ON THE INFECTIVITY OF THE MILK OF TUBERCULOUS COWS, THE BACTERIOLOGICAL DIAGNOSIS, AND THE PRACTICAL VALUE OF TUBERCULIN IN THE EXTIRPATION OF TUBERCULOSIS AMONG CATTLE.¹

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THE frequency and constant increase of tubercular disease among cattle has long ago drawn serious attention to the question whether the milk of tuberculous cows is capable of carrying infection to other animals. By means of minute and varied experiments it has been established beyond doubt that such is indeed the case: tuberculosis can certainly be produced in calves and swine through feeding them with infected milk. Nevertheless, it still remained to be shown how very common this mode of infection is under natural conditions. The magnitude of this danger—*viz.*, the spreading of tuberculosis among animals due to the consumption of milk—is best illustrated by the frequent occurrence of tuberculosis among calves, and by the alarming increase of the same disease among swine which has been observed within the last few years. About 90 per cent. of the latter cases have been proved to originate in feeding with infected milk.

The frequent occurrence of intestinal infection need not be wondered at when we consider the extent of tubercular disease among milch cows, as well as the fact that for a considerable length of time after birth the young calves are almost exclusively fed with their mothers' milk. The increase of tuberculosis among swine is further explained by the fact that the young pigs are likewise raised on cows' milk, the principal fodder of pigs in our days of large dairies consisting in the raw dairy residues, made up of the deposits and the lean milk left over from the centrifuging process.

The transmission of tuberculosis among cattle and pigs by means of milk being established, many years ago, it now remains to be seen whether the milk of all tuberculous cows contains tubercle bacilli, or whether the infectious properties are confined to special forms of tubercular disease.

The investigations of Bollinger, Bang, and a great number of other authors, have established, many years ago, that the milk of cows suffering either with general tuberculosis or with tuberculosis of the udder contains tubercle bacilli. A few observers, such as Hirschberger and others, have experimentally demonstrated the infectious quality of milk derived from cows with incipient tuberculosis and non-tuberculous udders.

Since the important discovery of tuberculin, however, there has been no examination into the question as to whether tubercle bacilli may not be present in the milk of animals which do not show any clinical signs of tuberculosis, but which nevertheless are shown, by their susceptibility to the tuberculin test, to be tuberculous. A positive answer to this question was furnished in 1899 by joint investigation of myself and Dr Kempner. We found that tubercle bacilli may be

¹ From the Transactions of the British Congress on Tuberculosis.

present in the milk in cases of tuberculosis, latent, but revealed by the tuberculin test. If, then, the milk of such cows contains tubercle bacilli, it must needs be regarded as infectious, and the consumption of such milk must be held no less dangerous to young animals than that coming from cows with advanced general disease or with diseased udders. In these, as well as in my subsequent experiments, I tested the presence of tubercle bacilli in the milk by means of intra-peritoneal injection on guinea-pigs, as I consider this method to be not merely satisfactory, but, indeed, the only safe method. The alternative way to prove the infectious character of milk, *viz.*, by feeding animals with it, is unreliable, since the quantity of tubercle bacilli thus introduced in the course of one or several experiments is greatly inferior to the amount which the animals would actually consume within a given period of time as part of their food. In the long run even a very slightly infectious kind of milk is bound to produce the disease.

The presence of tubercle bacilli in the milk of cows that respond to the tuberculin test without showing clinical evidence of tuberculosis was proved in the same year by the thorough experiments of Adami and Martin. A further proof in support of this theory was furnished by my last year's observations on milk supplied to infants in Berlin. I found no tubercle bacilli in the milk taken from cows which had been regularly subjected to the tuberculin test, while the supply obtained from animals not subjected to this test contained tubercle bacilli, though they showed no clinical symptoms of tuberculosis.

My own bacteriological observations of former years, as well as the more recent investigations concerning chiefly the milk of cows with suspected udder tuberculosis, established the following facts:—

The detection of tubercle bacilli by means of microscopical examination of cream and sediment specimens of centrifuged milk is unreliable, partly because the absence of acid-resisting bacilli in the examined specimens cannot be considered convincing, as their number may be so small as not to be found in every slide examined under the microscope, whilst even a very small number of tubercle bacilli can be detected by animal experiment. Besides, there occur in milk bacteria which, with regard to their growth, as well as to the effect they have on the animal organs, are very similar to the acid-resisting bacilli which were found by Koch in butter, and which, under the microscope, are unfortunately very difficult to distinguish from tubercle bacilli. These bacilli were not only found by Petri, Beck, Santori, myself, and others in the ordinary market milk, but we were also able to detect and isolate them by animal experiments from specimens of milk obtained from individual cows under aseptic conditions. The above-mentioned discovery of the acid-resisting bacilli, so similar in their nature and appearance to tubercle bacilli, in the milk of cows suspected of tubercular disease, made by our observations some years ago, has been recently confirmed by the experiments of Kühnau. This investigator was able, both in specimens of mixed milk and also in milk taken under aseptic conditions from individual cows, to prove by animal experiment the presence of those bacilli in 20 to 25 per cent. of the cases examined. Whether these bacilli are secreted by the mammary glands, or whether they enter the milk ducts of the frequently inflamed udders from without, is still uncertain, and is for the differential diagnosis of these bacteria without importance. In the

