THE RAPID DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* BY MICROSCOPIC EXAMINATION OF MILK FROM INDIVIDUAL COWS AND FROM GROUPS OF TEN COWS

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The success of detecting *Mycobacterium tuberculosis* in milk by microscopic examination of samples from each quarter of the udder and the use of this method in rapidly eliminating from a herd those cows which were excreting tubercle bacilli has been reported (Maitland, 1937). The advantages from the clinical point of view with illustrative clinical data have been recorded by Locke (1937), who collaborated in the investigation.

Quarter samples were examined microscopically from 950 cows and compared with the results of injecting guinea-pigs. The technique was fully described in the earlier paper. The main points were that the milk was centrifuged at a slow speed for a short time to deposit cell groups with a minimum of other cellular sediment. Films were made so that the cell groups were distributed around the edges of one end of the slide. Tubercle bacilli were sought for in the cell groups.

In only one case was the microscopic finding not the same as the result from biological examination of the milk or post-mortem examination of the cow. In this case cell groups were found microscopically, but no tubercle bacilli, although these were found later by another examiner. No false positive results were obtained.

It was possible to examine all the samples from one herd and frequently to detect the offending cow or cows within 2 days of taking samples. The maximum efficiency in applying this method requires close collaboration between the veterinary inspector and the laboratory worker.

During the years 1937–9 attempts were made to evolve a method which would make possible the microscopic detection of *Myco. tuberculosis* in milk from individual cows and in samples of mixed milk, i.e. milk from several cows. This eventually led to the development of a successful procedure, but the following five types of experiment are recorded although they turned out to be unsatisfactory for the purpose in view.

(1) *Phagocytosis of* Myco. tuberculosis. It was thought possible that small pieces of guinea-pig tissue (liver or spleen) if put into a suspension of *Myco. tuberculosis* would phagocyte the organisms, the tissue could be removed and films made from it and stained to show the *Myco. tuberculosis*. Suspensions of cultures of this organism were made and treated in this way. It was found that

* This work was supported and made possible by a grant from the Milk Marketing Board which is gratefully acknowledged.

phagocytosis did occur. Experiments were made to try to find the best size of tissue pieces to use and the best length of time to leave them in contact with the suspension. The results were then applied to the deposit, after centrifuging, from milk known to contain tubercle bacilli. It was found that there was so much phagocytosis of many sorts of organisms and of dirt that *Myco. tuberculosis* was seldom detected.

(2) *Concentration of* Myco. tuberculosis *by dissolving the deposit obtained after centrifuging.* If the milk is spun quickly for long periods in order to bring down as many of the tubercle bacilli as possible, the resulting sediment is so heavy that films made from it are too thick to show the organisms or the cell groups in which they lie. It seemed possible that this sediment might be dissolved, and leave unharmed the organisms we wished to detect, by using solvents that would not penetrate the fatty layer around the *Myco. tuberculosis*. Accordingly milk known to contain tubercle bacilli was spun for long periods at high speed and to the sediment was added (*a*) concentrated hypochlorite solution, and (*b*) concentrated Schweitzer's solution. This was left in the incubator and spun again at high speed for a long time. A great many speeds, times, concentrations of dissolving fluid, time and temperature of action of these solutions, were tried but none was satisfactory. The *Myco. tuberculosis* was found in comparatively few instances.

(3) *Adding a solvent to milk before centrifuging.* This series of experiments was the same as those in § 2, except that the solvent was added to the milk before it was spun in place of being added to the sediment after spinning. The results were in no way more reliable.

(4) *Cultivation of* Myco. tuberculosis *in guinea-pig embryo tissue.* Tissue cultures, similar to those described by Maitland & Maitland (1928), were made using minced guinea-pig embryo, guinea-pig serum and tyrode solution in small flasks. These were inoculated with cultures of *Myco. tuberculosis* and there was definite growth in a week. When attempts were made to cultivate *Myco. tuberculosis* from milk in this medium it was found that it was very difficult to kill other organisms in the milk and not leave sufficient of the destructive agent to destroy the cells in the tissue cultures. This problem was not studied exhaustively but sufficiently to indicate that it would probably have little value in a rapid routine examination of milk.

