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ABSTRACT

AN EPIDEMIOLOGIC STUDY TO EVALUATE METHODS
OF DETECTING BOVINE TUBERCULOSIS

By David Robert Towar

The object of this project was to determine if a combination of the presently available tests for tuberculosis would give results that could help differentiate animals infected with _Mycobacterium bovis_ from non-infected animals. The combination of tests included the comparative cervical intradermal tuberculin test and a passive hemagglutination test of serums collected before and following the caudal fold intradermal tuberculin test of all animals in all herds in a selected geographical area.

Results substantiated previously observed errors inherent in each of these methods used as a single or in a combined test of a single animal. Both supplemental methods reduced the number of animals having a false indication of infection with _M. bovis_. Neither method, however, was totally effective in identifying all animals with _M. bovis_ infection. There was too high a degree of error for their effective use as single tests for the individual animal. The comparative cervical tuberculin test identified a large percent of the _M. bovis_ infected animals with signs of progressive disease. It also demonstrated a certain number of probable false positive results. There was a significant increase in post tuberculin serum titers from animals in _M. bovis_ infected herds. A high percentage of false positive results, however, eliminates this method as a single test for a single animal. As a combination, the

supplemental test methods were found to be effective for differentiating herd infections with M. bovis.

Seventy-one of 92 herds receiving caudal fold tuberculin tests had one or more animals showing a tuberculin response. Supplemental testing procedures rapidly reduced the number of suspicious herds to twelve. Five of the 12 herds were found to be infected with M. bovis. A combination of a positive comparative tuberculin test and a post tuberculin injection hemagglutination titer of 640 or higher was highly specific for M. bovis infected animals with demonstrable lesions at post mortem examination. This combination of tests, however, did miss some animals with M. bovis infection.

The use of the supplemental tests resulted in earlier established knowledge on the presence or the absence of M. bovis infections within a herd. Despite increased effectiveness with these procedures, epidemiological investigative procedures still should play an important role in arriving at a diagnosis.

AN EPIDEMIOLOGIC STUDY TO EVALUATE METHODS
OF DETECTING BOVINE TUBERCULOSIS

By

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INTRODUCTION

Since the initiation of the bovine tuberculosis eradication program and the resulting reduction of bovine tuberculosis, increasing difficulties in the interpretation of the intradermal tuberculin test have been encountered. These difficulties can be classified under two general headings:

1. Some tuberculous animals do not respond to tuberculin. This factor makes the assured elimination of tuberculosis from a large herd of cattle through use of the tuberculin test very difficult. Examination of the tuberculin test history of animals found to be tuberculous at slaughter indicates that the percent failing to respond has remained about the same over the years. This error is referred to as a Type II error.
2. Some non-tuberculous animals do respond to tuberculin. These animals are generally reflected by the no-gross lesion rate of slaughtered reactors which has increased over the years. This is a Type I error.

This latter problem, the non-specific reactor becomes more apparent as the final phase of bovine tuberculosis eradication approaches. With modern means of rapid transportation, bovine tuberculosis is readily transported. To eradicate bovine tuberculosis, there must be an effective means of detecting all infected animals before spread of the disease occurs. At present, the two major methods of locating infection are dependent upon advanced tuberculosis. Tracing diseased animals found at slaughter means the animal has undoubtedly exposed many others. It is also hampered by a lack of a satisfactory system of identification of the cattle population. The second method, screening the cattle population with the intradermal test, theoretically detects cattle with tuberculosis earlier. If this test were 100% accurate, tuberculosis eradication would be simply a matter of testing all cattle and reservoirs of bovine tuberculosis, and eliminating the animals positive to the test. However, development of further criteria is necessary for interpreting the results of the caudal fold tuberculin test, because (a) only a portion of the cattle population is tested by any screening method; (b) a portion of those tested will show positive results due to non-specific causes; (c) not all tuberculous animals respond to the tuberculin test; (d) not all tuberculous animals will have gross lesions at slaughter.

Completely negative results to the test are not a problem unless occurring in a herd where tuberculosis is known to exist. In all other cases the veterinarian is called upon to decide if the response to the tuberculin test is due to _Mycobacterium bovis_ or due to some other cause. To do this he must consider the individual animal's and the herd's history, the number and size of responses observed in the herd, the animal's physical condition, and the environment. If a decision cannot be made, a retest 60 days later using the same type test is the only recourse.

A further diagnostic test is needed. If such a test could accurately differentiate specific from non-specific responses, it would contribute immeasurably to the eradication program even though it might be a complicated and time consuming procedure. No such method has yet been developed.

Many other methods have been developed to aid in the diagnosis of tuberculosis. Some are as accurate as the caudal fold tuberculin test method. None are as simple or sufficiently more accurate than the caudal intradermal tuberculin test and have not been used widely in this country. It could be that the area of error for these various tests do not completely overlap, so that certain results for a group of tests on the individual or herd may improve diagnostic efficiency. In this paper an attempt is made to define the non-specific problem which is defined as sensitization responsible for responses to mammalian tuberculin in animals free of tuberculosis due to M. bovis, and to apply certain other testing methods in an effort to differentiate the specific from the non-specific responses.

The intradermal tuberculin test is the most common method used throughout the world and is the official test in the USA. It is used as the basic test in this project. Other methods are added in an attempt to clarify the caudal test results. A comparative intradermal tuberculin test similar to the English single intradermal comparative method is used to retest all animals responding to the caudal fold test. Inclusion of this test in this project was prompted by the high correlation between progressive mycobacterial disease and a positive comparative test (McGavin, 1963). In the study of the pathogenicity of atypical mycobacteria by the tuberculosis project personnel at Michigan State University, it was demonstrated that some Group III mycobacteria can cause a progressive disease comparable to bovine tuberculosis, some cause a progressive disease that terminates in a non-progressive type, while others produce no disease. Comparative tuberculin tests were applied to these animals at two month intervals during the course of the experiment. When the disease was in a progressive state, the reaction to mammalian tuberculin was generally at least 5 mm greater than that of avian tuberculin. When the reaction to mammalian tuberculin was less than 5 mm greater, or less than that for avian tuberculin, the disease was generally in a non-progressive state. Later studies have revealed exceptions to these findings.

Serologic procedures are in many ways much more desirable as testing methods. Testing of a sample of blood eliminates any possibility of influencing an animal's immunologic response by injection of a foreign substance. Tuberculin may be capable of inducing antibody formation when injected intradermally. Mallmann _et_ _al_. (1963a) found that a single hemagglutination (HA) test as a diagnostic tool was not satisfactory due to an anamnestic-like response following a tuberculin test. Preliminary results of tests of serums from two gross lesion cows naturally infected and two no-gross lesion cows indicated a four fold increase occurred only in the gross lesion animals. It was not consistently true in calves experimentally infected. If this anamnestic HA test is consistent in this respect in naturally infected animals, it has great potential as a field test for tuberculosis.

Disclosure of the presence of an agent in a herd capable of producing a non-specific hypersensitivity can be a great aid in explaining tuberculin responses in such a herd when *M. bovis* is not evident. Non-specific hypersensitivity has been reported due to *M. avium*, *M. paratuberculosis*, *M. tuberculosis*. These are generally localized factors causing hypersensitivity while the non-specific problem is more ubiquitous in scope. One factor probably causing hypersensitivity and seemingly also world-wide in occurrence is tuberculoid skin lesions. Many non-specifically hypersensitive reactors have clinical signs of skin lesions. In unpublished studies Willigan, McGavin, and Goyings (1963) found microscopic lesions indicative of tuberculosis in the prescapular lymph nodes of reactor animals with skin lesions on the forelegs. Later, Mallmann *et al*. (1963) isolated atypical mycobacteria from lymph nodes of N.G.L. tuberculin reactors both with and without skin lesions. Some of the Group III organisms were capable of inducing varying stages of disease when introduced as an aerosol or per utero. The isolation of atypical mycobacteria from tuberculin positive cattle that can produce disease and sensitivity to tuberculin in experimental calves, plus the relationship of skin lesions and prescapular lymph node granulomas, introduces a new concept in non-specific hypersensitivity. A skin lesion-lymph node granuloma syndrome may be represented by many stages with clinical skin lesions representing one stage. Almost all reports of a connection between skin lesions and non-specific hypersensitivity are biased. Most reports on incidence of skin lesions are from tuberculosis reactor slaughter reports or from herds, cattle, or areas where a non-specific response or a skin lesion problem has arisen. This bias could be avoided by examining all animals in a geographical area for skin lesions.

The tuberculosis research team at Michigan State University proposed a field survey of the incidence of non-specific tuberculin reactions, the application of the comparative test for detecting progressive disease, and the application of the anamnestic hemagglutination test to both tuberculin-positive and negative animals. A conference was arranged with Dr. R. M. Scott[1], Michigan Bovine Tuberculosis Project Leader, to plan such a survey. Later, a second conference was held with Dr. A. F. Ranney[2] who gave his Department's support to such a project to be conducted by the author and Dr. C. A. Niles[3] subject to approval of the Federal Veterinarian in Charge, Dr. Asa Winter[4], and the State Veterinarian, Dr. John F. Quinn[5].

The data and conclusions drawn from the resulting project are applicable only to a small portion of Michigan. Conditions throughout the country where tuberculin test problems exist are sufficiently similar that collection of these data could establish a base of normal information that is essential for further epidemiological investigations. This project was undertaken in an effort to answer questions regarding tuberculin hypersensitivity and to establish an improved testing procedure to help guide the veterinarian in the evaluation of situations where the caudal fold test needs to be supplemented.

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Figure 1. Tuberculosis reactor rate since 1917.

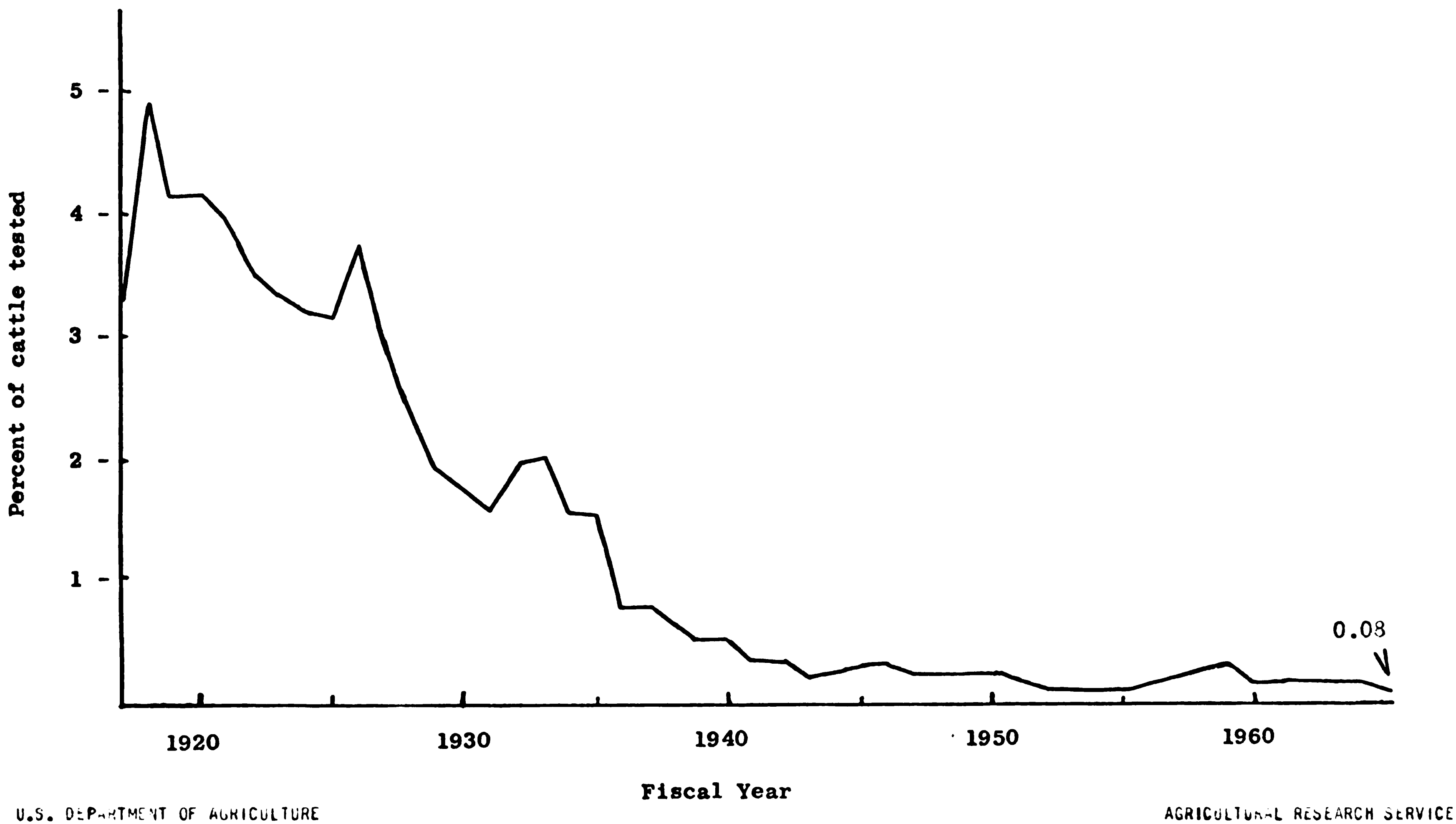


Figure 2. Progress in tuberculosis eradication since 1916
as shown on logarithmic graph

