STUDIES ON NO VISIBLE LESION REACTOR

I. CORRELATION OF FASCIOLIASIS TO TUBERCULIN REACTION

Motoji YAMAGUCHI, Ryoza AZUMA, and Yasuo KAWANISHI

National Institute of Animal Health, Ministry of Agriculture and Forestry, Tokyo, Japan.

(Received for Publication Sept. 27, 1955)

The so-called no visible lesion reactor (N. V. L. R) among cattle caused by tuberculous and none-tuberculous diseases, which causes questionable tuberculin reaction. DITTMAR⁽¹⁾ and FEDYUSHIN⁽²⁾ reported the fascioliasis as one of these conditions. If this be true, the reliability of tuberculin reaction will be lowered due to the frequency of N.V.L.R. in Japan, where there is a high percentage of liver fluke, throughout the country. Furthermore, the intradermal injection of fasciola hepatica antigen is made at the caudal fold in cattle in order to diagnose the fascioliasis parallel with the examination of egg incidence in the feces. Unfortunately this site is used for tuberculin injection in Japan, so that the presence of any mutual influences among these allergic antigens would occur in a restricted area.

This paper describes the results of studies on the correlation between tuberculin reaction and fascioliasis or fasciola hepatica antigen (F. H. A.).

Materials and Methods

1. Animals

Twenty-five male white rabbits weighing about two kilograms, and in nutritionally good conditions, were divided into four groups and infected with tubercle bacilli or metacercaria of liver fluke as described in each experiment.

Field trials on cattle were done in C prefecture of the Kanto district.

2. Metacercaria of liver fluke.

In the first half of the experiments, metacercaria were cultured in this laboratory and in the last half they were kindly given by Dr. Syozo WATANABE, Hokuriku Branch of the National Institute of Animal Health.

The method of keeping the metacercaria is as follows:

About fifty imago were collected from the liver of infested cattle at the slaughter house in Tokyo, and the eggs were placed in water of Petrie dishes after the incision of the ovaries. They were incubated at 25°C for 10 to 14 days and hatched to miracidium. The shell of *Lymnaea truncatula* collected from a brook neary this laboratory, were infested with these miracidium for a period of 40 to 80 days, in a water tank. Several corn leaves were thrown into the tank to enable them climb up and encapsulate on the leaves.

These metacercaria were inserted in carrots and optionally fed to rabbits.

3. Fasciola hepatica antigen

Lot No. 20 of ONO's diagnostic antigen for fascioliasis made by Tokyo Viscera

Chemical Company were used for the experiments.

4. Tuberculin

Two strains of bovine and one strain of human tubercle bacilli were used for preparing Sauton-tuberculin.

5. Tubercle bacilli

A four-week growth on Löwenstein-Jensen's medium of the strain "Bovine 10" was injected into the rabbits, intraperitoneally.

Methods used in the experiments will be mentioned under each experimental result.

Results

A. The two intradermal reactions in the rabbits infested with liver fluke

Nine of the rabbits were made to swallow about twenty-five to seventy metacercaria, floating in saline solution, through a pipet, and the transition of both intradermal reactions by F. H. A. and tuberculin were observed. The hair on the back of the rabbits was clipped off and 0.1cc tuberculin diluted with 100 volumes of saline was injected into the skin on the left side, and F. H. A., on the right side.

Both reactions were measured twenty-four hours after injection, and the average titer of each was taken into the readings.

To reduce the remaining effect of both allergic antigens, the injection site, on the back, was changed weekly.

After nine weeks the rabbits were sacrificed and the visceral changes due to the of liver fluke infestation were examined. The result of the experiment are shown in Fig. 1.

Fig. 1. Intradermal Reactions of Tuberculin and *Fasciola hepatica* Antigen in Lapine Fascioliasis.

Notes: 1. Curves are shown the average induration of 9 rabbits.
2. Tuberculin and F.h. antigen are injected on both sides of rabbits' backs.

The tuberculin reactions in both the experimental and the control groups remained almost negative throughout the period. On the other hand, the reaction of F.H.A. in the experimental group was stronger than in the control group, but this reaction was irregular, even in non-treated control animals.

Then the precipitin reaction described by URQUEHART⁽³⁾ was used to follow the infestation of

liver fluke, but the specificity of the reaction was, unfortunately, not observed.

