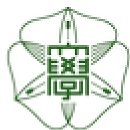

HOKKAIDO UNIVERSITY

Title	PRELIMINARY INVESTIGATIONS ON COMPLEMENT-FIXING ANTIGENS IN BOVINE TUBERCULOSIS
Author(s)	SHIMIZU, Kiheiji
Citation	Japanese Journal of Veterinary Research, 2(4), 157-167
Issue Date	1955-01-31
DOI	10.14943/jjvr.2.4.157
Doc URL	http://hdl.handle.net/2115/1663
Type	bulletin (article)
File Information	KJ00002372908.pdf

Instructions for use

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

PRELIMINARY INVESTIGATIONS ON COMPLEMENT-FIXING ANTIGENS IN BOVINE TUBERCULOSIS

Kiheiji SHIMIZU

Laboratory of Veterinary Hygiene and Microbiology,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan

(Received for Publication, October 2, 1954)

INTRODUCTION

In accordance with the decrease of tuberculin-reacting cattle, the problem of the no-lesion reactors has been frequently reported in almost the whole world. Also in Japan, especially in Hokkaido and in several prefectures in Honshu, the number of known no-lesion reactors has recently begun to increase. Consequently interest in the differential diagnosis between tuberculosis and so-called no-lesion reactors has been stimulated by the circumstance that the no-lesion reactors have caused some objections to the campaign against bovine tuberculosis.

Nowadays, however, excepting the intradermal tuberculin test, the complement-fixation test (C. F. T.) seems to be the most promising diagnostic method.

As to the diagnostic value of the C. F. T. in bovine tuberculosis, not many investigations have been performed. In 1944, HOLTH performed experiments on the utility of the C. F. T. in man and animal and concluded that it was inadequate as a diagnostic procedure. In 1949, TILGNER, however, reported that the C. F. T. gave satisfactory results in animal with advanced bovine tuberculosis, while of little use in animal with tuberculosis of non-severe stage. PROKŮPEK tested 540 blood sera of intradermal tuberculin positively reacting cattle by C. F. T. and recognized that 83.33% of C. F. T. positive cattle indicate the tuberculous lesions by postmortem examination. He considered the C. F. T. to be valuable in the detection of tuberculosis in cattle.

The author is confident that it depends upon the antigen and technique employed whether the C. F. T. indicated the excellent diagnostic value or not. In human tuberculosis, TAKEDA and SUGAI reported on the superior antigenicity of protein-nature substance from tuberculin and the increasing antigenicity by addition of lecithin. Also in bovine tuberculosis, recently KAMOCHI reported on the C. F. antigen in which he expressed himself on the superiority of the lipoidal substance of tubercle bacilli obtained by ether or absolute alcohol treatment. How-

ever, the titre of C. F. antibody obtained by the latter antigen seems to be not very high. These results may be due to the low antigenicity of the antigens. Accordingly further studies on the more specific C. F. antigen and suitable technique are needed.

Recently, the author made some comparative studies on the antigenicity of the several kinds of fraction of tubercle bacilli and recognized the C. F. T. as one of the good diagnostic measures for bovine tuberculosis. The results obtained from these observations seem to be of some value in several respects.

MATERIALS AND METHODS

1. Antigen

Antigens employed were prepared from dried tubercle bacilli of human and bovine type which were collected from glycerin broth and KIRCHNER or SAUTON media 30~40 days culture, after inactivation in waterbath at 70°C for 40 minutes (low temperature inactivation) and also from the bacterial residue of KOCH's O. T. (high temperature inactivation).

In this report, the antigenicities of the tuberculin, bacterial carbohydrate, protein, lipoidal substance and boiling supernatant were tested. Protein and carbohydrate antigens were dissolved in physiological saline in the proportion of 0.25 and 0.5 mg per ml respectively and used as the original fluid.

FIG. 1.

