔犬の気管内に虫体を注入して観察を行つたが、感染は成立しなかった。

2. 出産後16時間に互り病母犬と接触した仔犬の毛包内に、成虫のみあるいは成虫ならびに卵を認めた。感染部位は頭部、前肢、腹部、腰部、腿、肛門周囲であつた。

3. 皮膚感染を認めたこれ等の仔犬は、肉眼的には無症状であつたが、組織標本では、一様に皮下血管の拡張、一部棘細胞の腫脹、マルピギー層における水泡形成、極めて稀に皮下出血が認められた。また乳頭層および毛包周囲には組織球、リンパ球ならびに少数の好中球を主とする細胞浸潤、一部毛包破壊が認められた。概して虫体はこれ等の病変部に關係なく寄生しているが、上記の病変部においては破壊した毛包が多く、この毛包は虫体寄生する例が多くあつた。

4. 本虫の体内移行性は極めて弱いものであつて、前述した病変が存在して始めて、本虫の毛包破壊が可能となるものと考えることが妥当であらう。リンパ節およびその他の臓器に検出される本虫の侵入方法は、破壊された毛包より本虫が真皮内に移行した後、破壊されているリンパ管および血管あるいは前述病変のため脆弱化した諸管の管腔に偶発的に迷入し、リンパ節あるいはその他の臓器に移行するものと考えられる。

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