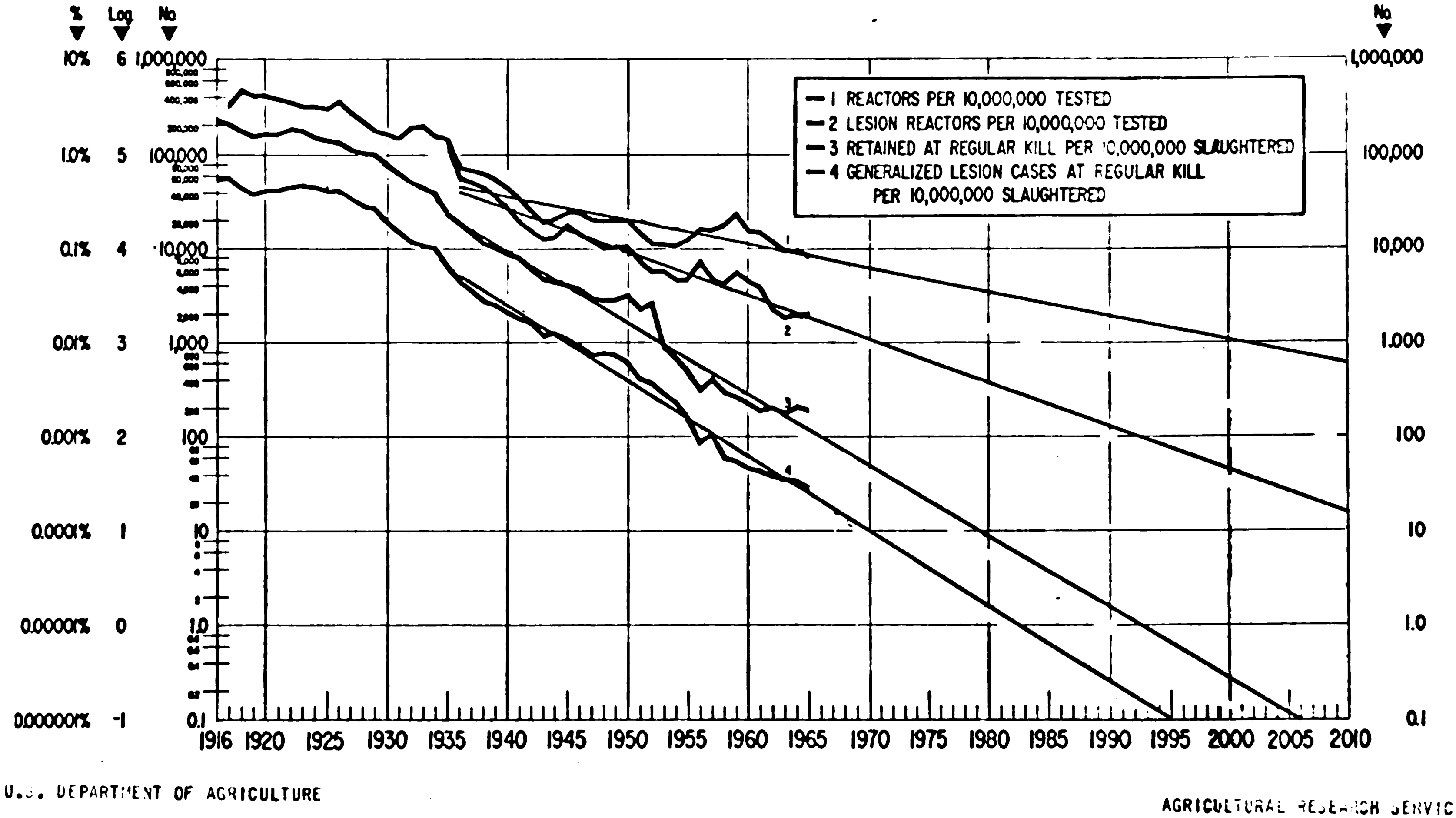
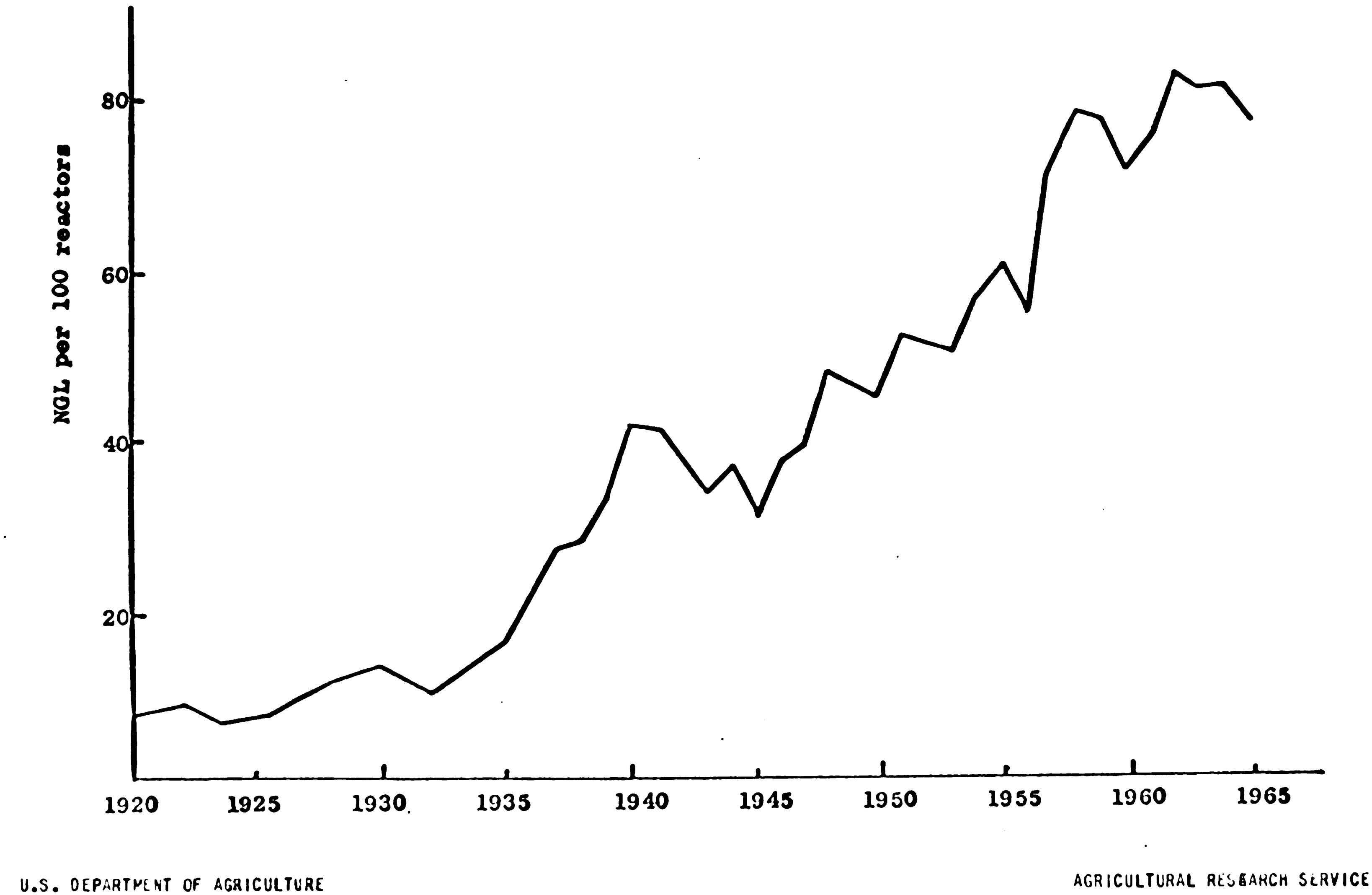


Figure 3. No gross lesion rate of tuberculin test reactors since 1920.



LITERATURE REVIEW

Tuberculin has been used as a diagnostic agent in the U.S.A. for tuberculosis in cattle since 1892, just two years after its discovery by Robert Koch. At that time, Pearson (1892), who had worked in Koch's laboratory, tuberculin tested a herd of cattle in Pennsylvania. His subcutaneous injection of Koch's old tuberculin resulted in an elevation of temperature of many animals, all of which showed signs of tuberculosis when slaughtered. Further testing indicated a high correlation between an animal's response to tuberculin and its infection with *Mycobacterium bovis*. Many other methods of applying the tuberculin test have since evolved; however, for ease and convenience the intradermal test has been shown to be superior to other methods.

By 1917, the U. S. Government was sufficiently convinced of the efficacy of the tuberculin test to inaugurate a bovine tuberculosis eradication program based on this test. The subcutaneous thermal test was used in the early 1920's but the intradermal test applied to the caudal fold was initiated which facilitated a more rapid tuberculin test coverage of cattle herds without sacrificing reliability. In 1934, the Bureau of Animal Industry introduced a heat inactivated, concentrated tuberculin, the filtrate of organisms grown on synthetic media. This replaced the animal peptone medium tuberculin and resulted in a more standardized product. This product and method of test is still used today.

Before the United States bovine tuberculosis eradication program started in 1917, the incidence of tuberculosis in the cattle population was estimated from slaughter reports to be about 5%, though some estimates ranged as high as 8 percent. It can be seen from Figure 1 that there was a rapid decline in the reactor rate until the early 1940's. Since that time the rate appears to have assumed an endemic plateau of about 0.2 percent. Figure 2 is a logarithmic graph illustrating numbers of animals retained and condemned due to tuberculosis at slaughter since 1916. The reactor rate has had little effect on the rate of reduction of animals found with lesions of tuberculosis on slaughter. If infection with M. bovis were the only cause of tuberculin hypersensitivity and if it could be assumed that the proportion of animals in early and later stages of the disease remained roughly constant, then these two graphs should be quite similar.

The reactor rate is based on tuberculin hypersensitivity. However, M. bovis is not the sole agent responsible for inducing hypersensitivity, some diseased animals do not react to tuberculin and some animals reacting to tuberculin were found with no-gross lesions (N.G.L.) of tuberculosis. The early concern was with the failure to disclose all diseased animals, but as the N.G.L. rate began to rise (Figure 3), it was improbable that all N.G.L. reactors represented early tuberculosis infection.

In 1914, Hastings reported an increase in the N.G.L. rate in Wisconsin reactors when the testing program was widened to include more than just suspect herds. He also noted that the N.G.L. rate was inversely proportional to the number of reactors found in a herd. His conclusion at this time was that most of the no-lesion reactors probably had tuberculosis lesions not detected on routine autopsies. By

1924, he believed the accumulated evidence pointed to sensitization by something other than *M. bovis*. Hagen (1930) reviewed 10 years of the eradication program and stated that one would expect the decrease in bovine tuberculosis to be accompanied by a similar decrease in N.G.L. reactors but instead, the latter was increasing. The N.G.L. rate was not consistent throughout the U.S.A. It was quite low in some areas and as high as 32% in others. By 1965, the reactor rate had decreased to 0.08% while the N.G.L. rate for these reactors had risen to over 75 percent. This latter figure is misleading. Morehouse (1963) reports that about 30% of the lesions from tuberculin reactors submitted to the National Animal Disease Laboratories are due to causes other than tuberculosis. Many workers have thought that the increased numbers of N.G.L. reactors indicated an increased rate of non-specific hypersensitivity or a change in the test. To the contrary, Robertson (1963) has shown that the proportion of tests negative for animals actually infected with *M. bovis* (Type II error) and tests that are positive for non-infected animals (Type I error), is similar to that seen in early phases of the program. The increased "percent" of N.G.L. reactors is an incorrect conclusion based on improper statistical sampling. With the proper procedure the Type I error, the proportion of non-tuberculous animals that react, can be defined as a percent of the normal (non-tuberculous) population. Thus, it is evident that even after the last *M. bovis* infected animal is slaughtered there will be a certain percent of the cattle population that will still react to tuberculin. This has been observed by Stenius (1949) in Finland where *M. bovis* has been considered eradicated, yet 3% of the cattle population still react to tuberculin.

Determination of the cause or causes of the Type I error is necessary for determining the nature of non-specific hypersensitivity but not necessary for the eradication of tuberculosis. As already mentioned, Finland can claim eradication of bovine tuberculosis with an unexplained 3% level of hypersensitivity.

Many possible causes of non-specific hypersensitivity have been suggested. Hagen (1945) points out that infection with *M. avium*, *M. paratuberculosis*, *M. tuberculosis* have all been shown to produce cross reactions with mammalian tuberculin. Granulomatous lesions with acid fast organisms found in the skin and subcutaneous tissues of the legs were at one time thought due to *M. bovis*. Later they could not be associated with *M. bovis*, were labeled tuberculoid skin lesions and incriminated as a cause of non-specific hypersensitivity (Traum 1959). Karlson (1959) in a review of the literature found at least 25 different mycobacteria capable of sensitizing animals to tuberculin. Mallmann, *et al*. (1964) isolated many unclassified mycobacteria from bovine and swine tissues. Inoculation of experimental animals with these organisms led them to the conclusion that tuberculosis or tuberculosis-like disease in domestic animals may be caused by a range of acid-fast organisms. Further, when infection occurred, sensitivity was induced which confirmed the heterogencity of the isolants. Paterson (1956) gives an excellent review of reports of suspected causes of non-specific hypersensitivity but evidence incriminating organisms other than the mycobacteria is scant and circumstantial at best. Considering the opportunities cattle have of contacting the genus *Mycobacteria*, it is no wonder that there is a certain portion that react to the wide spectrum antigenicity of mammalian tuberculin. Attempts to develop a more specific agent such as purified protein derivative (P.P.D.) tuberculin have fared little better. Francis (1958) points out that no tuberculin has so far been developed that will detect all tuberculous animals without eliciting a non-specific reaction in a varying but high proportion of animals.

The level of non-specific hypersensitivity in the cattle population reported in the literature varies. McLaughlin (1962) tested 2,686 cattle in 86 herds in a Wisconsin township and found 6.1% with a detectable response to tuberculin in 60% of the herds tested. Tervola and Berman (1959) found 12.6% responding cattle in 75% of the herds tested in six townships. In Outagammie County, Wisconsin, 1.5% of 42,850 cattle were reactors. Ranney (1966) reports that in Cache County, Utah, 591 (3%) of 20,440 cattle tested were classified reactors or suspicious by the use of the tuberculin test. Chaloux (1964) reports that 18,202 cattle tested in 23 states by regulatory veterinarians disclosed 3.2% with tuberculin responses. Reports from many areas of the world present similar findings. These data are difficult to compare as the populations tested were usually biased and frequently not fully defined. In many of these samplings, only adults were tested or calves below 6 months of age were excluded. In most, the definition of a reaction is nebulous as it takes into account a qualitative judgement by many different veterinarians and frequently, the population tested is selected due to an apparent high degree of tuberculin hypersensitivity.

Undeniably the best answer would be the development of a simple test capable of detecting all stages of M. bovis infection. This goal has thus far eluded the grasp of research workers. Personnel responsible for conducting the program, meanwhile, must make the best use of the tools already available. This is difficult due to the conflicting data and claims for the different testing methods.

Many different forms of retesting questionable animals have arisen. In the U.S.A. a second caudal fold intradermal test, 60-120 days after the first test is the primary method of retesting questionable animals. The veterinarian is required to determine subjectively if the response to the second test is similar to, or larger than that of the first test. If this criterion is met, the animal is considered infected with M. bovis. This second test, being the same as the first, gives little additional information to the investigator as to the nature of the hypersensitivity. Gregory (1949) suggested that the short thermal test could be used as a check on intradermal reactors. This method consists of the subcutaneous injection of tuberculin and a subsequent body temperature elevation in M. bovis infected animals. Robertson (1963) found the short thermal test ancillary to the subcaudal to be superior to a repeat subcaudal test given 60 days after the initial test. Larson & Kopecky (1964) developed a modified thermal test in which a quantity of tuberculin is injected directly into the vein of an animal. A rise in temperature is considered indicative of specific M. bovis infection. They concluded that this method could not replace the standard skin test but was useful as a supplemental tool in detecting TB-carrier cattle.

Northern Ireland favors the Stormont technique, a double injection, using P.P.D. tuberculin. A second intradermal injection is made in the site of the first, seven days later. It is their belief that an M. bovis infected animal will show a further increase in skin thickness within 24 hours after the second injection. Kerr, Lamont and McGirr (1949) reported a more reliable specificity by this procedure than by either single or comparative intradermal tests.

In the U.S.A. an attempt was made to reduce non-specific responses by decreasing the dose of tuberculin in units given per dose. It was found that many tuberculous cattle would not respond to these decreased doses due to a low level of hypersensitivity. For similar reasons, selecting animals with responses over a certain size as being specifically infected gives no better results. The degree of hypersensitivity in both the specifically and non-specifically infected animals varies widely. Many animals actually infected with _M. bovis_ but with a low degree of hypersensitivity are not identified, while too many highly hypersensitive non-specifically infected animals will be slaughtered.

The English use what they call a single intradermal comparative test which is based on the assumption that despite cross reactions, homologous antigens elicit a larger response in infected animals than those elicited by heterologous antigens. In their test a mammalian P.P.D. is compared with an avian P.P.D. for capacity in producing responses in the same animal. It is assumed that the antigens responsible for non-specific reactions are more closely related to avian P.P.D. than to the mammalian P.P.D. Thus, a specifically infected animal will respond more to mammalian P.P.D. while the non-specifically infected will respond more to avian P.P.D. or equally to both. This is the method used in many countries today; however, just as with other intradermal methods it is evident that it also confuses specific with non-specific responses.

Van Waveren (1953) using the single intradermal comparative test in Holland found that 96.5% of cattle subsequently found to be tuberculous, had responses to the mammalian P.P.D. When mammalian and avian tuberculin reactions were compared, only 86.3% would have been detected by the comparative test criteria. He concluded that the comparative test should not be used in heavily infected herds or on animals of unknown origin, but that it is very valuable in clean herds as it prevents the condemnation of many no-lesion reactors.

During studies of the pathogenicity in cattle of some Group III mycobacteria, McGavin, et al. (1964) noted a relationship between progression of a mycobacterial disease and the single intradermal comparative test. Animals in which the Group III organisms caused a progressive disease indistinguishable from bovine tuberculosis, reacted to the comparative test while those with non-progressive infections did not. These animals with non-progressive lesions at time of slaughter had reacted early after the introduction of the organism but became non-reactors on later tests.

Many serologic tests have been applied to the diagnosis of tuberculosis in cattle with variable success. Middlebrook & Dubos (1948) described a hemagglutination test subsequently modified by Smith and Scott (1950) and by Rheins and Thurston (1955) which detected specific antibodies in the serums of tuberculous cattle. Vardman (1962) found the hemolytic modification of this hemagglutination test to be superior. Grey (1953) meanwhile, reported that neither the H.A. test or its hemolytic modification were superior to the intradermal test.