(5) *Reduction of the volume of milk by clotting and by drying.* These experiments were an attempt to reduce the volume of milk to be examined without reducing the number of *Myco. tuberculosis.*

(*a*) Milk was clotted with concentrated hydrochloric acid and filtered. The clot containing the organisms was then dissolved with trypsin and spun for a long time to deposit the *Myco. tuberculosis.*

(*b*) Milk was dried *in vacuo* from the frozen state. The powder was dissolved with trypsin and spun for a long time at high speed.

Although variations of time of clotting, drying, dissolving and spinning were tried with variations of quantities of hydrochloric acid and trypsin, and temperature of clotting and dissolving, no results were comparable in accuracy with the direct examination of films of sediment from the untreated milk.

EXAMINATION OF A LARGE VOLUME OF MILK FROM INDIVIDUAL COWS AND MIXED MILK BY SLOW-SPEED CENTRIFUGATION

For examination of quarter samples 15–50 ml. or even less were spun. The centrifuge buckets in use held, when full, approximately 50 ml. First attempts to examine larger quantities of milk were made by filling eight of these buckets, spinning as usual, pouring off the top milk and putting small amounts of the milk with the sediments into another tube, thus 'bulking' the sediments. This was spun again and the sediment examined as for quarter samples.

This technique was applied to mixed milk prepared in the laboratory by diluting a known positive sample with milk which was microscopically negative for *Myco. tuberculosis*. It was possible to dilute the milk very considerably and still find cell groups and *Myco. tuberculosis* when this large amount, 400 ml., of milk was used for the examination.

Finally, a centrifuge fitted with large round-bottomed glass tubes holding at least 160 ml. was used. The milk was spun at about 2500 r.p.m. for 3 min. and the slides made and examined as before. A number of samples of milk (that is milk from one cow, not quarter samples) known to contain *Myco. tuberculosis* were diluted with milk microscopically negative for tubercle bacilli and examined. The organisms were always found up to a dilution of 1 in 32, and the highest dilution giving positive results was 1 in 128. The milk used for diluting the known positive samples was one which gave very little sediment even when large quantities were spun. This, of course, made the examination of the films easier than it otherwise would have been, because the cell groups were not masked by other material. It was thus evident that *Myco. tuberculosis* could readily be detected microscopically in milk from individual cows, and that there was good reason for believing that it should be possible to apply microscopic examination successfully to mixed milk from several cows.

The only way to determine the general usefulness of the method seemed to be to get samples direct from farms where the milk would be mixed. As a beginning it was thought that the milk from ten cows should be mixed so that there would not be too much sediment even if one or more cows in the group gave milk with an abnormally large amount of sediment.

As with previous work, Mr Locke gave his willing co-operation. When he had a report that milk from a particular farm was positive for *Myco. tuberculosis* by biological test he examined the herd clinically and took single samples from individual cows which might be the source of infection, and bulk samples from the remainder of the herd in groups of ten as nearly as practical. During the year April 1938–April 1939 we examined 108 herds in this way with control examination by guinea-pig inoculation. This was an attempt to clear rapidly herds (known to be producing tuberculous milk 3–6 weeks earlier) by examining milk microscopically from individual cows or groups of ten. When the microscopic examinations were completed all milks were sent for guinea-pig inoculation. During the interval required to obtain the result of guinea-pig inoculation, some suspicious cows were sold or had gone dry, so that some animals may have escaped detection. This

occurred with twenty-five herds which now showed no *Myco. tuberculosis* present in the milk on microscopic examination, confirmed by guinea-pig inoculation. The results from the remaining farms are considered in three groups as follows:

(*a*) *Microscopic examination of individual samples which confirmed clinical evidence of tuberculosis of the udder.* On fifty-nine farms there was clinical evidence of tuberculosis in the udder of one or more cows. Milk from these individual cows showed tubercle bacilli microscopically and in every case there was a tuberculous lesion in the udder post-mortem.

(*b*) *Group samples found to be positive microscopically leading to detection of the individual cow.* Thirteen samples of milk from groups of cows were found to contain *Myco. tuberculosis* on microscopic examination. The milk from each cow in the group was examined microscopically and the cow giving the milk containing tubercle bacilli was killed, and in each case there was some tuberculous lesion of the udder. These cows were not obviously tuberculous clinically and were not therefore sampled individually in the first instance, but owing to the rapidity of microscopic examination it was possible to take individual samples 24–48 hr. later.

