# Bovine Tuberculosis: 1ts Presents Status of Occurrence and Diagnosis in Japan

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Bovine tuberculosis, an important infectious disease of cattle caused by *Mycobacterium bovis*, is not only a potential menace for animal industry, but also a zoonosis transmissile from animal to man. From these reasons, the eradication program has been advanced to control this insidious disease in many advanced countries.

In Japan, the law for the prevention of bovine tuberculosis was enacted in 1901, employing test and slaughter method by means of the subcutaneous tuberculin test as a diagnostic method. In 1948 the intradermal caudal fold test was employed instead of the above-mentioned method.

The results shown in Table 1 indicate that the incidence of tuberculin positive reactor has reduced from 0.39 per cent in 1952 to less than 0.02 per cent at present, and the promising advance in the eradication program.

Nevertheless, a few cases of the outbreak of this disease have sometimes been reported because it is impossible to detect all tuberculous cattle with the diagnostic methods now available.

This brief report describes the problems in reference to the recent cases of the outbreak and diagnosis of bovine tuberculosis in Japan.

**Table 1. Number of cattle examined and removed by tuberculin test in Japan (annual results)**

| Year | Total number examined (A) | Number of reactors removed (B) | B/A% |
|---|---|---|---|
| 1952 | 258653 | 1001 | 0.39 |
| 1953 | 312091 | 885 | 0.29 |
| 1954 | 415834 | 947 | 0.23 |
| 1960 | 778179 | 1173 | 0.15 |
| 1961 | 817032 | 987 | 0.12 |
| 1965 | 997853 | 764 | 0.08 |
| 1966 | 995255 | 508 | 0.05 |
| 1967 | 1105619 | 313 | 0.03 |
| 1968 | 1217020 | 261 | 0.02 |
| 1969 | 1328124 | 270 | 0.02 |
| 1970 | 1421874 | 277 | <0.02 |

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## Recent cases

Contrary to the alleged assumption, bovine tuberculosis spreads rather rapidly as indicated in the following cases:

In a herd, newly built in September 1965, 20 positive and one doubtful reactors among 32 cattle were detected in the tuberculin testing in April of following year, and in the retesting carried out 21 days later six positive reactors became negative and four negative ones became positive.

From these findings, all of the 32 cattle of this herd were condemned to be slaughtered.

As shown in Table 2, the post-mortem examination revealed the presence of tuberculous lesions in 28 cases including five negative reactors. In this outbreak, a doubtful reactor with extensive lesions was regarded as the source of infection.

In another outbreak in Osaka Prefecture,

**Table 2. Results of tuberculin test in an infected herd of Gunma Prefecture**

| | | Results of retest | | | Total |
|---|---|---|---|---|---|
| | | − | ± | + | |
| Results of first tuberculin test | − | 7*(5)** 1*** | · | 4(4) | 11( 9) 1 |
| | ± | · | 1(1) | · | 1( 1) |
| | + | 6 (4) | 4(4) | 10(10) | 20(18) |
| Total | | 13 (9) | 5(5) | 14(14) | 32(28) 1 |

*: Number of cattle slaughtered
**: Number of cases in which microscopic tuberculous lesion was proved
***: Number of cases in which suspected lesion of tuberculosis was proved
Judgment of tuberculin test was described in the text

23 positive and 11 doubtful reactors were present among 88 cattle in a herd of having been free from tuberculosis in February 1969.

According to the owners' pleading, he introduced a cattle into his herd in September 1968 from a herd where all of the 28 cattle were slaughtered due to tuberculosis. Extensive tuberculous lesions were proved in this doubtful reactor which was regarded as the source of infection.

The two above-mentioned eases indicate that the disease spreads rapidly when cattle in an advanced tuberculous stage are introduced into the herd, and sometimes an intolerable economic damage to the owner is incurred.

The bronchial and mediastinal lymph nodes were the main site of tuberculous lesion in the above cases, and this fact suggested the infection via the respiratory route. From the viewpoint of epidemiology cattle in an advanced tuberculous stage with an extensive lesion of lung are a very dangerous source of infection.