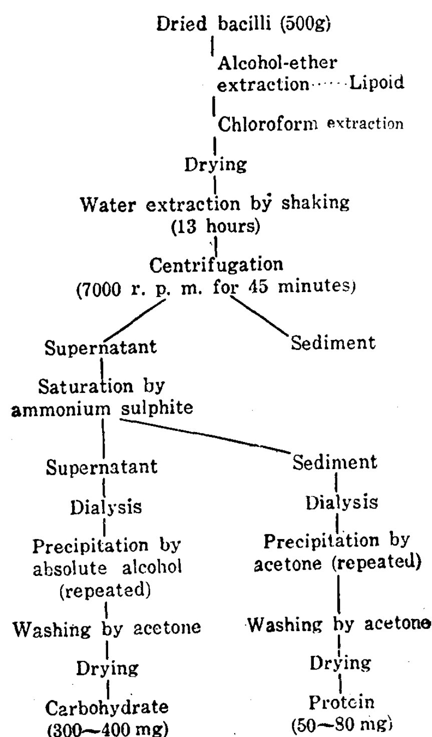


Fig. 1 indicates the method of preparing these antigens.

Tuberculin samples used in this experiment were synthetic ones which were obtained by courtesy of the Government Experimental Station for Animal Hygiene.

Boiling supernatant antigen was prepared as follows: the dried bacilli were emulsified in saline in the proportion of 20 mg per ml and centrifuged at the speed of 5,000 r. p. m. for 30 minutes, after boiling for 10 minutes and the supernatant fluid was used as the original one.

Also 2 kinds of lipoid antigens were prepared. The one was extracted by ether only, the other by alcohol and ether which were equally mixed. These were prepared following KAMOCHI's method: viz. 8 ml of the centrifuged supernatant ether, which were added to 0.2 g of the dried bacilli and extracted during 5 days period in room temperature, were poured

into 100 ml of physiological saline and homogeneous suspension were made by shaking as fast as possible.

2. Sera

Two tuberculous rabbit sera were employed. K 43 serum was obtained from the rabbit which was intravenously inoculated with 0.1 mg of living tubercle bacilli of bovine type and killed on the 44th day after inoculation. K 45 serum was also obtained on the 45th day after inoculation from the rabbit which received 1 mg of living human type of tubercle bacilli. These sera were used after inactivation at 56°C for 30 minutes in waterbath.

3. Techniques

Numerous methods of performing the test have been reported. In this country, IMAIZUMI has already reported the excellency of the BROWNING method. However, in almost all reports, there seems to be something lacking in their considerations on the techniques. In the course of the present study, the author made some comparison to find out which techniques are most suitable. It was recognized that CASALS' method is superior to KOLMER'S and 1 hour sensitization in 37°C waterbath, and gave the best results, so that the experiments reported in this report were entirely performed by CASALS' method.

EXPERIMENTAL RESULTS

First of all, in order to see the antigenic power, the titrations were carried out regarding each antigen. These results are stated as follows:

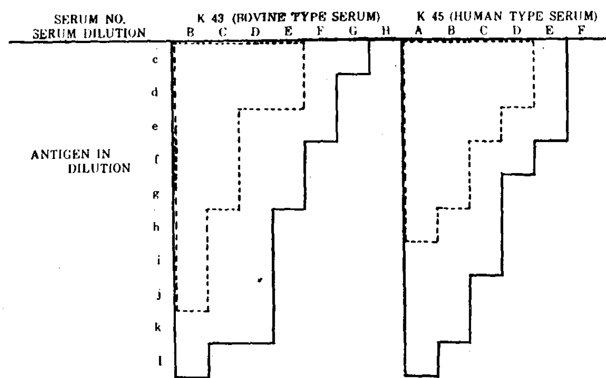
1. Antigenicity

Boiling Supernatant Antigens: The antigenicity of this kind of antigens seems to be very excellent. There are some interesting differences which may be seen from table 1 A and B. The inactivating temperature of bacilli exerts some important influences upon the antigenicity of human type antigen. High temperature induces a remarkable decrease of its antigenicity while such differences as caused by inactivating temperature were not at all observed in the case of bovine. This fact seems to be somewhat interesting as if it might suggest in part the essential differences between human and bovine type bacilli.