light of recent investigations we certainly need not be surprised to find these bacilli in the milk. Already years ago Severin found the same kind of bacteria in cow-dung, while Olt demonstrated their presence in the contents of the intestine of tuberculous as well as of healthy cattle. Moëller further succeeded in obtaining isolated cultures of these acid-resisting bacteria from the cow-dung as well as from the timothy grass used as fodder. The same kind of acid-resisting bacteria, or similar ones, have been recently detected by Cowie on the udder, and by Ludwig Neufeld in the mammary gland of the cow. Besides these acid-resisting bacteria, Nocard and Mollereau, Bang, and others, detected, some years ago, saprophytic as well as pathogenic bacteria, esp. streptococci, in the udder of cows suffering from mastitis. These discoveries were recently confirmed by the experiments of Ward as well as by those of Reed and Ward. The last-named authors have even isolated streptococci, along with harmless bacteria, from the milk of cows which were apparently healthy, showing clinically no signs of mastitis. When the udder of the slaughtered animals was examined, streptococci of the same kind were found to be present in all parts of it. The other bacteria, too, which were found in the udder after the cow was slaughtered, proved identical with those previously isolated from the milk. That streptococci may be present in the milk a long time after the mastitis has disappeared, has recently been proved by the experiments of Lameris and van Harreveld.

In view of these facts, the presence in cows' milk of the bacteria which resemble tubercle bacilli is, after all, not difficult to explain, as these microbes, as well as streptococci, can either enter the udder from the outer world through the teat, or they can be taken in with the food and absorbed by the blood and lymph-stream from the intestinal canal, and carried thence to the udder. In the present state of our knowledge it is not yet certain whether these bacteria can be the cause of the mastitis, or whether the mastitis existed already previously, the inflamed tissue furnishing a suitable nourishing medium for the development of the microbes. As these acid-resisting bacilli are sometimes visible in clusters in a specimen of centrifuged milk, a microscopical differentiation of them from the genuine tubercle bacilli, especially when both kinds are present mixed in one and the same specimen of milk, is rendered extremely difficult or even impossible.

The only and the safest way to prove the presence of tubercle bacilli in milk is, and remains therefore, the animal experiment, namely, the intra-peritoneal injection into guinea-pigs of a mixture of the cream and sediment of thoroughly centrifuged milk. The following interesting experiment made by Ostertag shows how very susceptible the guinea-pig is when subjected to the intra-peritoneal inoculation. The milk of a cow with tuberculous udder which, when 20 ccm. of it were given through the mouth to a guinea-pig, produced intestinal tuberculosis, was able to produce general tuberculosis when injected into the peritoneal cavity of guinea-pigs, even after it had been diluted a hundred thousand times. By comparative experiments I was, indeed, able to prove that the last milk obtainable from the udders of suspected cows gave positive results in regard to the presence of bacilli, whilst the first milk obtained from the full udders was not able to convey tuberculous infection in the animals.

It must further be noted that it is necessary to examine the suspected milk repeatedly, and at various times, as a single negative result cannot be considered finally convincing. Although it is easy to prove the presence of a small number of tubercle bacilli by means of inoculation, the result of the experiments may be obscured in cases where changes are produced in the tissues resembling tubercle when examined with the naked eye, but which in reality have been caused by the action of the above-mentioned acid-resisting bacilli. Their pseudo-tubercular nature can easily be proved when examined under the microscope. But, as we have already seen, real tubercle bacilli may be found in the tissues along with the acid-resisting bacilli; it is, therefore, advisable to carry on, in addition to a histological examination, inoculation experiments with parts of the suspected tissues, until positive results are obtained. Thus the process of diagnosing tubercle bacilli by means of experiments on animals may become very tedious, and two or even three months may elapse before we are able to decide whether the milk of the cow suspected of tuberculosis really contains tubercle bacilli.

The presence of tubercle bacilli in the milk does not as a rule correspond to the extent of the disease in the animal, as we had already occasion to mention. It must be admitted, though, that in cases of extensive general tuberculosis or udder tuberculosis the presence of tubercle bacilli in the milk may be looked for. How very often the clinical diagnosis of udder tuberculosis meets with great difficulties, even in the hands of the most competent observers, I had an opportunity to convince myself in the course of my last year's observations. The milk of the cows in question, as well as the results of the clinical examination, were kindly placed at my disposal by Dr Schütz, Professor at the Veterinary School in Berlin, and Dr Schroeder.