## Diagnosis

*Tuberculin test*

Tuberculin test has been employed universally as the most reliable diagnostic method easy to perform. The single caudal fold method is used in the test decided by law in Japan.

Heat concentrated synthetic medium tuberculin (OT) which is currently used for diagnosis of this disease in Japan contains 50,000 international units in 1.0 ml.

After intradermal injection with 0.1 ml of OT into caudal fold, the reading of skin thickness at the injected site is made at 72nd hours. The reactor with the increase of 5 mm or more in skin thickness is judged to be positive and the one with the increase of less than 3 mm is negative. The other reactor judged doubtful is retested two months later.

The positive reactor is ordered to be killed and the money for compensation of the animal is paid by law in Japan.

False positive and false negative reactors may be mentioned as the troublesome, annoying problems encountered in the practice of tuberculin testing. It is a well known experience that the incidence of false positive reactors among tuberculin positive ones increases when bovine tuberculosis has been successfully eradicated.

In relation to the non-specific reactor problem, the possibility of sensitization of cattle with aicid-fast bacilli other than *M. bovis* has been pointed out. At present situation in Japan there is no outbreak of avian tuberculosis of fowls and a few cases of the outbreak of Johne's disease is different from that of foreign countries.

Skin lesion, cited as an important cause of false positive reactions, was not reported in Japan until Shimizu et al.[7] recently observed two cases of false positive reactors in Hokkaido.

The significance of atypical mycobacteria as potential pathogens for domestic animal was emphasized by Kazda[2], and these microbes are stated to be a part of the normal soil flora.

From these reasons, much attentions must be paid to the role of atypical mycobacteria which exist in the surrounding of cattle as a potential tuberculin sensitizer.

Sometimes, false positive reaction occurs in connection with the disease having granulomatous lesion such as actinomycosis, actino-

bacillosis, other fungus diseases or abscess.

In order to differentiate specific reaction from non-specific ones the employment of diluted tuberculin has been recommended, but this problem must be treated cautiously after extensive field tests. At present, the comparative cervical test is recommended for this purpose.

As summarized in Table 3, the results of

**Table 3. Results of comparative method in tuberculous cattle at Sado district**

| Caudal method | Cervical method | Comparative method 0 | 0–0.9 | 1.0–1.9 | 2.0–2.9 | 3.0–3.9 | 4.0–4.9 | 5.9–5.9 | 6.0* |
|---|---|---|---|---|---|---|---|---|---|
| | − | · | · | · | · | · | · | · | · |
| − | ± | · | · | · | 1/1 | 0/1 | · | · | 1/2** |
| | + | · | · | · | 0/2 | 1/5 | 2/6 | · 0/1 | 3/14 |
| | − | · | · | · | · | · | · | · | · |
| ± | ± | · | · | · | 2/2 | 1/1 | 0/1 | · | · 3/4 |
| | + | · | · | · | 2/3 | 1/1 | 2/6 | 3/6 | 3/5 11/21 |
| | − | · | · | 0/1 | · | · | · | · | · 0/1 |
| + | ± | · | · | 1/1 | · | 1/1 | · | · | · 2/2 |
| | + | 1/1 | · | 2/2 | 0/1 | · | 1/3 | · | 11/12 15/19 |
| | | 1/1 | · | 3/4 | 5/9 | 4/9 | 5/16 | 3/6 | 14/18 35/63 |

* Increasement in skin thickness (mammalian-avian) mm

** Number of tuberculous cattle/number of slaughtered cattle

the comparative test applied in the outbreak in Sado district were in accordance with the results by Jong et al.[1] who pointed out that each test has some limitations in its efficiency as a diagnostic method.

False negative reaction is a perplexing problem for the eradication program because the detection of false negative reactor is difficult employing the tuberculin test now available. In this meaning we must use some ancillary methods as described in the following.