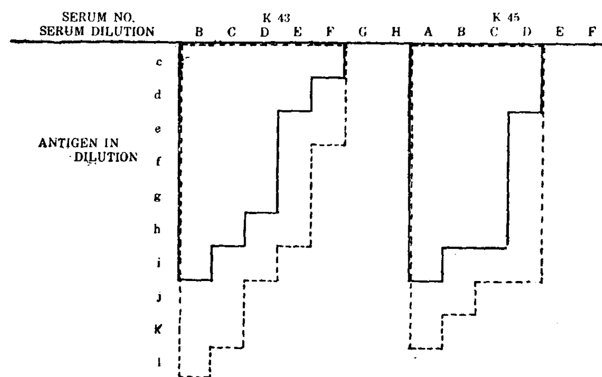
Protein and Carbohydrate Antigens: Table 2 indicates the antigenicities of the bacterial proteins. These antigens are also excellent but they are not so good as the boiling one because of the easy decrease of antigenicity even in the comparatively low dilutions. The influence

TABLE 1. *Titration of the Boiling Supernatant Antigens*

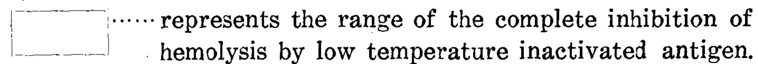
A. Human Type Antigen

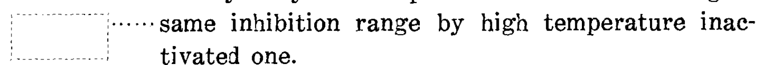


B. Bovine Type Antigen



Remarks A, B, C, D······indicate 1 : 8, 1 :16, 1 : 32······respectively.
a, b, c, d······indicate 1 : 1, 1 : 2, 1 : 5, 1 :10, 1 : 20, 1 : 40······respectively.


······represents the range of the complete inhibition of hemolysis by low temperature inactivated antigen.

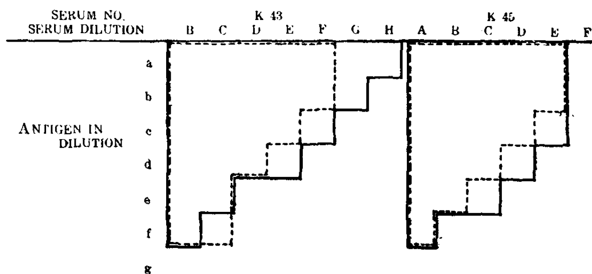

······same inhibition range by high temperature inactivated one.

by inactivating temperature in human type of antigen was also recognized but not so intense as in the boiling supernatant antigen of human type. In bovine type of antigen, conversely to the boiling supernatant antigen of the same type, some antigenic decrease by high temperature inactivation was seen, especially in the case of the homologous reaction.

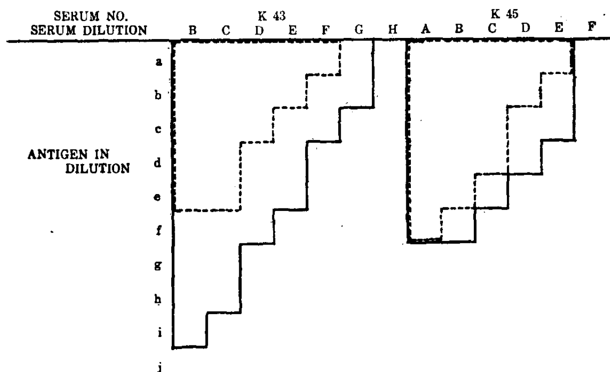
Bacterial carbohydrate fractions of the tubercle bacilli do not indicate any sign of antigenicity. Tests were repeated three times but no antigenicity could be demonstrated.

TABLE 2. *Titration of the Bacterial Protein Antigen*

A. Human Type Antigen



B. Bovine Type Antigen



Explanation of signs: As in table 1.

Lipoid Antigens: The results of titration are listed in table 3. From this, it may be seen that the antigenicities of the lipoidal antigens are not good, comparing with above-stated antigens. The influence of temperature upon antigenicity was also examined only in the ether extracted sample. A great decrease of antigenicity caused by high temperature inactivation was also recognized and especially in human type antigen, no sign of antigenicity was recognized at all when use was made of such dilution of sera in which the other antigens showed always very strong positive reactions.