Many researchers have worked on a complement-fixation test. In 1945 Johnson found 71% of tuberculosis reactors with lesions positive to the C.F. test. There were also 75% of reactors with only skin lesions and 51% of N.G.L. reactors that had positive C.F. tests. Seven percent of normal cattle were positive. Vardman and Larsen (1964) after testing over 2,000 cattle for C.F. antibodies against tuberculosis concluded that this test was of no value for detecting infected cattle on an individual basis; however, they did find a good correlation on a herd basis between M. bovis infection and high serum titers. They found only 2 of 28 tuberculosis free herds (7.1%) had animals with diagnostic titers, while 14 of 15 M. bovis infected herds (93.3%) had such animals.

In recent years much work has been done on gel and double gel diffusion precipitation tests. These seem to offer more hope as a diagnostic test than other serologic tests. The double-diffusion gel precipitin test could, when fully exploited by use of multiple antigens in serial dilution, be of critical value in distinguishing tuberculosis from non-tuberculous disease (Alshabkhoun et al 1960). Parlett (1959) found the double-diffusion method to be highly specific with only a 2.1% non-specific response rate. He noted that most of these false positives actually resulted from the presence of antibodies against mycobacterial antigens in the serum of these reactors but that some may have been precipitation artifacts.

Mallmann, _et_ _al_ (1963) found the hemagglutination methods of Takahashi, and of Middlebrook and Dubos to have little value in differentiating those animals reacting to the tuberculin test due to _M_. _bovis_ infection from non-specifically reacting animals. They did note an anamnestic-like rise in antibody titers in tuberculin positive animals from gross lesion herds, three to fifteen days after tuberculin testing. They suggested that a differential test might be possible utilizing this anamnestic-like response.

It can be readily noted in a brief review of the literature that comparisons of similar testing methods is difficult if not impossible. Various details of the testing procedure change, the population tested is frequently not adequately defined, and what each author means by reactor, suspect or deviator is subject to question. In 1956, Paterson suggested, "A valuable study from the epizootilogical aspect would be to carry out standardized tests in different world areas on randomly selected tuberculosis-free herds of comparable productivity and geographical status. Such investigations would give little information on causes of sensitization, but would permit quantitative comparison of reactivity in a way at present impossible, and eliminate much subjective opinion on the incidence of non-specific sensitization."

DEFINITIONS

ADULT CATTLE: Two or more years of age

CAUDAL FOLD TEST: An intradermal tuberculin test applied to the fold of skin at the base of the tail.

CERVICAL TEST: An intradermal tuberculin test applied to the skin of the lateral aspect of the neck.

COMPARATIVE TEST: The simultaneous intradermal injection of the same animal with two different tuberculins for the purpose of comparing the degree of hypersensitivity of one to the other. In this report A.R.S. mammalian and avian tuberculins were used.

GROSS LESION: Visible signs of tuberculosis at post mortem inspection.

H.A. TEST: Hemagglutination test. In this report refers to Mallmann, *et al* (1963) modification of Middlebrook and Dubos (1948) procedure.

HERD: A group of cattle belonging to one owner.

HYPERSENSITIVITY: Any reaction elicited at the site of the intradermal injection of an amount of tuberculin inocuous to the normal individual visible or palpable at the official time of reading.

N.G.L. No Gross Lesion. The lack of any visible signs of tuberculous disease at post mortem inspection.

NON-SPECIFIC: In this report, refers to sensitization which is responsible for responses to mammalian tuberculin in the animals believed to be free of tuberculosis due to *M. bovis*.

P.P.D.	Purified protein derivative tuberculins. P.P.D.-S is an antigen made from M. tuberculosis, while P.P.D.-B is made from Batty type organisms. Lydia Edwards (1960).
REACTOR:	An animal with a comparative cervical test having a mammalian response as defined below, 5 mm or more than the avian response.
RESPONSE:	Any visible or palpable sign of hypersensitivity at the reading time for the test. This was at 72 hours for the caudal test and 48 hours for the comparative test. Minimal response--those classified less than P1, X2 in size. See Appendix 1.
SKIN LESIONS:	Clinical signs of a nodular disease of the skin or subcutaneous tissues usually involving the lower forelimbs of cattle. Atypical mycobacteria have been incriminated as the causative agent.
TYPE I ERROR:	The error of a test that denotes bovine tuberculosis in an animal or herd when it does not actually exist.
TYPE II ERROR:	The error of a test failing to detect bovine tuberculosis when it actually occurs.

PROCEDURE

The initial tuberculin testing and investigation were conducted under actual field conditions. The purpose being that results obtained would be applicable to the present testing methods in the U.S.A. Further, the additional differential methods used in this project are familiar procedures and if successful, could be easily included in the present bovine tuberculosis eradication program.

To obtain results without the bias that would be introduced by spot selection of certain types of herds all resident herds were tuberculin tested in one entire township. Herds representing all three types of tuberculin test problems were to be found in the area to be investigated. These three types were (1) herds infected with *M. bovis*, (2) herds with animals with tuberculoid skin lesions, and (3) herds with animals with tuberculin hypersensitivity not caused by *M. bovis* infection (non-specific hypersensitivity). The selection of this area was governed primarily by the presence of known *M. bovis* infected or possibly infected herds. The other two types of herd problems are ubiquitous. In only one area of Michigan were there several adjacent townships that seemed to meet these criteria. One of these townships was chosen. It was Marion Township, in Sanilac County, 36 square miles of undulating to hilly land in the center of Michigan's primary dairy region. The general soil description is well to imperfectly drained, slightly acid to neutral, sandy loams to loam. One small town, Deckerville, with a population of about

800 is located in the southwest corner of the township. The total human population in the township is about 1550.

At the time of this investigation there were two herds under quarantine due to *M. bovis* infection. Herd histories indicated that tuberculin reactors had been removed from 39 of the herds in this township in the previous 12 year period. Gross lesions, characteristic of bovine tuberculosis, had been found in animals from 14 of these herds. Tuberculoid skin lesions were present in some herds, and some herds had animals with tuberculin responses not due to *M. bovis* infection.

The proposed program was submitted to the State and Federal disease control officials and to the Director of the Michigan Department of Agriculture for approval. Upon receipt of this approval, the cooperation of State and Federal stockyard and meat inspection personnel was obtained for aid in post mortem examinations and collection of tissue samples. The hemagglutination test on all blood samples and bacteriologic and histologic examination of tissue samples were made at the Michigan State University Tuberculosis Research Laboratories. The local veterinary practitioners and the County Extension Director were enlisted to acquaint the herd owners with the project and obtain their cooperation.

Dr. J. L. Isbister of the Michigan Department of Health[1] conducted tests on the human population using a comparative test comparable to that used for the cattle testing. The results of these tests might reveal some relationship or lack of relationship between human and animal non-specific tuberculin hypersensitivity

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[1]J. S. Isbister, M.D., Chief Cardio-Pulmonary Diseases Section

and would give the incidence, if any, of active human tuberculosis in the township. A double Mantoux test, incorporating P.P.D.-S and P.P.D.-B (purified protein derivative tuberculins, S made from M. tuberculosis and B from the "Battey" Mycobacterium) injected on opposite arms of each individual was used. The physician reading these tests recorded all changes and called any reaction over 2 mm in diameter a response.

The success of any field test program depends upon the cooperation of all individuals in the subject area. To gain the acceptance of the herd owners that would be involved, a meeting was held in the Deckerville High School to which all township residents were invited. In addition, articles were released to local newspapers explaining the program and its possible long range benefits to all cattle owners.

There were estimated to be about 100 cattle herds in this township, largely dairy operations. The area was divided in half. Each half containing approximately equal numbers of herds was assigned to a veterinarian trained in epidemiology who performed all tests and investigations in his respective half. All the herds in each section of the township were tested before proceeding to the next section, to avoid introducing bias from early selection of the more easily investigated herds or more cooperative farmers. A minimum of two visits to each farm was required. The first for injection of tuberculin and initiation of other investigations and the second to read the results of the test. All information was gathered during these two visits unless tuberculin hypersensitivity was found in the herd.

The first testing procedure was as follows:

> All cattle one day and older in age were inoculated intradermally in the caudal fold with 0.1 ml of A.R.S. standard mammalian tuberculin. Multiple dose, calibrated tuberculin syringes and 3/8 inch 26 gauge needles were used. Before this injection was made the animal's identification, ear tag number or tattoo was recorded and a 5 ml blood sample obtained from the adults. Three days later (72 hours) the animals were examined visually and by palpation for signs of tuberculin hypersensitivity. All changes from normal were recorded as a response. These responses were measured and recorded in millimeters across the greatest thickness with the Hauptner dermal thickness gauge. They were also classified by the A.R.S. method of P, hard and circumscribed, or X, soft and diffuse with no distinct demarcation.* All animals in the herd were visually examined and the legs of the adults palpated for signs of tuberculoid skin lesions. When found these were classified in one of three groups. These three groups were:
>
> I. Moderate to extensive lesions occurring in chains following the lymphatic drainage of the legs.
>
> II. One or a few well described typical nodules in the same locations.
>
> III. Not typical or questionable lesions occurring on the legs and those occurring high on the shoulder, flank or other body area.
>
> Due to the questionable nature of the latter, they were not used in compiling skin lesion data. All investigations were completed at this time for those herds in which no cattle with tuberculin response were found unless tests for other species were required.

During these two visits the epidemiologist gathered and recorded information about the animals with tuberculin hypersensitivity. Information about the herd was also gathered. General management, sanitation, herd health, feed practices, purchase and sales practices, use of communal pastures and possible associations with local past or present known *M. bovis* infected herds were noted.

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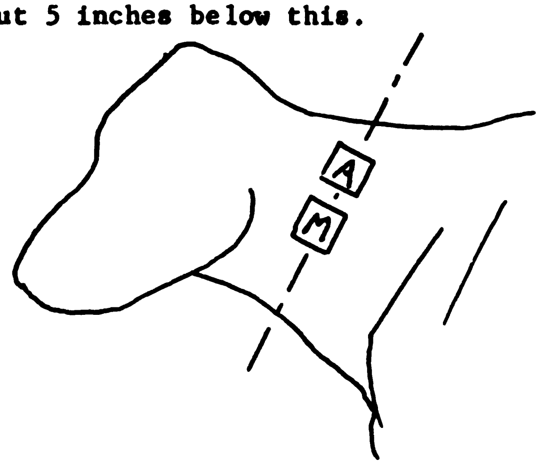
*See Appendix 1

Arrangements were made to test all chickens and hogs in lots of 40 or less and at least 10% of larger lots unless they were very large brooder type operations. Chickens were tested with 0.05 ml of A.R.S. standard avian tuberculin injected intradermally into the wattle. Responses at 48 hours were recorded. Hogs were inoculated with 0.1 ml of both avian and mammalian tuberculins in the skin over either ear. When possible other livestock and pets were tested.

Blood samples collected during the first visit and samples taken seven days after the caudal injection were tested by the hemagglutination test for tuberculo-polysaccharide specific antibodies in the Michigan State University Tuberculosis Research Project laboratory.

The second phase of work involved only those herds in which one or more of the animals had a caudal fold tuberculin hypersensitivity:

> One week after the caudal test injection of tuberculin all animals that demonstrated tuberculin hypersensitivity were again blood sampled. An equal number of non-responding animals from each of these herds were also bled. All caudal responding animals then received the cervical intradermal comparative test. Hair was clipped from two sites on the same side of the neck. These sites were located on a plane parallel to the shoulder, in the middle third of the neck. The upper site was placed 4 to 5 inches below the crest of the neck and the lower site about 5 inches below this.



A fold of skin was lifted in each site, measured and recorded in millimeters thickness with the Hauptner dermal thickness gauge. Avian tuberculin (0.1 ml) was always injected into the upper site and mammalian (0.1 ml) into the lower. Two days (48 hours) later these two sites were observed and palpated for signs of hypersensitivity. Responses at both sites were measured by lifting the response and determining in millimeters the greatest increase in skin thickness. The normal skin measurement at each site was then subtracted from its respective response measurement and the results for the two sites compared for a differential measurement. These were recorded as a mammalian response or avian response predominance by number of millimeters difference. Animals with a mammalian response 5 mm or larger than its avian were considered reactors if other circumstances indicated the possibility of M. bovis infection and sent to slaughter. Reactors were consigned to slaughter at Federally approved slaughter houses where a Meat Inspection Division veterinarian and an assigned stockyard veterinarian conducted a post mortem examination for the presence of lesions compatable with tuberculosis. Samples of all lesions were submitted to the M.S.U. tuberculosis research project. When no lesions were found, representative lymph node samples were submitted for complete histologic and bacteriologic examinations.

Agricultural Research Service avian tuberculin was used as the antigen to compare with mammalian tuberculin primarily because of its availability. The comparative test is based on the premise that an infected animal will react more to its homologous antigen and to a lesser degree to other antigens. If the animal's hypersensitivity is due to some agent other than those from which the two antigens are made, then its degree of sensitivity to both should be nearly equal and/or minimal.

Reactor, suspect, or negative status was assigned to the animals with caudal fold responses after the herds' history and caudal test results, individual animal's history, and results of the cervical comparative test were reviewed. Only the animals that the veterinarian considered likely to be infected with M. bovis could be condemned in this project. To obtain slaughter data on the non-reacting cattle, the owners were given pre-addressed postal cards with which they were asked to report prior to the slaughter of animals from their herd. A reporting system already existed in that most of the animals culled from herds in this area were sent to slaughter houses with meat inspection services. These services are required to report the occurrence of all tuberculous lesions and submit samples for confirmation. As long as no reports are received, theoretically, no tuberculous animals have been slaughtered. This gives a check on most of the herds that periodically cull some animals.

RESULTS AND DISCUSSION

Veterinarians conducting intradermal tuberculin tests agree that most tuberculous and some non-tuberculous animals will react to tuberculin. What constitutes a reaction is debatable. The size and consistency have been used as criteria in the past. However, some tuberculous animals have only a minimal or no response to the intradermal tuberculin test. Further, some non-tuberculous animals show more than minimal responses. Early in an eradication program it is the non-responding tuberculous animals that prolong and spread the disease. When a very low incidence of disease is reached it is the non-tuberculous animals that respond that show or defeat attempts of eradication by camouflaging responses due to bovine tuberculosis. It is not economically sound or feasible to remove a large number of non-specifically sensitive animals while searching for the bovine tuberculous animal or for a more ideal test for the presence of tuberculosis. Instead, present diagnostic methods and techniques should be further refined, or new tests devised, to give the veterinarian more aid in making a sound judgement.

One factor hampering the veterinarians' use of many of the diagnostic methods is a lack of organized data from the use of the tests. Non-specific reaction rates for the caudal intradermal test are ill-defined because minimal responses are frequently not recorded. As the difference between a minimal and full response is a subjective decision, one frequently cannot be sure what is reported.

In this thesis to avoid much subjective bias, any deviation from normal at the time of reading was considered a response. These were measured and recorded in millimeters skin thickness and were also recorded by the Agricultural Research Service method of X or P types*.

CAUDAL INTRADERMAL TUBERCULIN TEST

All cattle one day and older were tested in one township in Michigan. The area in which this township is located was not noted for being either especially high or low in non-specific responses. In the previous 12 years, 252 reactors had been removed from 41 herds in this township. Gross lesions had been found in 14 of these herds and two were still under quarantine at the start of this project.

There were 92 herds containing 3,972 cattle; seventy-two were dairy herds, ten were breeding beef herds and ten were feeder herds. Cattle less than two years of age numbered 1,441, more than 1/3 of the total number. Those six months and under comprised about 16% of the total. The results of the caudal test on these animals are recorded in table 1. Over 8% of the cattle responded to some degree to the caudal fold test. Only 0.25% were under two years of age and none were less than eight months old. The response rate for those cattle over two years of age is similar for each age group. See figure 4, page 31.

If only cattle two years and older were considered, as in the U. S. Uniform Method and Rules for area testing and the area test programs of some states, 12.8% responded which is more representative of the response rate likely to be encountered by veterinarians

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*Appendix 1

Table 1. Caudal fold intradermal test results.