In these instances the success of microscopic examination of group samples was demonstrated as well as the value of microscopic examination of individual samples in detecting tuberculous mastitis which was not apparent clinically.

(*c*) *Group samples which were negative microscopically but positive by guinea-pig inoculation.* This occurred with one group sample from each of nineteen farms. Each farm was re-visited, and more samples were examined, that is some 6–7 weeks after the first sampling. Milk from five of these nineteen farms was now found to contain tubercle bacilli on microscopic examination. No tubercle bacilli were found on microscopic examination of the milk from the remaining fourteen farms, and this was confirmed by guinea-pig inoculation. In some cases cows had been sold or gone dry, but from at least one farm the milk had come from the same cows as on the first examination.

Summary. In practice the results of using the microscopic method of examining milk from groups of cows as well as from single cows were such that 88% of the 108 farms in this series were clear of cows giving *Myco. tuberculosis* in the milk 24–72 hr. after Mr Locke's first visit to the farm, and the remaining 12% were cleared within 6–7 weeks, i.e. 24–48 hr. after a control sample of milk from the first visit was reported to contain tubercle bacilli as a result of guinea-pig inoculation.

ROUTINE EXAMINATION OF BULK SAMPLES

To carry the investigation a stage further still, it was decided to apply the microscopic examination of bulk samples to milk that was being tested in a routine way by local authorities. The Medical Officer of Health of Lancashire very kindly arranged for co-operation of the sanitary inspectors in this district. When these inspectors went to a farm to take samples of milk for guinea-pig inoculation they took samples from groups of ten cows (as nearly as possible) and sent these for microscopic examination as well as for inoculation into guinea-pigs. Milk from a series of farms has been examined in this way.

All but three farms were negative both microscopically and by guinea-pig

inoculation. Milk from two farms was found to contain *Myco. tuberculosis* on microscopic examination. Single samples were then taken and the cow was found and killed within 6 and 7 days respectively after the first sample. In the third case, when the sample was found to contain *Myco. tuberculosis* only on guinea-pig inoculation, the report was made in 5–6 weeks and the herd re-examined. No udder tuberculosis was found clinically, no *Myco. tuberculosis* were found in the milk on microscopic examination and a control inoculation into guinea-pigs gave negative results. Some of the cows in the herd had gone dry during the time taken for the first test.

CONCLUSIONS

The microscopic examination of milk from individual cows and mixed milk from groups of ten cows is a practical procedure. It can be applied to the routine testing of milk from herds without recourse to preliminary guinea-pig inoculation. No false positive results by microscopic examination have been noted.

If samples from individual cows only were examined, excretors would be detected within 24–48 hr. and very few would be missed.

If a combination of group and individual samples were examined as described in this paper, the majority of herds could be cleared of cows excreting *Myco. tuberculosis* within a week. It is a matter of opinion and circumstance whether guinea-pig inoculation of bulk milk should be carried out as a final check. Guinea-pig inoculation is the most sensitive method of detecting *Myco. tuberculosis* and the standard of reference by which the efficiency of other procedures should be judged.

It has been shown that the microscopic examination of quarter samples is practically as sensitive a test as guinea-pig inoculation. In our experience the microscopic examination of milk from individual cows is very little inferior provided the technique described is followed. The advantages and limitations of examining bulk samples microscopically have been indicated. The advantages greatly outweigh the limitations.

The maximum efficiency of microscopic examination of milk depends upon attention to details of technique, especially time and speed of centrifuging, method of making, staining and examining films. The successful application of the method in eradicating udder tuberculosis depends upon the close collaboration with the laboratory of those who take samples.

The collaboration of Mr R. D. Locke, M.R.C.V.S., D.V.S.M. (Vict.), formed an essential part of this investigation and is gratefully acknowledged. We are also indebted for the willing assistance given by Dr F. Hall, C.B.E., Medical Officer of Health in Lancashire, at the time of this investigation, and the sanitary inspectors who took part in its final phase.

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(*MS. received for publication* 14. II. 50.)