From this result, it was concluded that the infestation by liver fluke and the repeated intradermal injection of F. H. A. did not give sufficient influence to make the tuberculin reaction positive.

B. Intradermal reaction of the rabbits infected with tuberculosis after infestation with liver fluke

Five of the six rabbits were inoculated with a sterile saline emulsion of bovine tubercle bacilli, at the rate of two-tenth mg. per cc., by subcutaneous injection in the lower abdomen after they had been infested with liver fluke in the method described

above. The remaining one, was taken as a non-treated control.

The tuberculin reaction did not become positive until the injection of tubercle bacilli and the reaction of F.H.A. showed indefinite variations throughout the whole period.

C. Intradermal reaction of rabbits infested with liver-fluke after infection with tuberculosis

Five rabbits infected with tubercle bacilli (three died within one to two weeks after the inoculation) were later tested for both intradermal reactions up to four weeks

Fig. 2 Both Intradermal Reactions in Lapine Fascioliasis
Reinfected with Bovine Tubercle Bacilli

Notes: Same as Fig. 1

Fig. 3 Both Intradermal Reactions in Lapine Tuberculosis
Reinfected with Fasciola hepatica

Fig. 4 Both Intradermal Reactions in Rabbits
Infected with T.B. and F.h. Simultaneously

vious results, it may be supposed that the concurrency of tuberculosis and fascioliasis give an unfavorable influence to the vital condition of rabbits. The results are shown in Fig. 4.

E. Both intradermal reactions in cattle

Eighty-five cows in C prefecture were examined for parasite eggs in feces and both tuberculin and F.H.A. reactions were observed with the results as shown in Table 1.

Eggs of liver fluke were detected in one cow and paramphistomum in twenty cows. Although the tuberculin reaction was negative in all cows, neither interaction nor interference between the two antigens was observed.

thereafter. The surviving two were infested with liver fluke in the sixth week.

The tuberculin reaction progressed three to four weeks after the inoculation and continued throughout a period of ten weeks. The infestation by liver fluke, however, did not augment the tuberculin reaction and also, the intradermal reaction of F.H.A. showed varied results, the same as in former experiments.

D. Simultaneous infection of tuberculosis and liver fluke

It is regrettable that long term observation could not be made due to the death of two of the three rabbits simultaneously infected with tuberculosis and liver fluke, four weeks after the injection, and the remaining one, six weeks after.

From these and pre-

Table. I Both Intradermal Reactions in Cattle

Groups	Modes of Tests		Tuberculin		F. h. antigen		Egg of F. h.	Egg of Paramphistomum
	Left caudalfold	Right caudalfold	Neg.	Pos.	Neg.	Pos.		
1	Tuberculin single	F. h. antigen single	10	0	3	7	0	3
2	Same as above	T. B. lin F. h. antig. simultan. inject. (sites separated 4 inches)	12	0	5	7	0	3
3	Same as above	T. B. lin 30 min. after F. h. antigen (sites separated 4 inches)	12	0	8	4	0	3
4	Same as above	T. B. lin 30 min. after F. h. antigen (same site)	16	0	9	7	1	5
5	F. h. antigen 72 hours after T. B. lin	None	11	0	6	5	0	6
6	Same as above	F. h. antigen 72 h. after the inject. of Left caudalfold	24	0	16	8	—	—
Total			85	0	47	38	<1	<20

Notes: Figures are shown the heads of cattle.

From these examinations, however, we can observe some evidences that: (1) the tuberculin reaction did not become positive by the injection of F. H. A.; (2) the infestation by liver-fluke and paramphistomum did not interfere with the tuberculin reaction; (3) even if both reactions were done simultaneously, they did not show any interference; (4) in one group of cows, the F. H. A. reaction injected seventy-two hours after the tuberculin injection, at the same site on the left caudal fold, was stronger by about five to ten mm. in swollen size than a single F. H. A. reaction on the right caudal fold, and (5) there was no coincidence in the results of intradermal reaction of F. H. A. and excretion of the eggs of liver fluke, and moreover, F. H. A. reacted unspecifically even in the case of paramphistomiasis.