Among the lipoid antigens, the alcohol-ether extracted one possessed the better antigenicity than the ether extracted one and also the lipoidal antigenic substance of bovine type are more easily extracted by alcohol-ether mixture than by ether only. On the other hand, the alcohol-ether extracted one has a considerably strong anticomplementary action while not in the ether extracted lipoid antigen.

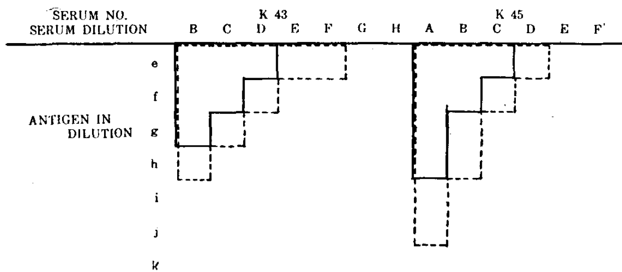
These lipoidal substances easily became granular and spontaneously precipitated, so it is difficult to keep homogeneous suspension, there-

fore, the antigenicities seem to be not stable.

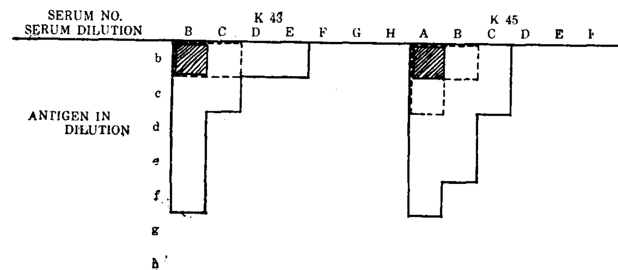
From these facts, the author considers that these antigens are of comparatively little significance.

TABLE 3. *Titration of the Lipoid Antigen*

A. Alcohol-ether Extracted



B. Ether Extracted



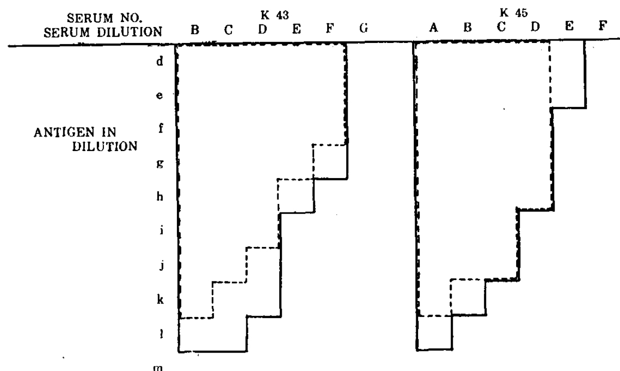
Remarks: ☐ ······ indicate the range for human type which show the complete inhibition of hemolysis (low temperature inactivation).

(dotted box) ······ same inhibition range for bovine type antigen (low temperature inactivation).

(hatched box) ······ same inhibition range for bovine type antigen (high temperature inactivation).

The other signs are as in table 1.

TABLE 4. *Titration of Tuberculin as a C. F. Antigen*



Remarks:

☐ ··· indicate the range for human type tuberculin which show the complete inhibition of hemolysis.

(dotted box) ··· same range for bovine type tuberculin.

The other signs are same as in table 1.

Tuberculin: As is indicated in table 4, both types of tuberculin represent good reactivity. In the dilutions lower than 1 : 20, they are slightly anticomplementary; however these antigenicities are fairly strong, almost the same as in boiling supernatant one.