When repeated tuberculin tests were carried out, the reaction pattern of the tuberculin test as the delayed type hypersensitivity sometimes changed into that as the immediate type hypersensitivity. This noticeable phenomenon[4] was

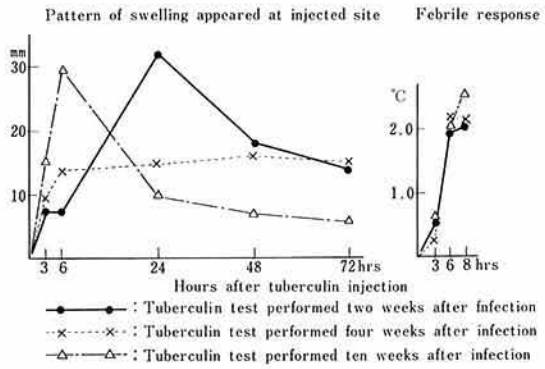


Fig. 1. Results of repeated tuberculin test carried out in experimentally infected cattle

observed experimentally and naturally infected cattle as shown in Fig. 1.

The febrile response with the rise of body temperature over 40°C was observed in about one half of the tuberculous cattle when tested intradermally. This febrile response appeared from 6 to 10 hours after tuberculin injection as in the case of the thermal tuberculin testing. This finding was useful for the detection of false negative reactors from infected herd.

As stated generally, tuberculin testing must not be employed as a diagnosis of individual cattle, but as a herd test. A practitioner must be experienced and have sufficient knowledge concerning the history of the herd examined, in order to increase the accuracy of the tuberculin test.

*Serological diagnosis*

Passive hemagglutination (PHA) test, its hemolytic modification (HL) test, and complement fixation (CF) test were examined by Nemoto et al.[5] for the diagnosis of bovine tuberculosis.

It has been proved that the diagnostic values of these tests are rather limited because serum titers of tuberculous cattle are rather low except in the advanced cases, and it is difficult to differentiate tuberculous cattle from healthy ones by means of these tests.

Recently Yugi et al.[10] examined the kaolin agglutination (KA) test[9] with the simultane-

**Table 4. Comparison of serological tests**

| Serological tests | | Number of cattle examined | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tuberculous cattle 47(%) | | Healthy cattle 134(%) | | No-visible leason reactor 27(%) | |
| PHA | Negative | 14 | 29.7 | 116 | 86.6 | 15 | 55.6 |
| | Positive | 33 | 70.3 | 18 | 13.4 | 12 | 44.4 |
| HL | Negative | 17 | 35.8 | 109 | 81.4 | 10 | 37.1 |
| | Positive | 30 | 64.2 | 25 | 18.6 | 17 | 62.9 |
| KA | Negative | 5 | 10.6 | 128 | 95.5 | 24 | 88.9 |
| | Positive | 42 | 89.4 | 6 | 4.5 | 3 | 11.1 |
| CF | Negative | 31 | 65.9 | 128 | 95.5 | 17 | 62.9 |
| | Positive | 16 | 34.1 | 6 | 4.5 | 10 | 37.1 |

ous application of other serologic methods as shown in Table 4. The results of PHA, HL and CF tests were in accordance with the ones by other investigators. KA test, which is promising in this study, must be examined further to achieve practical application.

Blood samples were collected before tuberculin testing and two weeks later to examine the effect of tuberculin injection upon serum titers. Low or negative serum titers of tuberculous cattle before tuberculin injection became to be positive showing about four fold rise in accordance with the phenomenon described by Mallmann et al.[3]

When the dramatic effect of tuberculin injection upon serum titers of tuberculous cattle became evident, this anamnestic-like reaction could be employed advantageously to detect false negative reactors or for the confirmation of the results of tuberculin testing.

*Post-mortem examination*

A detailed post-mortem examination must be carried out in order to prove the presence or absence of tuberculous lesion and to know the nature of the lesion. The information obtained from this examination is also useful for the reading of further tuberculin testings in the herd.

Microbiological examination should be carried out to elucidate the causal agent. The identification of the isolates is important from the viewpoint of epidemiology. When acid-fast bacilli other than *M.bovis* are isolated, the risk of further spreading of this disease would be limited.

For the isolation of *M.bovis* the egg media containing pyruvic acid[8] was reported, but the author[6] employed Ogawa media containing Tween 80 at the concentration of 0.5 per cent and has obtained satisfactory results.

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