Discussion

It seems that due to the mechanical disturbance and toxic effects of liver fluke and of tubercle bacilli, the vitality of the host declines, as shown in the experiment C and D. From these phenomena, we supposed that the so-called negative anergy in dermal reaction of the host would appear rather than hyperergy. It may, therefore, be assumed that the tuberculin reaction in cows, infested with liver fluke present only a few positive results.

It seems to be difficult to explain the so-called N. V. L. R. by the allergic relationship between tuberculosis and fascioliasis. Furthermore, N. V. L. R. may be blamed when no postmortem findings are shown in tuberculin positive cows besides the infestation changes due to liver-fluke.

However, no conclusion could be reached as to the absolute irrelativity between tuberculin reaction and fascioliasis, and non-specific reaction may occur in the cases of choledochitis, abscess, and purulent hepatitis in heavy infestation by liver fluke. In any case, the frequency of such phenomena are not so much in practical tuberculin field trials, and in this point, we agree to the opinion of BRATANOVIC⁽⁷⁾.

Summary

(1) Liver fluke infestation in rabbits did not afford any positive tuberculin reaction.

(2) Even in cows, correlation between fascioliasis or paramphistomiasis and tuberculin reaction did not seem to hold.

(3) F.H.A. does not seem to play any practical role in routine tuberculin reaction.

REFERENCES

- 1) DITTMAR, U.: *Inaug. Diss. Hannover*, 1952.
- 2) FEDYUSHIN, V. P., and A. I. UTESHEV.: *Veterinariya* (Moscow), **29**, (1952) Quoted by *J. Amer. Vet. Med. Ass.*, **122**, 58. (1953)
- 3) URQUHART, G. M., W. MULLIGAN, and F. W. JENNINGS.: *J. Inf. Dis.*, **94**, 2 (1954)
- 4) HEDSTRÖM, H.: *Studier över s.k. Hudtuberkulos Hos Nötkreatur*, Gernandts Boktryckeri, Stockholm. (1949)
- 5) HOLTH: Summary in *Vet. Bull.* 493. (1939)
- 6) HASTING, E. G.: *J. Amer. Vet. Med. Ass.*, **82**, 565 (1933)
- 7) BRATANOVIC, U. E.: *Bull. Off. Internat. Epiz.*, **42**, 278 (1954)

無病巢陽性反応に関する研究

I. 肝蛭症とツ反応の関係について

山口本治・東 量三・川西康夫

(農林省家畜衛生試験場)

(昭和 30 年 9 月 27 日受付)

ツベルクリン反応を行う際に極めて紛らわしい偽陽性反応を起さしツ診断上の一つの暗影を投じているものに所謂無病巢陽性反応なる概念がある。この中には各種結核菌に基くホモローガス・アレルギー、各種抗酸性菌に由るヘテロローガス・アレルギー及びミコバクテリウム性原因以外の各種疾病によつて生ずるとされている非特異性反応等が包含されている。これらの三者の中前二者に関しては抗原抗体反応としての現象論的説明は或程度首肯されるものがあるが後者の因果律的必然性には未だ納得のゆく説明が与へられていない現況にある。そこでこれらの非特異性反応が果して実際のツ反応上どれくらいの役割を演じているものかを知らんとして、先づその中の大綱をなすと思われる肝蛭症について研究を開始した。本症の無病巢陽性基因説はドイツ、ソヴィエト等で論ぜられており而も周知の如く本邦内畜牛の寄生率は極めて濃厚であり、若し真に肝蛭

症がツ反応に影響があるとすれば本邦のツ検診の信頼度は頗る稀薄となるからである。

先づ実験小動物中ツ反応の現れ方が比較的牛に類似する点の多い家兎を用い実験 A 乃至 D に示す様に結核・肝蛭の両疾患の各種組合せによる人工感染試験を行い主としてツ反応・肝蛭アンチゲン皮内反応の両者によりその相関作用を見出さんとしたが特に指摘する程の相乗・干渉等の現象は認められなかつた。

次で野外の実際の牛集団でこれらの関係を検討すべく実験 E に示す如き各種組合せによる両反応の相関を追究したがこの試験に於ても特に意義づける程の現象は確証出来なかつた。

以上の各種試験から肝蛭症は少くとも実際のツベルクリン検診の面には問題視する程の影響は及ぼさないものであるとの判決に到着せざるを得ない様な結果に終つた。