Data on these antigenicities prepared from the low temperature inactivated bacilli which possess superior antigenicity to that from the high temperature inactivated ones, are conveniently summarized in table 5 A and B, although, in accordance with their anticomplementary

TABLE 5. *Summarized Data on the Titrations of Several Antigens Prepared from the Low Temperature Inactivated Bacilli and Tuberculin*

A. Human Type Antigens

SERUM NO. K 43 K 45
SERUM DILUTION B C D E F G H A B C D E F
ANTIGEN IN DILUTION

B. Bovine Type Antigens

SERUM NO. K 43 K 45
SERUM DILUTION B C D E F G H A B C D E F
ANTIGEN IN DILUTION

······Tuberculin	Antigen in dilution begins with	d
······Boiling supernatant	”	c
······Ether extracted lipoid	”	b
······Protein	”	a
······Ether-alcohol extracted lipoid	”	e

Explanation of signs the same as in table 1.

action, the beginning dilutions of each antigen are not the same. Accordingly, in these cases, the comparisons are not necessarily appropriate. However, from these data it may be roughly estimated that tuberculin, bacterial protein of bovine type and boiling supernatant antigen have almost the same strong antigenicities with each other. Ether extracted lipoid one, however, has only a weak antigenicity which corresponds to one half of the boiling one and especially in bovine type, its antigenicity is very weak. Also the lipoid antigen of human type which was extracted by alcohol and ether mixture, gives the same results with the ether extracted one, while that of the bovine type indicates the better antigenicity than ether extracted one.

The bacterial proteins also have good antigenicity, however their reaction ranges are comparatively narrow and they easily lose their antigenicity even in the comparatively low dilutions; besides their preparation is not sufficiently easy for general use.

The above investigations have shown that the boiling supernatant antigen from human type by low temperature inactivation and from bovine by both low and high temperature inactivation, and also tuberculin are pretty efficient as C. F. antigen.

2. Specificity of Various Antigens

In this case, only three kinds of antigens were tested, viz., tuberculin, boiling supernatant antigen and ether extracted lipoid antigen. All these antigens were prepared from the low temperature inactivated human type organisms. As is indicated in table 6, the sera tested were obtained from tuberculous cattle, laboratory animals which received artificial inoculation, no-lesion reactors in Hokkaido and from healthy cattle which showed negative tuberculin tests. As to the no-lesion reactors in Hokkaido, the authors have already made bacteriological investigations and recognized the human type bacilli infection in 7 cases out of 46 as already reported in this Journal (a part of them are not yet published). Even in the cases from which the human type bacilli were not isolated, the some influences exerted by tuberculous care-takers, or owners were considered. The author is therefore confident that the great majority cases of no-lesion reactors in Hokkaido are subject to the sensitization by human type bacilli to some extent.

Employing such sera, the specificity of these antigens was tested. The results are summarized in table 6.

TABLE 6. *Specificity of Some Antigens*

Sera Originating from	Lesions	Isolation of Bacilli	Kinds of Antigens Tested		
			Lipoid Antigen (Ether Extracted)	Boiling Supernatant Antigen	Tuberculin
Tuberculous Cattle	+	+	6/9	8/11	
Tuberculous Rabbits and Guinea Pigs	+	+	2/8	14/14	8/11
No-Lesion Reactors	−	+	1/6	4/6	2/6
		−	13/36	21/36	12/34
Healthy Cattle	−		4/27	0/27	1/25 10/25#

Explanations: A fraction indicates No. of positive/No. tested.
# indicates No. of suspicious/No. tested.

Among these three kinds of antigens, the boiling supernatant one is considered the most specific because of the highest percentage of positive reactions with tuberculous cattle (73%), laboratory animals (100%) and no-lesion reactors (60%) and of the absolutely negative reactions with normal healthy cattle which indicate negative tuberculin reactions.

In Japan, the tuberculous bovine sera are difficult to obtain, so the sera used in this case, were not fresh and some contamination was recognized as a result of storage at 5°C for several months without any addition of disinfectant. Accordingly a larger number of positive reactions may be expected if the fresh tuberculous bovine sera were employed.

On the other hand, the ether extracted lipoid antigen and tuberculin do not give so definite positive reactions as the former antigen and moreover they present positive or doubtful reactions with apparently healthy tuberculin negative cattle to a certain extent. It is clear that these are not so sensitive and specific.

From these data, the boiling supernatant antigen seems to be much the best one.