Number of cattle Total - 3972 Adult- 2531	Number of herds	Number with caudal response	% of herd totals	Number of caudal responses P1, X2 or larger	% of herd totals	Percent of caudal responses P1, X2 or larger
From herds containing animals with caudal test responses.						
From *M. bovis* infected herds						
235	5	61	25.95	24	10.21	39.34
193			31.60		12.43	
From non-infected herds						
3241	63	249	7.68	87	2.68	34.93
2033			12.24		4.27	
Others						
54	3	12	22.22	4	7.40	33.33
36			33.33		11.11	
From caudal test negative herds						
442	21					
269						

conducting routine tuberculin tests. On a herd basis, responses were observed in 77.0% of the herds. Over 87% of the dairy herds, 64% of the breeding beef herds, and 20% of the feeder herds had one or more animals with a caudal response.

It is a tuberculin test rule of thumb that the large response is more likely to be due to M. bovis. At one time in the nation-wide testing program all responses X2 or P1 and larger were to be considered reactors and slaughtered unless the possibility of M. bovis infection was highly improbably. In this township, 36% of all animals with caudal response were classified P1 or X2 and larger. Over 50% of the total herds in the township had one or more of these animals. They represented 3.3% of all cattle tested and 4.5% of those two years and older.

The above figures included five herds that proved to be infected with M. bovis. All five had past histories of reactors with gross lesions of tuberculosis and included the two under quarantine at the start of the program. Mycobacterium bovis was isolated and identified at the M.S.U. tuberculosis research project, from animals slaughtered as the result of this program testing. Table 1 shows the caudal test results for the M. bovis infected herds, and herds from which M. bovis was not isolated. A third group of three herds with responding animals was not included in either set of data as they had probably been recently exposed to M. bovis. Nearly 26% of the animals in the M. bovis infected herds responded to the tuberculin test, while the average response rate in the non-infected response herds was 7.7%. In the former herds 12.4% of the animals had responses classed P1, X2 or larger. Only 4.3% had similar size responses in the non-infected responder herds.

There is a highly significant difference in the caudal response rate between M. bovis infected herds and non-infected herds, but no significant difference between the two in the percent of the caudal responses that are classified P1, X2 or larger in size. Appendix 2 contains X^2 values for these tests. The ranges of each group overlap considerably so that classification by the caudal test alone is very difficult. The range for the caudal response rate in M. bovis infected herds was from 14 to 36% of the animals in the herd. In the non-infected herds this was 1.4% to 54%. The ranges of P1, X2 or larger response rates for both groups were from about two to 19% of the herds.

OTHER INVESTIGATIONS

Other epidemiologic observations were made in addition to the cattle testing to detect possible causes of non-specific sensitization. There were 20 flocks of chickens, ranging in size from one to 7,800 birds associated by farm with 20 of the cattle herds. All or a representative sample from each flock were tuberculin tested. An injection of 0.05 ml of A.R.S. standard avian tuberculin was made into the wattle of each bird and the test was read at 48 hours. There were three reactors found in one flock. These birds and all birds in other flocks with any questionable test results and those with a physical appearance suspicious of avian tuberculosis were slaughtered. Tissue samples were examined bacteriologically and histologically at the M.S.U. tuberculosis research project. No indication of avian tuberculosis was found in any bird or flock.

There were only four pigs on two farms in this township. These were tested with both avian and mammalian tuberculins. The skin over the back of the ears was the testing site with 0.1 ml of mammalian tuberculin injected into one ear and 0.1 ml avian tuberculin into the opposite ear. All tests were negative.

Other livestock and pets were tuberculin tested when convenient or when the results of the tests of the cattle were highly suspicious. These tests included horses, ponies, dogs, and cats. All tests were negative.

No paratuberculosis has been reported from this area. There was no evidence of this disease being present in the cattle examined during the testing in this township. One animal with chronic diarrhea was slaughtered and examined for signs of paratuberculosis. No gross or histological pathology of paratuberculosis was found.

A tuberculin test survey of the human population of this township was conducted by the Tuberculosis Control Division of the Michigan Department of Health. A comparative type test was used on 1553 individuals. About 700 of these lived outside of the town of Deckerville. Each individual was injected with P.P.D.-S on one forearm and P.P.D.-B on the other forearm. A physician read the results of the test at 48 hours and recorded all responses observed at the injection sites. All changes from normal skin appearance over 2 mm in diameter were called responses. About 20% of those tested had a response at the P.P.D.-S site and about 17% at the P.P.D.-B site. A large number of those responding to one antigen also responded to the other. No evidence of active tuberculosis was found in the human population.

The preceding findings indicate that the non-specific hypersensitivity in cattle probably was not caused by *M. avium* *M. paratuberculosis*, or *M. tuberculosis*. The responses to both antigens in individuals without other evidence of tuberculosis demonstrates a similar problem of hypersensitivity in man and other animals.

SEVENTH DAY OBSERVATION OF CAUDAL FOLD TESTS

Consideration was given to the possibility that a portion of the responses seen at 72 hours might be due to other than a delayed type hypersensitivity. Duboczy (1965) reports that "residual" non-specific early or local sensitization both of which peak early between 24-48 hours might extend through 72 hours. If this were to account for a large portion of the non-specific responses seen at 72 hours, a later reading time might be beneficial. In some areas of the country, human tuberculin tests are being examined at seven days in an attempt to avoid some of the non-specific response problems. A 96 hour interval to observation has been recommended by some as just as accurate as a 72 hour interval in finding *M. bovis* infected animals without as high a non-specific response rate.

Approximately half of the cattle with caudal responses were examined at seven days post injection. About 20% of the responses seen at 72 hours had disappeared by this reading time, almost all of which were minimal responses. It would appear that further delay of the time of observation (until the seventh day) would give little help in differentiating between large responses caused by *M. bovis* infected and those resulting from other causes.

TUBERCULOID SKIN LESIONS

All animals were examined for the presence of tuberculoid skin lesions which were classified as follows:

I. Moderate to extensive lesions occurring in chains following lymphatic drainage of the legs.

II. One or a few well described typical nodules in the same locations.

III. Not typical or questionable lesions occurring on the legs and those occurring high on the shoulder or flank or other body areas.

This latter group, due to their more questionable nature were excluded from the skin lesion data.

Skin lesions of Group I and II were found on 117 animals in 44 herds. There were nine animals in the third group. An average of 5% of the animals in the 44 herds were affected with a range of 0.4 to 33.3%. All of these herds had one or more animals with a caudal response, and a herd response rate average of 10% with a range of two to 53.5%. See Table 2.

Nearly 80% of the animals with skin lesions, from 90% of the skin lesion herds, elicited a caudal response. These animals represented 29% of the total cattle with caudal fold response.

No tuberculoid skin lesions were found on any animals less than two years of age. Nearly eight percent of the adult population in the affected herds had skin lesions. The percentage affected for each age group over two years of age were approximately equal. (Figure 5.)

Approximately 14% of the cattle population were beef cattle.

No skin lesions were found on any beef breed animals. Personal communications with veterinarians in Michigan confirmed that skin lesions are either non-existent or are a rare occurrence in the beef breeds. It is interesting to speculate, if this is true, as to whether this is due to an environmental difference between beef and dairy types such as housing or feeding practices or to a genetic difference resulting in an increased resistance or susceptibility to the etiologic agent of skin lesions.

It has been stated by some that skin lesions cause only a low level hypersensitivity to tuberculin. Over 40% of the skin lesion animals in this study had caudal responses that were classified P1, X2 or larger. This represents a significantly higher rate of large responses in animals with a caudal fold test response and skin lesions than in animals with a caudal fold test response but with no skin lesions.

Fincher (1959) and others have reported a lack of skin lesions in *M. bovis* infected herds. It is quite likely in any infected herd that has been under tuberculin test for a period of time that better than 80% of the skin lesion animals will have been removed as reactors. There were 14 skin lesion animals found in four *M. bovis* infected herds in this study. These represented over 7% of the adults and only 23% of those with caudal responses in these herds. This can be compared with 5% of adults and 39% of the caudal fold responding cattle affected with skin lesions in their respective herds.

In this township, *M. bovis* infection in the herd did not preclude skin lesions from occurring on some animals in the herd or even reduce

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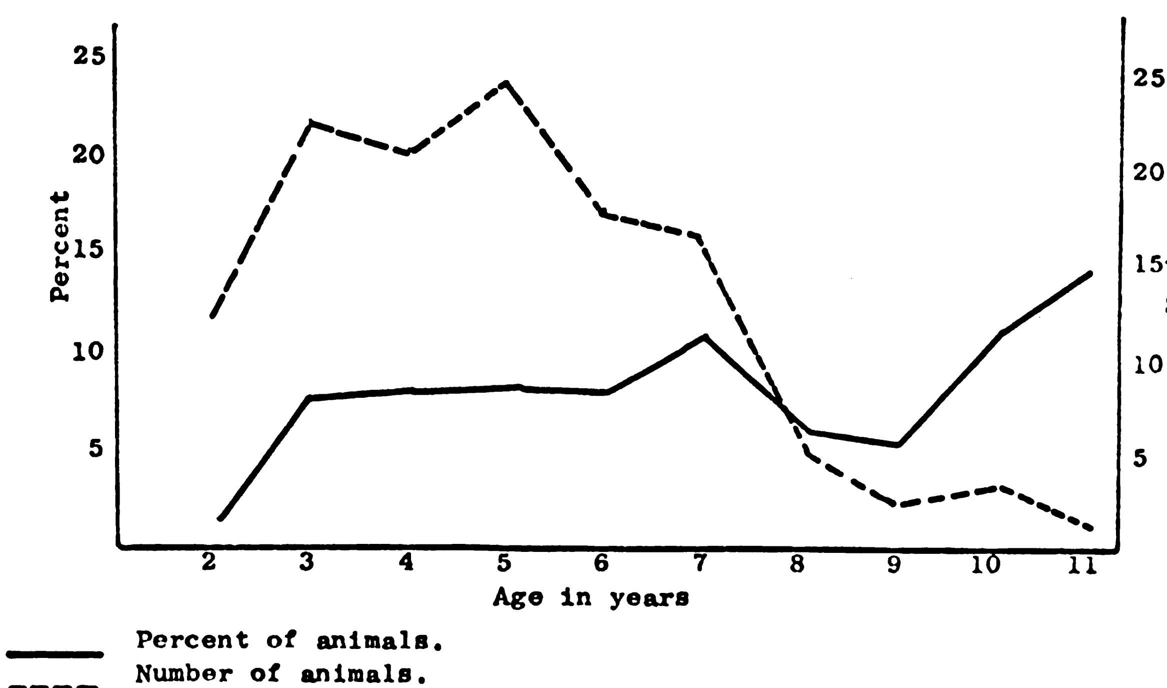
*The author has since observed typical skin lesion chains on two grade Angus cows in another part of the State.

Table 2. Cattle having a response to the caudal fold test and/or tuberculoid skin lesions.

Totals	Number with a response to the caudal fold test	Number with skin lesions	Number with both skin lesions and a response to the caudal fold test	Percent of caudal fold test responding animals with skin lesions	Percent of animals with skin lesions also having a caudal fold test response
Animals					
3972	332	117	93	28.01	79.48
% of total	8.35	2.94	2.34	-	-
Herds					
92	71	44	39	54.92	88.63
% of total	77.17	47.82	42.39	-	-

Figure 4. Marion township cattle with a caudal fold test response by age.

Figure 5. Incidence of tuberculoid skin lesions in Marion township cattle by age.



the likelihood. Two of the 14 skin lesion animals were found to have gross lesions at slaughter and therefore an M. bovis infection in an animal does not preclude skin lesions.

There is a highly significant relationship between the occurrence of skin lesions and caudal fold tuberculin hypersensitivity. Over one-third of the caudal test responding animals had clinical skin lesions. More than 80% of the samples of lymph nodes from N.G.L. reactors from Michigan, submitted to the tuberculosis research project at M.S.U. had microscopic granulomas, the majority were from pre-scapular lymph nodes. The suggested relationship between the granulomatous lymph node and clinical skin lesions suggests that non-specific hypersensitivity in cattle may be due to a skin lesion syndrome of mycobacterial etiology which involves the lymphatic drainage of the legs primarily.

COMPARATIVE TEST

The cervical comparative intradermal test using standard A.R.S. mammalian and avian tuberculins was used to retest all of the 322 animals responding to the caudal test. Results for this test are listed in Table 3a and 3b.

Nearly three-fourths (72%) of the animals tested with the comparative procedure had a response to mammalian tuberculin that was larger than the comparative avian tuberculin response. Twenty-six animals in twelve herds had a response at least 5 mm larger to the mammalian tuberculin than to the avian tuberculin. These are called comparative test reactors in the remainder of this thesis. Fifteen were from the five M. bovis infected herds. Of the latter group, one reactor was found in each of the two herds that had been under quarantine and tested at the start of this program. In a third herd, the one comparative reactor was originally from one of the quarantined herds and has been the only gross lesion, M. bovis isolation case from this herd. The other 12 came from the two herds which had not been tuberculin tested during the previous two years. Three of the comparative reactors from M. bovis infected herds had minimal caudal responses. It is probable that the previous tuberculin tests in the quarantined herds had removed many of the animals that would have shown a positive comparative test. The comparative test would have great value in testing herds in which M. bovis is suspected and which have not been recently tested, but less relative value in retesting long quarantined herds which have had a number of tuberculin tests.

The comparative test was also run on 45 caudal fold negative adults in three of the M. bovis infected herds. One comparative reactor was found in one of the previously unquarantined herds. The animal had thoracic lesions from which M. bovis was isolated. Gross lesions were found in 44% of the comparative test reactors in M. bovis herds. Mycobacterium bovis was isolated from each.

This gives much support to a claim that this testing method is more efficient than other testing or retesting methods in locating the tuberculous animal. Comparative test reactors while associated with M. bovis infection, unfortunately, are not limited to M. bovis infections. Eleven other comparative reactors were found in seven other herds. One herd had three reactors, two others had two each, and the remainder, one each. There was no history or other evidence of bovine tuberculosis in any of these seven herds. No association between the reacting cattle or herds and other M. bovis infected animals or herds could be established.