Consideration

In human and bovine tuberculosis, C. F. antigens of every kind are under examination, however insofar as the present writer is aware, no detailed systematic and comparative studies on these antigenicities according to type, bacterial fraction or influences by inactivating

temperature of original bacilli, etc. have ever been performed.

As to the boiling supernatant antigen of the bacterial body, there is no more than the one report by IMAIZUMI as far as the writer knows. In this report, there is offered no special criticism on this antigen.

Since many years ago, in our Laboratory, the boiling supernatant antigen was generally used as C. F. antigen of equine paratyphoid and good results were always obtained. Accordingly the author intended to apply it in the C. F. antigen of tuberculosis and at the same time to make some detailed experiments.

Recently, TAKEDA and SUGAI reported on the excellent antigenicity of protein-nature substances from tuberculin. From the writer's experiment, the bacterial protein was also recognized to be excellent, however, the best results were obtained by only the boiling supernatant of tubercle bacilli. This boiling supernatant antigen seems to be very excellent from the view points of the simplicity of preparation method, strong specificity and sensibility.

Ether or alcohol-ether extracted lipoid antigens are not excellent.

Although the test cases are not sufficient, it was with considerable interest that the author found the bovine type bacilli to have the strong antigenic principle which was not destroyed by the preliminary boiling treatment while this was not the case in the human type.

As to the specificity of the boiling supernatant antigen, it indicates considerably good result as far as tested, but more large scale experiments on the naturally infected or healthy or other diseased cattle sera are needed.

SUMMARY

The principal subject discussed in the present paper is the antigenicities of several kinds of antigens for the C. F. T. in bovine tuberculosis.

1. The antigenicities of next-mentioned human and bovine type of antigens were examined; Boiling supernatant, bacterial carbohydrate, bacterial protein, ether-extracted, ether-alcohol-extracted lipoids which were prepared from the dried bacilli preliminarily inactivated by temperature of 70°C and 100°C, and tuberculin.

2. The boiling supernatant antigens from the human type bacilli by low temperature inactivation and from the bovine type bacilli inactivated by both low and high temperature, equally indicated very

good antigenicity and specificity.

3. Bacterial proteins possess considerably good antigenicity, however, their reaction ranges was not so wide but that they easily lost their antigenicity by comparative low dilutions.

4. Tuberculin also indicated excellent antigenicity but the non-specific reactions were sometimes recognized.

5. Ether-extracted lipoid antigens are not recommendable ones on account of their weak antigenicity, especially in the one from bovine type, and on account of non-specific reactions.

6. Bacterial carbohydrate antigens do not have any antigenicity at all.

The author is debted to Professor HIRATO, the chief of this Laboratory for his helpful advice and many suggestions in this work and also would express his thanks to the members of the Government Experimental Station for Animal Hygiene and Dr. M. ENDO, the member of the National Institute of Health of Japan, Tokyo, for their willingly supplying materials.

REFERENCES

1) HIRATO, K. & K.SHIMIZU (1953): *Vet. Res.*, **1**, 1 & 148 (Japanese with English summary).
2) HOLTH, H. (1944): *Skand. Vet. Tidskr.*, **34**, 577 & 641 [*Vet. Bull.*, **19**, 645 (1949)].
3) IMAIZUMI, T. (1936): *Hokkaido J. Med. Sci.*, **14**, 897 (Japanese).
4) KAMOCHI, G. (1951): The Newest Veterinary Science (Edit. by OCHI, Y. & Y. OKUBO), Tokyo, p. 145 (Japanese).
5) PROKŮPEK, K. (1946): *Cas ceskoslovensk Vet.*, **1**, 449 [*Vet. Bull.*, **17**, 584 (1947)].
6) TAKEDA, T. & T. SUGAI (1934): *Jap. J. Exp. Med.*, **18**, 1353 (Japanese).
7) TAKEDA, T. & T. SUGAI (1935): *Ibid.*, **19**, 1665 (Japanese).
8) TILGNER, K. (1949): *Mh. prakt. Tierheilk.*, **1**, 221 [*Vet. Bull.*, **21**, 656 (1951)].