Table 3a. Comparative cervical test results

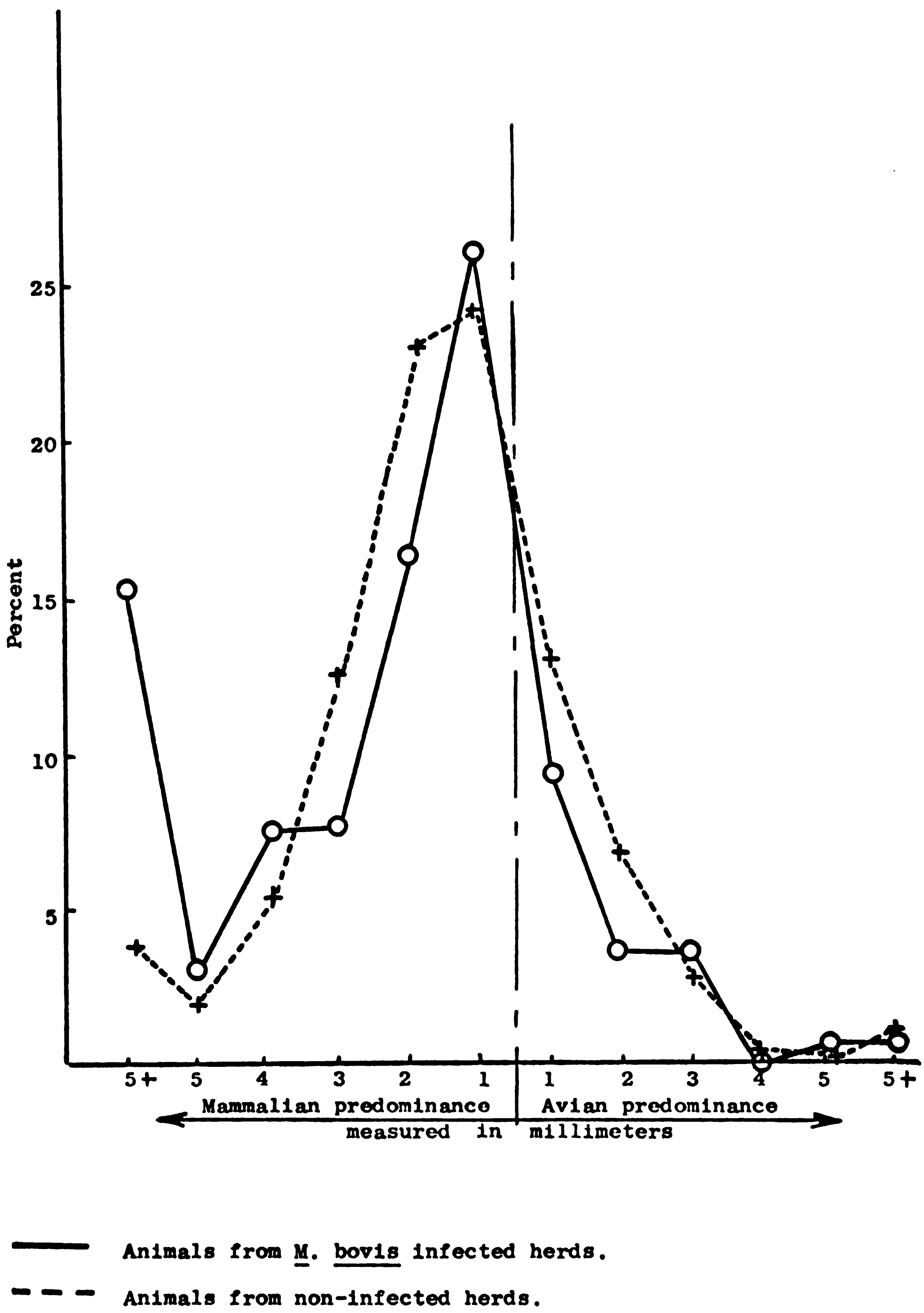
Number of animals in comparative reactor[1] herds	Number of herds	Number of comparative reactors	%	Number of caudal test responding cattle in these herds	%
M. bovis infected					
235	5	16	6.8	61	25.96
Non-infected					
332	7	11	3.3	31	9.33

Table 3b. Comparative cervical test results

Caudal test responding cattle also comparative test reactors	Number of comparative reactors[1] with skin lesions	% of comp. reactors	Number of comp. reactors with H.A. titer increases			Comparative reactors with H.A. titers 640 or greater
			4 fold	8 fold	16+ fold	
15	3	20.0	3	4	3	5
11	7	63.6	1	3	1	0

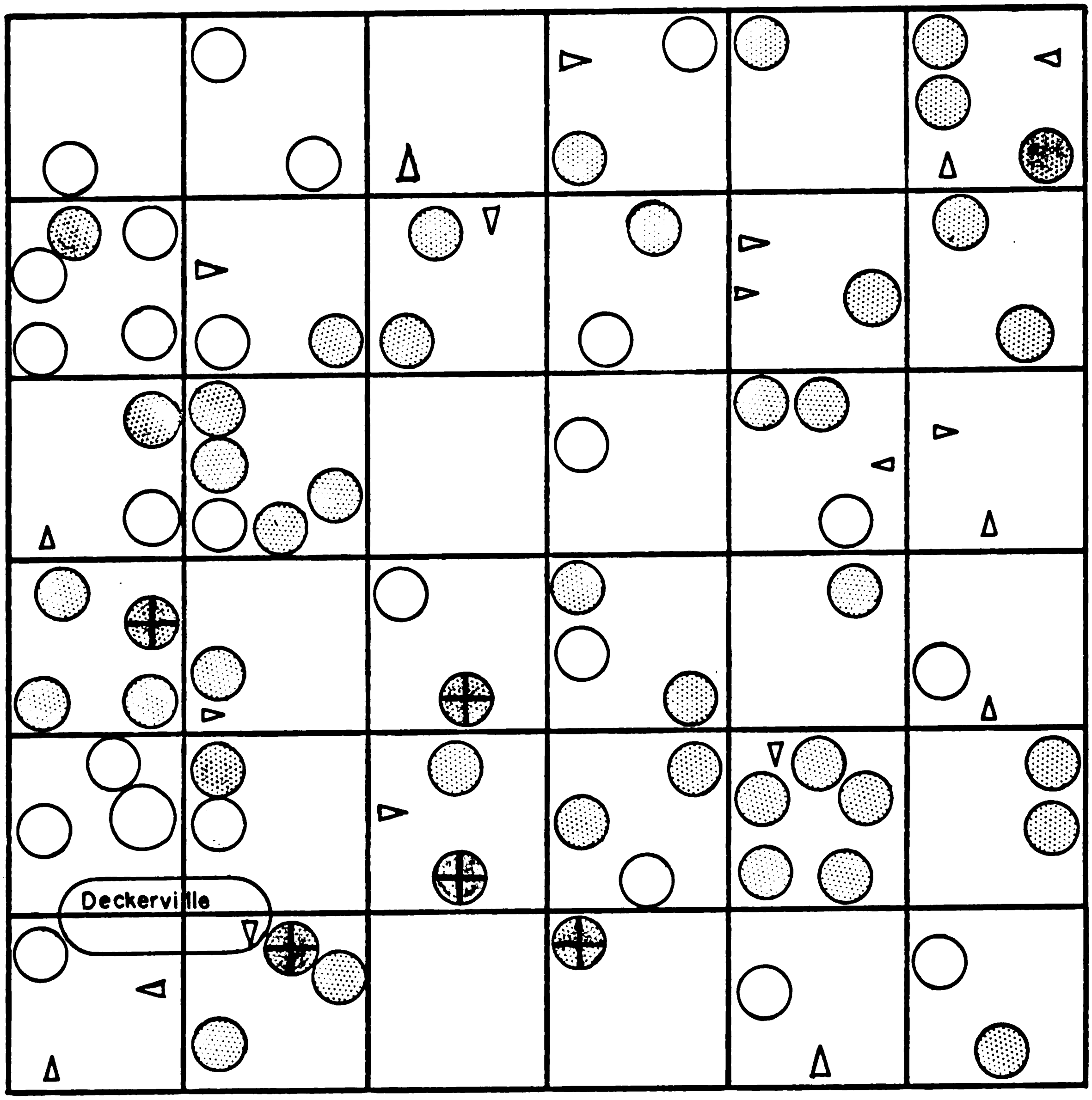
1. Comparative test reactor: An animal that had a comparative cervical test response 5mm or larger to mammalian tuberculin than to avian tuberculin.

Figure 6. Comparative cervical test results.



Eight of these reactors were slaughtered. No gross lesions were found and no M. bovis was isolated from tissue samples from these animals. This would not seem to support the work of McGavin et al that a positive comparative test indicates progressive mycobacterial disease. Seven of the eleven reactors (64%) had clinical tuberculoid skin lesions. Three of the 16 comparative reactors (19%) in M. bovis herds also had skin lesions. Thus over 60% of the comparative reactors had visible lesions of mycobacterial disease. Ten had tuberculoid skin lesions and seven had thoracic lesions from which M. bovis was isolated. It could be, as reported by McGavin et al that a comparative reaction is specifically indicative of a progressive mycobacterial disease. As previously mentioned, the pathologists, Willigan, McGavin and Goyings, in the tuberculosis research group at M.S.U. found that a high percent of N.G.L. reactors had microscopic granulomas. It is possible to speculate that a large proportion of the non-specific caudal fold sensitivities are due to some phase of atypical mycobacterial infection, then it is also possible to speculate that the comparative reactors could be animals with presently active or progressive mycobacterial disease. The research group found that animals with primary complex disease, i.e. granulomas in prescapular lymph nodes only, were not comparative test reactors. It may be that these lesions were in a static or regressive state and no comparative differentiation would be expected. Unfortunately, complete histologic and bacteriologic examinations were not possible on all responding animals in this project and the number with or without progressive mycobacterial disease is not known.

Figure 7. Cattle farm locations - Marion township.



Herds with one or more animals with a caudal test response.

Herds with one or more animals with a caudal test response and one or more animals with skin lesions.

Herds infected with *Mycobacterium bovis*.

Caudal test negative herds.

Herds with comparative test reactors

A total of 6.8% of the animals in *M. bovis* herds were comparative test reactors which represented over 26% of these herds' caudal responding animals. Only 3.3% of the animals in non-infected, comparative test reactor herds had comparative reactions, but represented over 35% of the animals with caudal fold test responses in these herds. The actual caudal response rate of these two groups shows 26% responses with a range of 14 to 36% for *M. bovis* infected herds and 11% with a range of 5 to 19% for the non-infected, comparative reactor herds. Thus, there are significantly more caudal test responding animals in *M. bovis* infected herds than in non-infected herds which contain false comparative test reactors.*

The comparative test method, like the caudal test, has a high degree of both Type I and Type II error when used on individual animals. These errors also occur on a herd basis but the test greatly reduces the number of herds which are considered suspicious by the caudal fold test without missing *M. bovis* infected herds.

SEROLOGY

Blood samples were obtained before and after tuberculin testing from 518 adult cattle. Approximately half of the animals had responded to the caudal test and the other half were negative animals from the same herds. The first sample was collected prior to the caudal fold injection of tuberculin and the second seven days later. Serums from these samples were tested by the Michigan State University (MSU) Tuberculosis Research Group for tuberculopolysaccharide-specific antibodies by the modified passive hemagglutination test (Mallmann *et al*

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*Appendix 2

1964). The titers were recorded as the reciprocal of the highest dilution of the serum which yielded a positive test. The pre and post tuberculin injection titers for each animal were compared for an anamnestic-like response as indicated by a 4 fold or greater increase in titer.

There were 109 tests from M. bovis infected herds and 409 from the non-infected. Titers for 215 caudal test responders were also compared with 202 caudal test negative animals (Tables 4a and 4b).

Preinjection titers for all animals averaged slightly more than the 1/40 dilution (Figure 8). Only 12.1% of the samples had no detectable antibodies at the 1/20 dilution. There was no significant difference in preinjection titers among animals from M. bovis infected herds and non-infected herds or from subsequent caudal test responding and caudal test negative animals. Animals with tuberculoid skin lesions had significantly higher pre and post tuberculin injection titers than did those without skin lesions.

In all of these groups there was a post injection titer increase ranging from 1.8 to 5.8 times the preinjection titer level (Table 4a). Post injection titers are compared for M. bovis infected herds and non-infected herds, and for animals with and without caudal responses (Figures 9, 10). The mean titer increase for animals from M. bovis infected herds with caudal responses was nearly six fold. This compared with a mean increase of 2.4 fold for animals from non-infected herds with caudal responses. Statistically this increase is significant only to 10%. All five M. bovis infected herds and seven non-infected herds had comparative test reactors. Titers from these two groups were compared and the results were approximately the same for both.

Table 4a. Mean increase of post tuberculin injection hemagglutination titers.

	Number of samples	Mean increase of post injection titers[1]
All samples	518	2.5
Caudal test responding animals	267	2.9
Caudal test negative	251	2.0
M. bovis infected herds	101	4.4
Caudal test responding animals	52	5.7
Caudal test negative	49	2.6
Non-infected herds	417	2.2
Caudal test responding animals	215	2.4
Caudal test negative	202	1.8
Animals with skin lesions	82	3.1
Animals with no skin lesions	436	2.4

1. Increase in multiples of the mean pre tuberculin injection titer.

Table 4b. Hemagglutination (H.A.) titers from herds with comparative test reactors[1]

Number of animals [2]	Increases in H.A. titer after tuberculin test			Number with a post tuberculin titer of 640 or more
	4 fold	8 fold	16+ fold	
M. bovis infected herds				
52	14	10	5	6
%	26.92	19.23	9.61	11.53
Non-infected herds				
26	6	3	4	3
%	23.07	11.53	15.38	11.53

1. H.A. titer: reciprocal of highest serum dilution in which tuberculo polysaccharide specific antibodies were detected.
   Comparative reaction: Cattle which had a cervical 5 mm response of or greater to mammalian tuberculin than to avian tuberculin.

2. Animals had responded to tuberculin injected into the caudal fold and the pre and post tuberculin H.A. titers determined.

Nearly 30% of the animals bled had titer increases of four fold or greater (Figure 11). There were a significantly greater number with a four fold or greater increase in the *M. bovis* infected herds. Comparing the number of titer increases that were 16 fold or greater, however, shows no significant differences in high titers between *M. bovis* infected and non-infected herds. Due to the lack of a clear cut separation, a four fold anamnestic-like increase in titer has little value as a differential test under field conditions. However, 6 of the 21 animals with 16 fold or greater titer increases were from *M. bovis* infected herds and 4 of these were found to have gross lesions at slaughter. A number of non-infected animals will have these high titers, but their occurrence in a herd should alert the veterinarian that there is a greater possibility of *M. bovis* infection.

Table 4a shows that the mean increase of post tuberculin injection titers of animals with skin lesions falls near the middle of the range of the other means listed. Appendix 2, however, shows it to be a significant difference. As the preinjection titers also demonstrate a significant difference, we conclude that skin lesion disease itself is responsible to some degree for the higher initial H.A. titers but that the anamnestic increase is actually no greater than it is for the other samples. Only 9.5% of animals with very high post injection titers had skin lesions. This compares with 7.8% skin lesion incidence rate in the total population. The lack of a high titer increase would seem to indicate that skin lesion disease is not responsible for the very high levels of circulating, tuberculopolysaccharide-specific antibodies.

A decrease in titers between the first and second samples was observed in 5.20% of all samples. In *M. bovis* infected herds only 1.9%

of the samples decreased, while in non-infected herds, 6% decreased. Investigation of non-infected comparative reactor herds showed 6.5% of their samples decreased in titer. Statistical analyses indicated there was no significant difference in these reversal rates between caudal test responding animals and caudal test negative animals or between *M. bovis* infected and non-infected herds.

Five of 16 animals with post injection sample titers of 640 or higher, were also comparative test reactors. Their caudal test responses were all X2, P1 or larger in size. These five were all found to have gross lesions at slaughter, and *M. bovis* was isolated from each. Non-infected animals while meeting one or two of these criteria failed to meet all three. Further investigation of this facet may indicate that the combined use of the three tests will effectively increase the detection of *M. bovis* infection with less time, effort, and error.

Figure 8. Pre and post tuberculin injection hemagglutination titers. All samples.

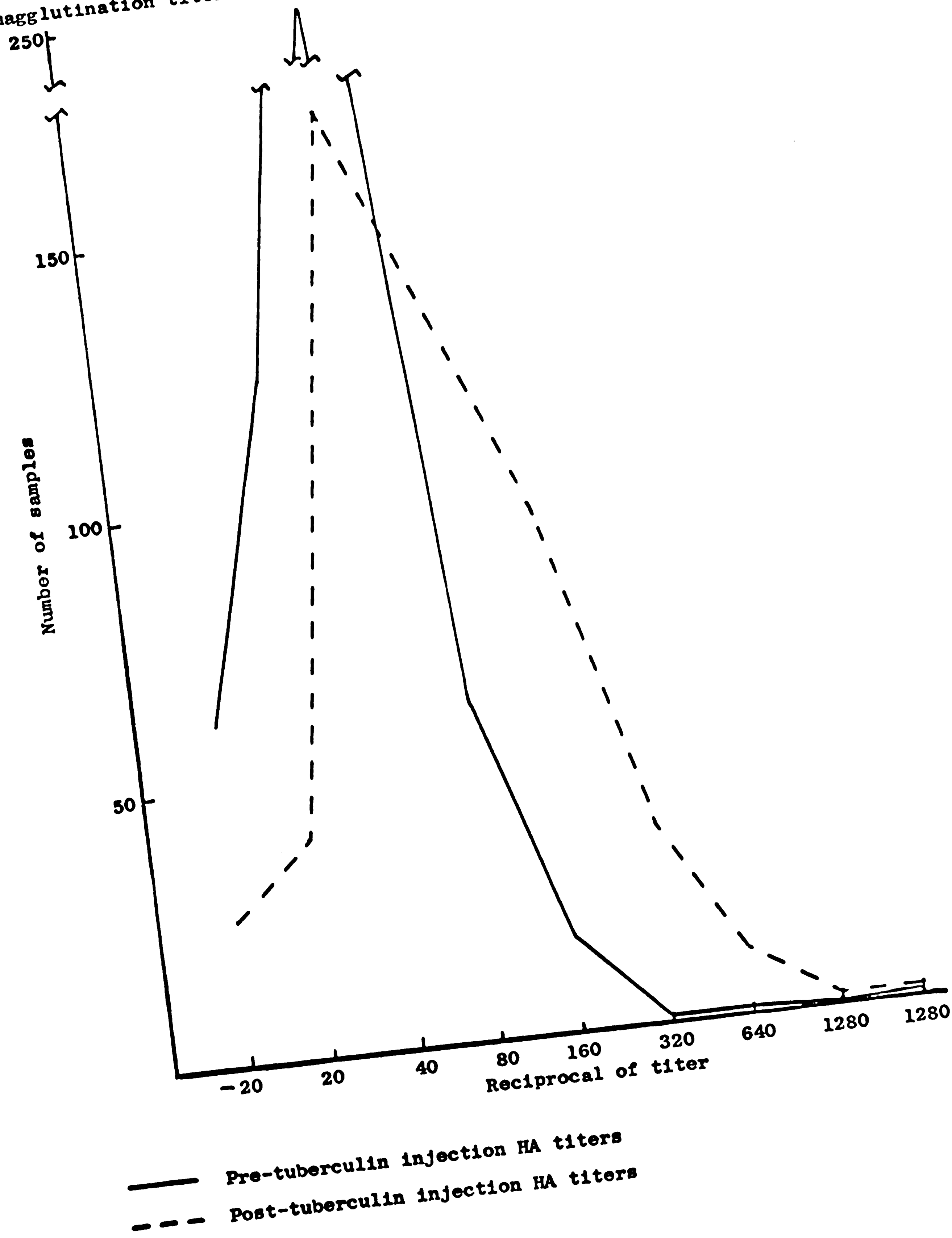


Figure 9. Post tuberculin injection hemagglutination titers. Animals from Mycobacterium bovis infected and non-infected herds.

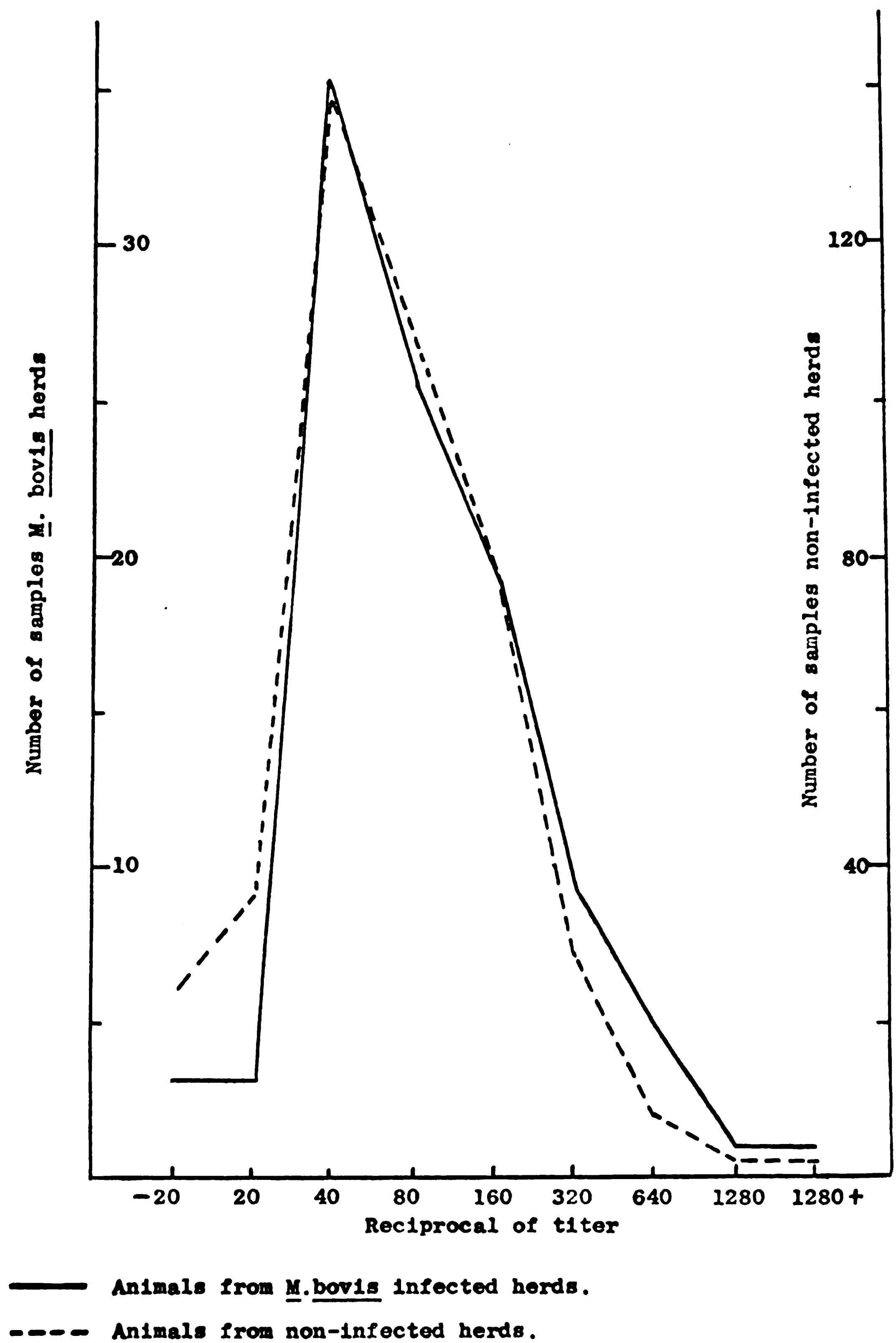
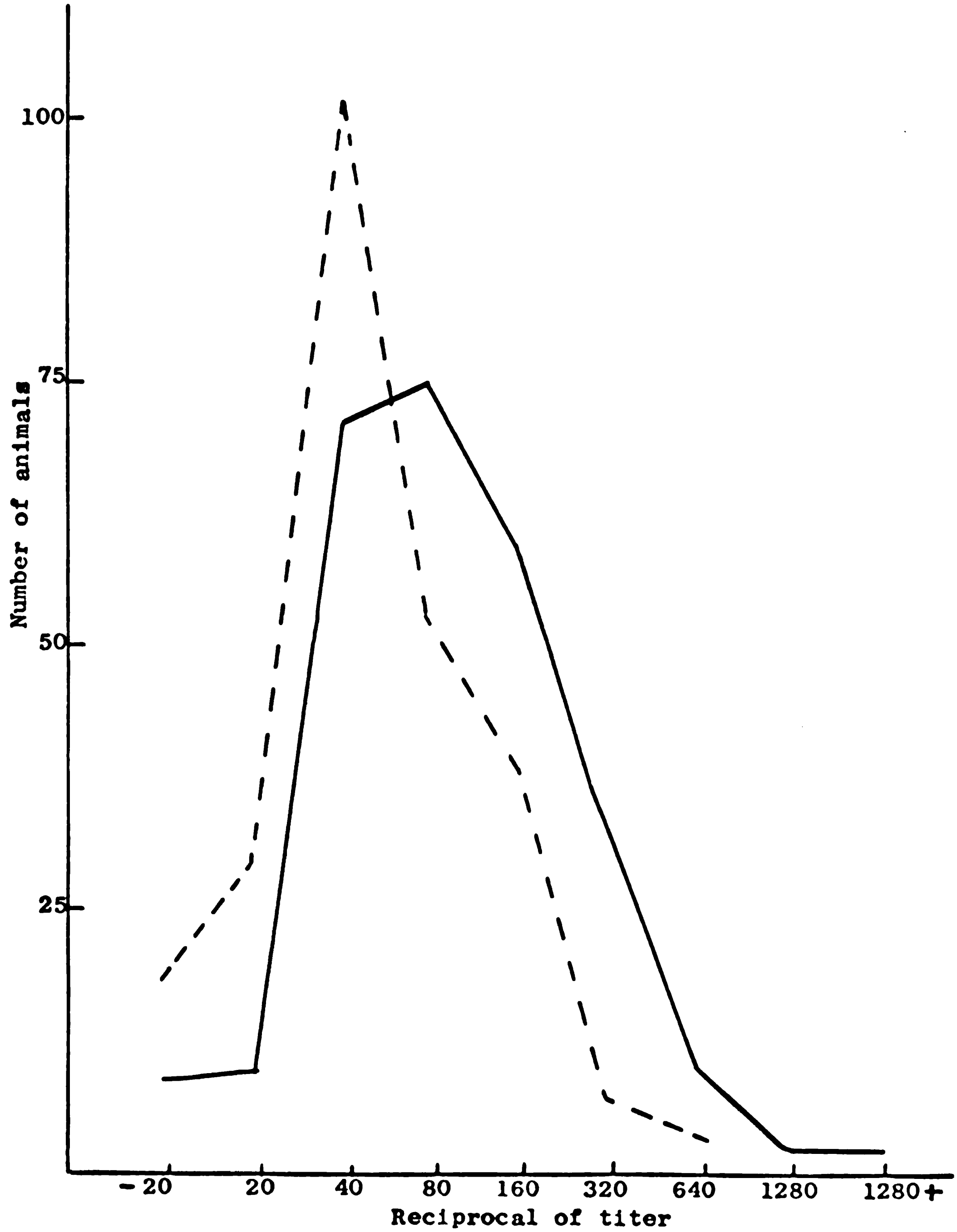


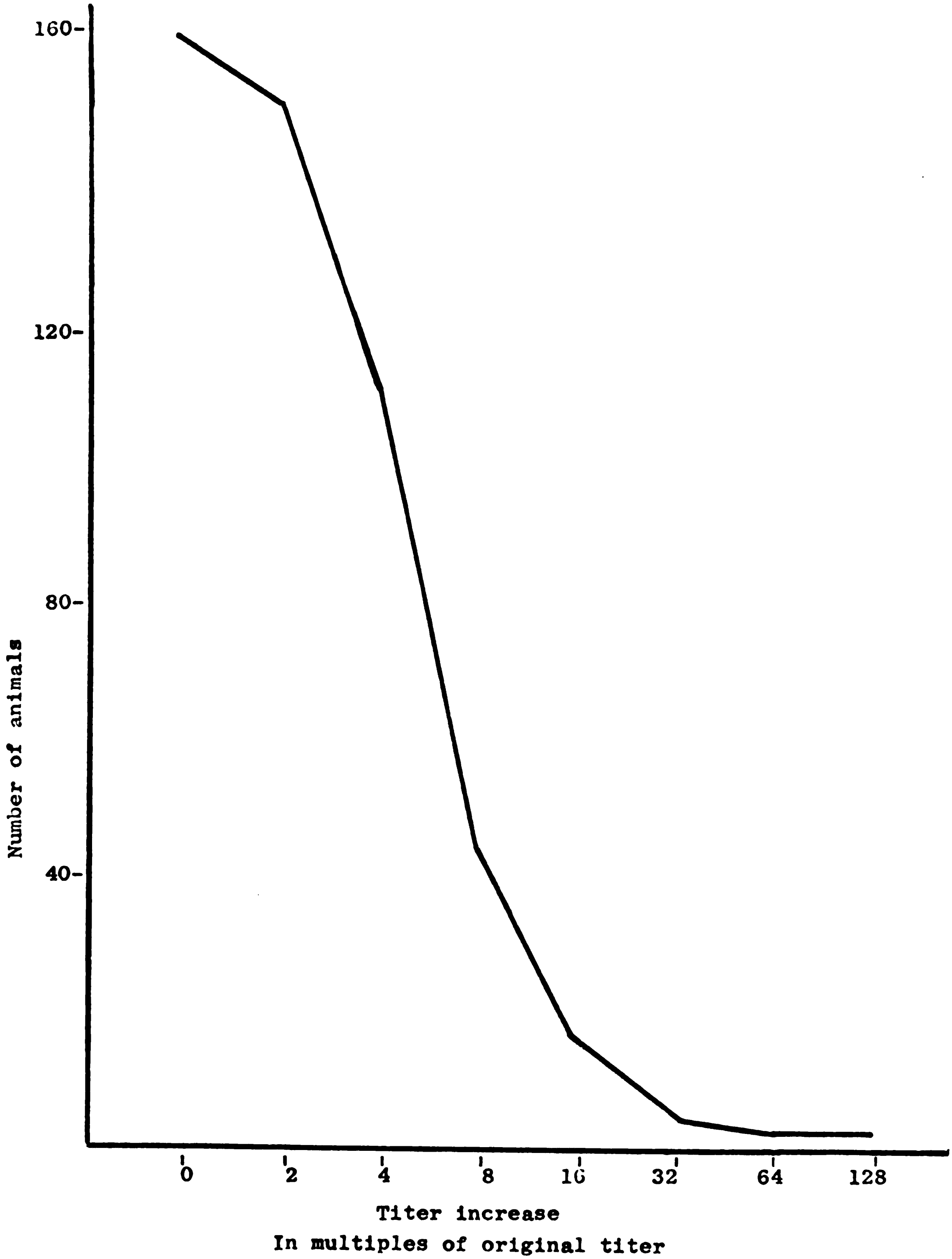
Figure 10. Post tuberculin injection hemagglutination titers. Animals with caudal test responses and caudal test negative animals.



_____ Caudal test positive.

\- - - - Caudal test negative.

Figure 11. Post tuberculin injection hemagglutination titer increases.



CONCLUSIONS

The caudal fold test of *M. bovis* infected herds in this township was found to miss over 36% of the animals with tuberculous lesions (Type II error). Not all of these lesions have been proved by laboratory work to be due to *M. bovis*, so this figure may be high. Other workers have reported as much as a 19% Type II error (Robertson 1936b). There were over 8% of non-specific positive tests (Type I error) which is approximately in the middle of a range from other reports of Type I error.

It is obvious that such a test is ineffective as an individual animal test in detecting less than 0.01% infection in a population of 100 million. None of the tests for tuberculosis presently available, when used singly, are any more reliable. Further, it is unlikely that any ante-mortem test will be devised that is totally reliable. However, the effects of both types of error can be minimized by considering *M. bovis* infection on a herd wide basis.

A high percentage of animals responding in a herd tested by the caudal test indicates possible *M. bovis* infection. There is a portion of non-specifically infected herds that will also show similar results to this test. Therefore, an adequate history of the herd and individual animal is invaluable, although not infallible, in differentiating specific from non-specific infection.

A veterinarian finding caudal fold suspicious results while testing a herd has at present four alternatives:

1. He can decide that there is *M. bovis* infection in the herd and remove all responding animals. This will probably remove a large percent of the productive animals. The owners economic loss is great, and perhaps needless if the infection is non-specific.

2. Some of the responding animals can be slaughtered. If they have lesions of tuberculosis, the other animals can be removed. However, an infected herd can be sampled many times without disclosure of lesions at slaughter.

3. The herd can be retested in 60-90 days using again the caudal fold intradermal test. The results of such a retest will be no more definitive than the original test.

4. The responses can be considered to be non-specific due to the large number of skin lesions and the herd called negative. The danger here, of course, is the possibility of leaving an animal infected with M. bovis which can be spread from the animal or herd to others.

Supplemental tests, when their limitations are recognized, can be used with a higher degree of confidence to help differentiate those herds with suspicious caudal fold test results. Individually, the two supplemental tests used in this project gave overall results similar to those of the initial caudal test. Each test had some degree of Type II error but the errors did not entirely overlap. Thus, the use of the combined results from the three tests provided a more specific diagnosis than possible with any of the tests individually.

The comparative test had a high degree of both Type I and Type II error but as a follow-up to the caudal fold test, reduced the Type I error appreciably. The sizes of comparative test responses cannot be used as a single determinant. However, the occurrence of a number of mammalian responses in a herd approaching or exceeding the 5 mm measurement indicates that an M. bovis infection more probably exists.

A four fold increase in hemagglutination titer was not found to be specific for *M. bovis* infection in individual animals. There was a significant four fold rise in titer seven days after the injection of tuberculin overall samples collected from animals in *M. bovis* infected herds. There was, however, no significant difference between *M. bovis* infected herds and others in the occurrence of post tuberculin titers 16 fold or greater. A specific rise in titer cannot be used as a determinant. As the comparative test, this test has some degree of Type I error, yet is helpful in supplementing the results of the caudal fold test.

To illustrate the value of these two supplemental tests, the testing results from two herds in this project are presented in Table 5. The caudal fold testing of all animals in each herd revealed responses in 33% of herd A, and 53% of herd B's animals. If the caudal test had been conducted as in the routine screening method only on animals two years and older, then responses would have been found on 48% of the animals tested in herd A, and 65% of those in herd B. Further, 24% of the adults in both herds had responses classified P1, X2 or larger. Examination for tuberculoid skin lesions revealed 17% of herd A and 38% of herd B had clinical skin lesions.

Reviewing the history of each herd revealed that herd A had extensive tuberculosis infection several years before and had been released from quarantine two years before this test. Herd B had two negative tuberculin tests in the preceding six years and had no indication of *M. bovis* infection at any time in its history. The veterinarian conducting these tests should have little trouble in concluding that herd A was probably infected with *M. bovis*. The tuberculin test results indicated that

Table 5. Comparison of testing results from one *Mycobacterium bovis* infected and one non-infected herd.

Herd	Number in herd	Number with caudal response	Caudal response P1, X2 or larger	Comparative test reactors	Number with 8 fold or greater hemagglutination titers	Number in herd with skin lesions
A	48	16	7	7	2	5
%		33.3	14.6	14.6	4.2	10.4
B	43	23	8	0	0	13
%		53.5	18.6	0	0	30.23

M. bovis infection is present in Herd B also. However, the history and presence of a large number of skin lesions indicate that more probably a non-specific sensitivity exists. This is substantiated by the results of cervical comparative tests of animals in herds A and B responding to the caudal fold test. There were six reactors in Herd A and none in Herd B. All adults in both herds were tested with the comparative procedure. Over one-third of the adults in Herd A had mammalian responses 4 mm or greater than the avian response, while no animal in Herd B had more than 3.3 mm difference.

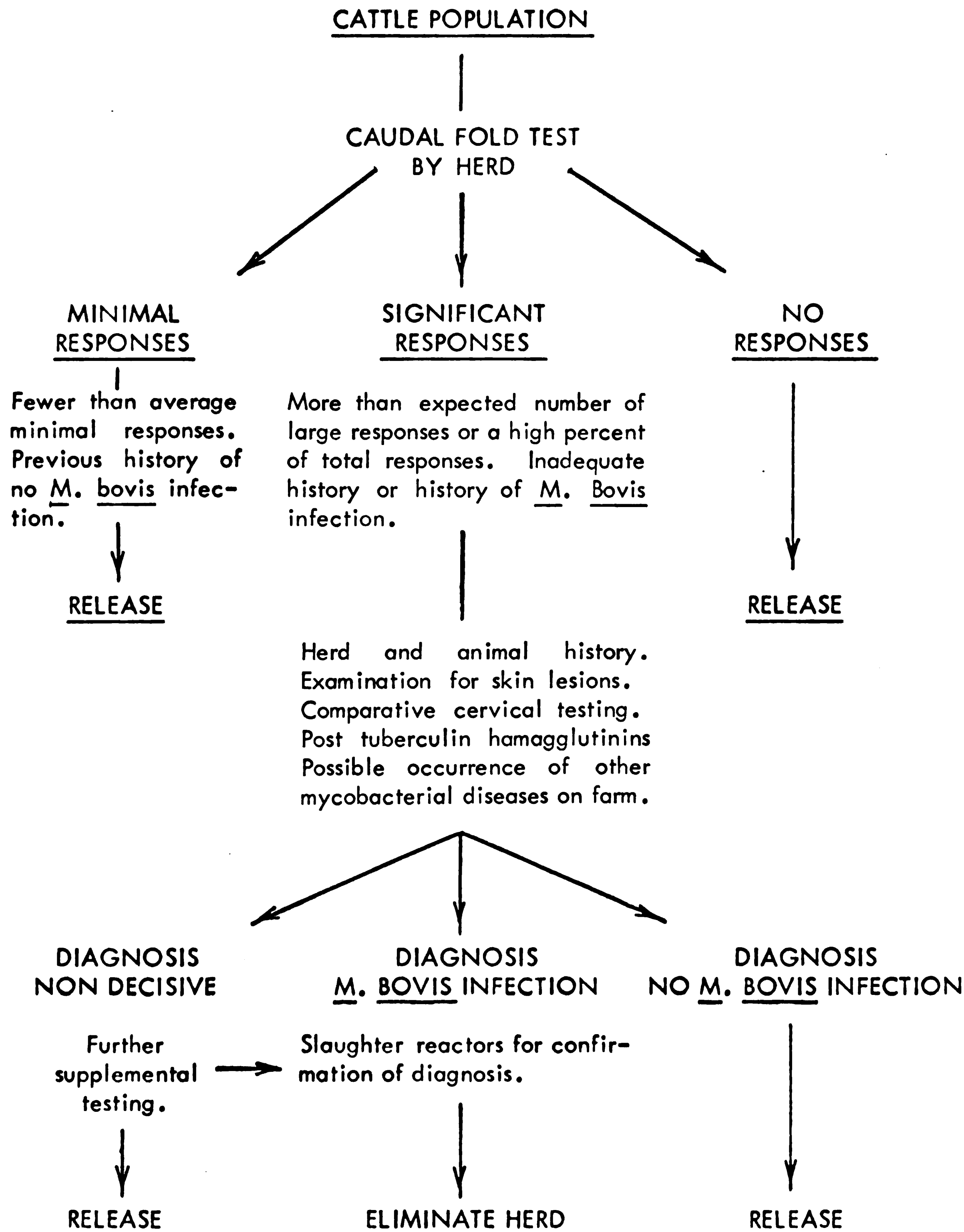
Blood samples were collected from all adults in both herds and the serums tested by the hemagglutination test. In Herd A, 23% of the samples from animals responding to the caudal fold test had anamnestic-like titer increases of 8 fold or greater. Only 5% of the corresponding animals in Herd B were this high. More significantly, three animals of Herd A and none in Herd B had post tuberculin injection titers of 640 or greater. These three in Herd A each had comparative test results with a mammalian response 4 mm or more larger than the avian response and all three had gross lesions of tuberculosis from which *M. bovis* was isolated. Of two caudal fold negative animals in Herd A, later demonstrated to have lesions of tuberculosis, one had a positive comparative test and one had a 16 fold titer increase to 640.

The results were not this clear cut in all instances. Nearly half (11 of 26) of the caudal fold test responding animals that were found to be comparative test reactors came from herds not infected with *M. bovis*. Comparison of the combined results for each of the testing methods did reveal a significant difference in caudal fold test responses and post tuberculin H.A. titers of 4 fold or more between M. bovis infected herds and non-infected herds that had comparative test reactors. Therefore, it can be seen that the combination of tests gives more accurate information about the herd than any of the methods singly.

The most definitive combination was a caudal response P1, X2 or larger, a comparative test mammalian response 5 mm or more larger than the avian, and a seven day post tuberculin H.A. titer of 640 or greater. There was no Type I error for M. bovis in this small sample. It did not detect all those in which tuberculous lesions were found or from which M. bovis was isolated. Its major value is in identifying the most likely tuberculous animals in a suspicious herd.

Table 6 outlines a suggested procedure for determining the presence or absence of M. bovis infection within an established herd of cattle. Some modifications and other supplemental tests may further increase the accuracy of the procedure. In addition to the supplemental test procedure, the epidemiologic observations and interpretations of the veterinarian conducting the test must play a major role in the final decision. Once it is established that a herd is infected with M. bovis and the responsing animals removed, the effectiveness of epidemiological investigations is reduced. Subsequent efforts to remove all animals infected with M. bovis from the herd involves repeated testing and removal of new responders. State laws and regulations generally specify a given number of negative herd tests as the basis on which the herd can be released from quarantine. The knowledge that Type II error occurs and may extend through several tests, and that such herds may be released from quarantine demonstrates a potential threat to the cattle industry.

Table 6. Suggested epidemiologic procedure for detecting *Mycobacterium bovis* infection



Once a herd infection with M. bovis has been established, it is more economical both to the herd owner and to the testing agency to eliminate the herd at that time. If the herd is allowed to continue, reactors are replaced with new purchases. Some may become infected, are infected, or may later respond non-specifically further compounding the problem. The savings achieved by the immediate elimination of the herd are recognized in time and further testing expense both to herd owner and veterinarian, and in good will between the testing agency and the cattle industry.

MODIFICATIONS

The described procedure has value and should be used; however, further studies should be made.

Berman, et al (1959) showed in a similar project in Wisconsin, that substitution of Weybridge P.P.D. tuberculins for the ARS tuberculins resulted in a decrease in the non-specific reactor rate without missing more M. bovis infected animals. Substitution of P.P.D. tuberculins for the ARS tuberculins in the comparative testing of herds with suspicious caudal fold test results should further reduce the Type II error of the caudal fold test and add to the effectiveness of this testing procedure.

Davenport (1963) demonstrated a closer antigenic relationship between skin lesion isolants and some antigens from other than those from M. bovis, M. tuberculosis, or M. avium. Skin lesions may be responsible for a large percent of the non-specific hypersensitivity. Substitution of antigens obtained from skin lesion isolants for the avian tuberculin may reduce the Type II error of the comparative test.

The English use the comparative test but read the tests at 72 and 96 hours. It has been demonstrated that delayed hypersensitivity in some *M. bovis* infected animals does not arise until 72 hours or later and persists for some period of time. Some non-specific responses are due to an immediate type hypersensitivity that may still be evident through 48 hours. Therefore, a later reading time for the comparative and perhaps even the caudal test should be investigated.

More work is required to determine the interval after the injection of tuberculin at which the maximum hemagglutination titer increase occurs and whether or not the cervical intradermal test a few days after the caudal test has any further effect on the tuberculopolysaccharide specific antibody levels.

The results of this study indicate that the caudal test may be effectively supplemented with the comparative test and the hemagglutination test; however, further studies are needed.

SUMMARY

All cattle in one Michigan dairy farming township were tuberculin tested using the method required by the State-Federal bovine tuberculosis eradication program. A large number of the responses to this test could not be attributed to infection with *M. bovis*. Two supplemental testing procedures were examined both as an individual test and as herd test procedures. Combined results of caudal tests, comparative tests and hemagglutination tests were examined to determine if the combined results would differentiate caudal test hypersensitivity due to *Mycobacterium bovis* infection from hypersensitivity due to other undetermined causes (non-specific sensitivity). All tests and procedures were used in an actual trial situation in one township. The non-specific response rate for the caudal tuberculin test was approximately 7% of the township's cattle population or 11.2% of cattle two years or over in age. Over one-third of the caudal responses were more than minimal size (classified as P1, X2 or larger).

All animals were examined for tuberculoid skin lesions. Signs of skin lesions were detected in 117 animals of which 93 were hypersensitive to tuberculin. There was a significant relationship between the occurrence of skin lesions and tuberculin hypersensitivity.

All caudal test responding animals were retested one week after the caudal test injection with a comparative cervical test using mammalian and avian tuberculins. The animals on which the response was at least 5 millimeters larger to the mammalian tuberculin than to the avian tuberculin were slaughtered unless the epidemiologic investigation had indicated that most probably no *M. bovis* infection existed. There were 26 (8%) of the caudal test responding animals

that were comparative test reactors. The 26 animals came from 12 herds, five of which were subsequently demonstrated to be infected with *M. bovis.* Fifteen of the 26 animals were from the five infected herds and half of these were found to have lesions of tuberculosis when slaughtered. The comparative test can be used after the caudal test to reduce its Type I error (indication that *M. bovis* infection exists when it does not). The Type II (indication that *M. bovis* infection does not exist when in actuality it does), and I errors of the comparative test are too high to use as an initial individual animal test. However, a number of comparative test reactors within a herd is strongly indicative of specific infection.

Blood samples were collected from all animals prior to the caudal test. One week after the injection of tuberculin blood samples were collected from all animals responding to the caudal test and an equal number of caudal test negative animals in the same herds. The serums were tested by the passive hemagglutination test for tuberculopolysaccharide specific antibodies. There was no significant difference in pre-tuberculin injection titers between animals from *M. bovis* and non-infected herds or caudal test responding and caudal test negative animals. Animals with tuberculoid skin lesions had significantly higher titers than those without. Serums from animals in each of these groups had an increase in post injection titer levels. Significant differences were found only between animals with skin lesions and those without.

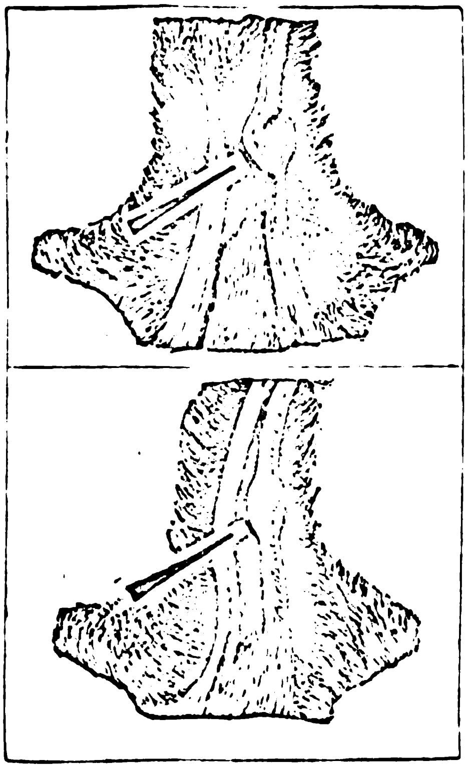
A combination of the three tests reduced both types of error. It is believed that fewer infected animals were missed and fewer non-infected animals were erroneously condemned using this procedure than would have occurred using routine tuberculin test procedures.

The results of the testing in this township indicate that a combination of certain results can identify the infected herd and some diseased animals. In six instances, *M. bovis* diseased animals were identified by a P1, X2 or larger caudal test, a comparative test mammalian response 4 millimeters larger than their avian response, and a post injection H.A. titer of 640 or greater. In the small sample tested, the combined tests failed to detect some diseased animals but identified all of the infected herds. It also falsely identified six non-infected herds as being infected with *M. bovis*. Despite this latter error, this testing procedure reduces greatly the number of epidemiologic investigations of herds that the veterinarian would be required to make, reduces the number of return trips for retesting the herd, and shortens considerably the time required to establish the presence or absence of infection in a herd.

The results of this study emphasize the characteristic shortcomings of all the present tuberculin tests. In view of the multitude of these shortcomings, and as the number of tuberculosis infected herds in the U.S.A. are quite low, it is recommended that once infection has been established within a herd that the entire herd be eliminated. Further investigative procedures could be more efficiently concentrated on finding the source of infection and possible routes of dissemination from the infected herd.

Appendix 1

USDA-ARS-ANH. Method of classifying responses to the caudal fold intradermal tuberculin test. Determined by palpation.



Caudal fold
Ventral side of tail

Circumscribed Swelling

P = Pea size 3/16" diameter.

P2 = 2 times pea size.

P3 = 3 times pea size, etc.

PP = pea type less than 3/16 dia.

Diffuse Swelling

X = thickness of caudal fold.

X2 = 2 times thickness.

X3 = 3 times thickness, etc.

Appendix 2. X^2 Analysis of data

Surveys cannot be regarded as satisfactory substitution for experiment; nevertheless, they are of value in situations in which experiments are difficult or impossible, though in such cases all conclusions must be tentative. They are also of value as a preliminary to experimental work, since they frequently indicate the factors that are likely to be most worth investigating.

Yates (1934)

X^2 analysis of the relationship between HA titers from animals in M. bovis infected herds and those in non-infected herds. Differences in the number of classes is due to grouping to obtain sufficiently large expected values.

	Pre injection HA titers	Post injection HA titers	Difference between pre & post injection titers
All Samples	X^2 8.78 N 518 df 4 Sig. N.S.	X^2 10.47 N 518 df 6 Sig. N.S.	X^2 198.22 N 1036 df 8 Sig **
Samples from animals with caudal fold test response	X^2 3.66 N 267 df 4 Sig. N.S.	X^2 8.93 N 267 df 6 Sig. N.S.	X^2 112.48 N 534 df 7 Sig. **
Samples from animals with no caudal fold test response	X^2 8.01 N 251 df 4 Sig. N.S.	X^2 4.91 N 251 df 5 Sig. N.S.	X^2 65.18 N 502 df 6 Sig. **

X^2 analysis of the relationship between HA titers from animals with skin lesions and animals without skin lesions.

	X^2 12.43 N 82 df 4 Sig. **	X^2 21.54 N 82 df 6 Sig. **	X^2 44.08 N 164 df 5 Sig. **

* .05
** .02

Appendix 2 (continued)

One degree of freedom	X^2 ANALYSIS OF THE RELATIONSHIP BETWEEN M. bovis infected herds / Non-infected herds	M. bovis infected herds / Non-infected comparative reactor herds	Animals with skin lesions / Animals with no skin lesions
Number of caudal responses	X^2 86.12 N 3550 Sig. **	X^2 26.65 N 557 Sig. **	X^2 815.12 N 3972 Sig. **
Number of caudal responses P1, X^2 and larger	X^2 0.41 N 310 Sig. N.S.	X^2 0.13 N 92 Sig. N.S.	X^2 3.97 N 322 Sig. *
Number of comparative reactors	X^2 27.70 N 322 Sig. **	X^2 3.37 N 557 Sig. N.S.	X^2 1.81 N 380 Sig. N.S.
Number of HA titer increases of 4 fold or greater	X^2 4.13 N 518 Sig. *	X^2 7.32 N 147 Sig. **	X^2 2.29 N 518 Sig. N.S.
Number of HA titer increases 16 fold or greater	X^2 1.16 N 518 Sig. N.S.		X^2 2.27 N 322 Sig. N.S.
Number with skin lesions	X^2 5.49 N 3530 Sig. **	X^2 0.75 N 557 Sig. N.S.	
Number of HA titers that decreased	X^2 2.65 N 518 Sig. N.S.		X^2 0.03 N 518 Sig. N.S.

* .05
** .02

Appendix 3 - Post Mortem and Laboratory Results

Herd No.	Animal Identification	Tissue Processed	Gross Pathology	Histological Pathology	Tissues From Which Organisms Were Isolated	Culture Identification
1	91-64	ABCDEFH	NGL ex. F	NML ex. F caseation & giant cells	1 AF on plate	Not obtained in pure culture
5	92-64	ABCDEFH	NGL ex. F	NML	A & F mxd	IFT
8	(6074)	No Sample	NGL	No Sample		
14	239-64	ABCD	NGL		A	IFT
	85-65	D(supramammary)	NGL	NML	D	probable III
						GP NGL
	91-65	ABCD I-adrenal	NGL	NML ex.adrenal	None	GP NGL
						No isolations
	183-65	ABCD	NGL	NML	A	Group III
	186-65	A-B pool, CD	NGL	NML	C	in process
18	(3088)	No Sample	NGL	No Sample		
24	(25344)	No Sample	NGL	No Sample		
	(2986)	No Sample	NGL	No Sample		
	(2987)	No Sample	NGL	No Sample		
26	(4777)	No Sample	NGL	No Sample		
	(2763)	No Sample	NGL	No Sample		
38	42-64	ABCDE	Some GL	AF seen in E some NML	BCE	*M. Bovis*
	43-64	I	GL	AF seen	I	*M. Bovis*
	44-64	I	GL	AF seen	I	*M. Bovis*
	45-64	I	GL	ML,AF not seen	I	*M. Bovis*
	99-64	I-node, E	abscess	NML,AF not seen	I	IFT
	100-64	I-node	NGL	NML	None	
	101-64	F, I-liver	GL	ML,AF not seen	F	IFT,GP
						No isolations
	102-64	I-node	NGL	NML	None	
	103-64	I-tongue	GL	AF not seen	Histo. only	
	104-64	F	open Cyst	thickening	Histo. only	
	105-64	F, nodes	F-GL, Nodes	Node-NML		
		muscle	Muscle-NGL	AF not seen	I F	*M. bovis*

Appendix 3, Continued

Herd No.	Animal Identification	Tissue Processed	Gross Pathology	Histological Pathology	Tissues From Which Organisms Were Isolated	Culture Identification
38	106-64	E, nodes	NGL	NML	I-nodes	IFT GP A.F. isol.-IFT
	107-64	Nodes	NGL	NML	None	
	108-64	E-histo.nodes	Nodes-GL E-NGL	ML	I-nodes	*M.bovis* or III
	109-64	nodes, I-liver	GL	Liver-ML Node-NML	None	
	110-64	E, nodes	E-GL Nodes-NGL	E-ML Nodes-NML	None	
	111-64	I-nodes	Nodes-NGL	NML	None	
	112-64	I-node	GL	ML	Histo.only	
	113-64	I-node	NGL	NML	None	
44	40-64	ABCDEHI-liver	NGL	NML	D I-liver	IFT
62	53-64	ABCDEH	A & Med rest NML	Mediastinal GL rest NML	ABC	*M.bovis* or III GP, probable *M.bovis*
	15-65	ABCDEH	prescap GL rest NGL	prescap. ML	AEH	GP NGL,AF Isolated,IFT GP NGL,No isolation
	16-65	I abscess	NGL	NML	I	GP NGL, No isolation,IFT
	129-65	ABCDEH	NGL	NML	H	mixed-not typed
	130-65	D-prescap	NGL	NML	None	
	135-65	D, I-liver	I-GL D-NGL	NML	D	Rapid-Grower
80	182-65	ABCDEH	NGL	NML	None	
	215-64	ABCDEH-histo only	NGL	NML	BE	Rapid Grower
83	(0018)	No Sample	NGL	No Sample		
	(2940)	No Sample	Skin Lesions only	No Sample		
	(4611)	No Sample	Skin Lesions only	No Sample		
	(4706)	No Sample	NGL	No Sample		
87	(5405)	No Sample	NGL	No Sample		
	54-64	C	GL	Lesions but may not be due to TB		

Appendix 3, Continued

Herd No.	Animal Identification	Tissue Processed	Gross Pathology	Histological Pathology	Tissues From Which Organisms Were Isolated	Culture Identification
87	505-64	ABCDEH	C & Mam GL	NML	AB not pure culture	Not typed
	208-65	I-liver		NGL	not completed	
	60-64	ABCDE	NGL ex E	NML	B	_M._bovis or III GP NGL
88	59-64	B-mediastinal	GL	ML	B	GP GL, Probable _M._bovis
	238-64	Pooled nodes	NGL		C	IFT
89	58-64	ABC-hepatics DE only	A-GL rest NGL	A-ML rest NML	AC	GP-GL Probable _M. bovis_
	506-64	ABCDEH	NGL	NML	ABDEH	IFT

Code:

1. Tuberculosis project case number or animal's ear tag number

2. A - Cervical - Head & Neck
   B - Thoracic
   C - Mesenteric & hepatic
   D - Body (pre fem
   Sup. mamm
   pre scap)
   E - Lung
   G - Mammary gland
   H - Peyers Patches
   I - Lesions or other
   F - Skin
   IFT - Insufficient growth for typing
   NGL - No Gross Lesion
   GL - Gross Lesion

NML - No Microscopic lesion
ML - Microscopic lesion
AF - Acid fast organism
GP - Guinea pig
III - Runyon group III mycobacteria

LITERATURE REVIEW

Alshabphoun, A., Chapman, P. T., White, M. F. and Degroat, A. 1960. A study of the double-diffusion gel precipitation test in tuberculous patients. Am. Rev. Tuberc. Pulmonary Dis. 81:704.

Berman, D. T., Tervola, C. A., and Erdmann, A. A. 1959. Preliminary report of investigations of tuberculin sensitivity in Wisconsin cattle. Proc. U. S. Livestock San. Ass'n., 63rd Ann. Meeting, p. 197-204.

Bodie, G. F. 1962. Diagnostic methods in veterinary medicine. 5th ed., p. 289. J. B. Lippincott Co., Philadelphia.

Chaloux, P. 1964. Editorial. Tuberculosis Review of the USDA, ARS, An. Disease Erad. Div. 1: p. 8.

Crawford, A. B. 1936. Tuberculin reactions in cattle showing no visible tuberculosis lesions on post mortem. J. Am. Vet. Med. Assoc. 89: 562-583.

Davenport, C. A. 1963. The precipitinogen relationships among mycobacteria of bovine, porcine, human, and soil origins. Ph.d. Thesis, Michigan State University, East Lansing.

Duboczy, Ela. 1965. Nonspecific early type of tuberculin reaction. Am. Rev. Resp. Dis. 92: 1 55-63.

Edwards, P. Q. & Edwards, L. B. 1960. Story of the tuberculin test. Am. Rev. Resp. Dis. 81(1): 1-47.

Fincher, M. G. 1959. Proc. Tuberculosis Eradication Conference, Kansas State Univ. 142-144.

Franchis, John. 1958. Tuberculosis in animals and man. 1st ed., Cassell & Co. Ltd., London.

Gregory, T. S. 1949. The accuracy of diagnostic methods used in the detection of tuberculous cattle. Aust. Vet. J. 25(7): 138-152.

Grey, D. F. 1953. The significance of hemagglutination titers in bovine tuberculosis. Aust. Vet. J. 29: 293.

Hagan, W. A. 1931. The no-lesion case problem in the tuberculosis eradication campaign. Corn. Vet. 21:163.

Hagan, W. A., Feldman, W., Traum, J. Schoening, H. W., Wight, A. E., Hastings, E. C. 1945. Proc. U. S. Livestock San. Ass'n. 49th Ann. Meeting. Report 1944 of committee on tuberculosis. p. 142-144.

Hastings, E. C., Wisnicky, W., Beach, B. A., & McCarter, J. 1933. A detailed study of no-lesion tuberculin reacting cattle. J. Am. Vet. Med. Ass'n. 82: 565.

Hastings, E. G. 1914. What has been done with the tuberculin test in Wisconsin. Bull. Univ. Wisconsin Agr. Exper. Station. No. 243.

Hastings, E. G., Beach, B. A., and Weber, C. W. 1924. No-lesion and skin-lesion tuberculin reacting cattle. J. Am. Vet. Med. Assoc. 66:36.

Hebert, C. N. & Paterson, A. B. 1955. Tuberculin reactivity in a sample of attested herds. Vet. Rec. 67:1143.

Henley, R. R. 1951. History of the bureau's work with tuberculin. Proc. 55th Ann. Meeting. p. 273.

Johnson, R. B. 1945. Studies on the complement-fixation reaction as applied to bovine tuberculosis. Corn. Vet. 35: 52-60.

Karlson, A. G. 1959. Reciprocal or cross sensitivity reactions to tuberculin in cattle. Tuberculosis Erad. Conference, Kansas State University. 48-60.

Kerr, W. R., Lamont, H. G. & McGirr, J. L. 1949. Further studies on tuberculin sensitivity in the bovine. Vet. Rec., 61: 466.

Larson, A. B., Kopecky, K. E. Sept. 1964. New bovine tuberculosis test. Ag. Res. p. 14.

Mallmann, V. H., Robinson, P., McGavin, M. D. 1963a. An anamnestic like reaction elicited by tuberculin. Am. J. Vet. Res. 25 (106): 693-698.

Mallmann, V. H., Mallmann, W. L., & Robinson, P. (1964) Relationship of atypical bovine and porcine mycobacteria to those of human origin. Health Laboratory Science, 1(1): 11-20.

Mallmann, W. L., Mallmann. V. H., Ray, J. A., McGavin, M. D. Ellis, D. L. 1963b. Infectivity of Atypical Group III mycobacteria of NGL cattle, Swine & Soil origin. Proc. Am. Vet. Med. Ass'n. 100: 265-267

McGavin, M. D., et al. 1964. Experimental inoculation of calves with unclassified mycobacteria by the intradermal route. Final report of the Michigan State University bovine tuberculosis research contract with the USDA. 9-46.

McLaughlin, A. R. 1962. A study of tuberculin sensitivity in Wisconsin cattle. M. S. Thesis, Univ. Wisconsin, Madison.

Middlebrook, G., & Dubos, R. J. 1948. Specific serum agglutination of erythrocytes sensitized with extracts of tubercule bacilli. J. Exptl. Med. 88: 521.

Moorehouse, L. G., et. al 1963. Examination of bovine specimens suspected of being tuberculous, Proc. U. S. Livestock San. Ass'n. 67th Ann. Meeting. pp. 430-437.

Munce, T. E. 1920. Tuberculosis eradication in Pennsylvania. J. Am. Vet. Med. Ass'n. 9: 14.

Parlett, R. C., Youmans, G. P. 1959. An evaluation of the specificity and sensitivity of a gel double-diffusion test for tuberculosis. ART Res. 80: 153-166.

Paterson, A. B. 1956. The incidence and causes of tuberculin reactions in non-tuberculous cattle. Adv. Tuberc. Res. 7: 101-129.

Plum, N. 1937. Studies on tuberculosis and tuberculin tests. Skand. Vet. Tidskr. 27: 589.

Ranney, A. F., Chaloux, P. A., & Schwindaman, D. 1964. Comparative field studies using dilute and standard tuberculins. Tuberculosis Review 1: p. 1.

Ranney, A. F. 1966. Personal communication.

Rheins, M. S. and Thurston, J. R. 1955. Additional tuberculous antibodies demonstrated with erythrocytes sensitized by successive adsorptions of OT and further studies on the adsorptive capacities of tanned cells. Am. Rev. tuberculosis. 72: 210-217.

Ritchie, J. H. 1959. Tuberculosis eradication, p. 713-739. In Stableforth & Galloway (ed.), 1959. Diseases due to bacyeria. Vol. 2. Academic Press, Inc. N. Y.

Robertson, T. G. 1963a. Evaluation of the short thermal and repeat caudal fold tests used as ancillary tests on initial reactors to the caudal fold test. New Zealand Vet. Journ. 11: 11-15.

Robertson, T. G. 1963b. The valuation of tuberculin tests. New Zealand Vet. Journ. 11: 6-10.

Sjollema, P. 1954. Causes of non-specific reactions to the tuberculin test in cattle. Tydschr. Diergeneesk. 78. p. 761. 1953.

Smith, D. T. and Scott, N. B. 1950. Clinical interpretation of the Middlebrook-Dubos hemagglutination test. Am. Rev. Tuberculosis 62: 121.

Stableforth, A. W. & Galloway, I. A. 1959. Diseases due to bacteria Vol. 2. Academic Press, Inc. New York.

Stenius, R. 1949. Experience gained in the control of bovine tuberculosis in Finland. Proc. 15th Int. Veterinary Congress, 2: 221-224.

Tervola, C. A. 1960. A study of the transitory nature of tuberculin hypersensitivity in Wisconsin cattle. Unpublished report. USDA ARS, ADED-Wisc.

Traum, J. 1959. Skin lesions and their significance in tuberculosis eradication. Proc. Tuberculosis Eradication Conference, Kansas State University. pp. 60-67.

USDA, ARS, ANH. 1965. Tuberculosis eradication progress report. p. 39.

VanWaveren, G. M. 1953. Proc. XVth Int. Vet. Congre. Stockholm, Part I, p. 140.

Vardman, T. H. & Larsen, A. B. 1962. A comparison of the hemagglutination, hemolytic, and complement-fixation tests on serums from intradermal bovine tuberculosis reactors. Am. J. Vet. Res. 23(93): 274.

Vardman, T. H. & Larsen, A. B. 1964. The complement fixation test for detecting tuberculosis in cattle. Am. J. Vet. Res. 25 (106): 690.

Walker, C. C. 1920. Tuberculin Tests. J. Am. Vet. Med. Ass'n. 9:5-13.

Wight, A. E. 1939. Tuberculosis in livestock. U.S.D.A. Farmers bulletin No. 1069. p. 16.

Williams, J. E. 1958. Our present tuberculin. Proc. Tuberculosis eradication conference, Michigan State University. 41-44.

Willigan, D. A., McGavin, M. D. & Goyings, L. S., Unpublished data. MSU Tuberculosis Project.

Yates, F. 1949. Sampling methods for censuses and surveys. p. 128. Chas. Griffin & Co. Ltd. London.