I made it my task this time to carry on my experiments on the occurrence of tubercle bacilli in milk only of such cows as have been proved by repeated clinical examination to be suspicious or to show udder tuberculosis. These ten cows, the milk of which was repeatedly examined microscopically and by animal experiment, were not submitted to the tuberculin test. In our this year's experiments, we merely wished to ascertain whether the clinical examination of a cow, especially of her udder, allows the inference that the milk of the animal is infectious.

It would lead us too far to enter here into details of our experiments. I shall only mention the results of the examination of those cows which throw most light on the subject in question.

In one case, where the clinical diagnosis pronounced the udder to be tubercular, we detected the presence of tubercle bacilli only with the aid of animal experiment, and failed to do so with the microscope. In another cow with the same diagnosis the microscopical examination of the milk repeatedly revealed a great number of acid-resisting bacilli, while the animal experiment gave negative results. The autopsy of this cow did not confirm the clinical diagnosis of tuberculosis of the udder; it revealed merely the presence of chronic interstitial mastitis. Inoculation experiments with portions of the suspected udder also failed.

In two other cows, in one of which the diagnosis of udder tuber-

culosis was made with great certainty, while in the other a strong suspicion was entertained, microscopic examination repeatedly detected in the milk a great number of acid-resisting bacteria, of the nature of which we could not be sure: we thought them at times to be real tubercle bacilli, at other times they seemed more like rod-shaped bacilli merely resembling tubercle bacilli. Inoculation experiments with the milk of these two cows produced in the animals experimented upon suspicious changes, which were due to acid-resisting bacilli resembling the tubercle bacilli. Further inoculation with suspected tissues of these animals gave a negative result.

Of special interest was a case where the clinical diagnosis of udder-tuberculosis was confirmed by the *post-mortem* examination. Neither the microscope nor the experiment on animals could show the presence of tubercle bacilli in the milk. The microscopical examination of sections of the tuberculous udder revealed numerous clumps of tubercle bacilli in the tuberculous interstitial tissue, while there were none to be found in the milk ducts.

From a review of these cases we arrive at the conclusion that the clinical diagnosis of tuberculosis, especially of udder tuberculosis, does not enable us to judge whether tubercle bacilli are secreted along with the milk of the cow in question. For, on the one hand, our previous investigations, as is seen in the protocol of the same, have shown tuberculous disease of the udder sometimes to escape detection altogether, or simple inflammation may be diagnosed clinically, while later on the histological examination, as well as the results of inoculation experiments with the milk and with portions of udder tissue, prove that it was clearly a case of udder tuberculosis. Delépine arrived at the same result in the examination of a cow whose udder was not found to be tuberculous till examined histologically. The milk of this cow contained tubercle bacilli, although repeated clinical examination, as well as the observation of the udder with the naked eye after the autopsy, yielded no grounds for suspicion of tubercular disease. Of course, it is not to be wondered at that the commencement of udder disease escapes detection when palpation is used.

On the other hand, the udder may be clinically pronounced tubercular, while both the autopsy as well as the histological examination clearly show that we have merely to deal with a case of interstitial mastitis or of cyst formation in the udder. This fact was not only proved by ourselves, as may be seen from the above synopsis, but the investigations of Adami and Martin, as well as those of Delépine, illustrated by a series of interesting experiments, led to the same results.

Kühnau, in his recent article on the "Extermination of Tuberculosis among Cattle," has further shown that streptococci, which not infrequently invade the udder, as seen before, are occasionally capable of producing changes closely resembling tuberculosis.

A third possibility has been also demonstrated in the present year by my own investigations, in which I proved that even repeated inoculation of the milk may fail to show the presence of tubercle bacilli, though the clinical diagnosis of udder-tuberculosis was confirmed by the autopsy, and though great numbers of tubercle bacilli could be detected with the microscope in the tuberculous interstitial tissue (though not in the milk ducts).

The presence or absence of tubercle bacilli in the milk of cows with udder tuberculosis is, in our opinion, greatly dependent on the extent of the disease in the organ. It is easy to understand that in a case where the tubercular foci in the udder are circumscribed and encapsuled, the tubercle bacilli will not be able to enter the milk ducts with the same ease as they may do when the disease of the organ is diffused. From examination with the naked eye and under the microscope, as well as histologically, of a number of tubercular udders which were kindly placed at my disposal by Dr Reissmann, director of the Berlin slaughter-houses, I was enabled to convince myself how very much the occurrence of tubercle bacilli in the milk depends on the extent and the local seat of the tubercular changes in the udder tissue. In many cases of far advanced disease I was only able to detect the presence of tubercle bacilli in the fluid expressed from the milk ducts after having examined a great number of specimens. In several cases I failed to detect tubercle bacilli in the capillary milk ducts under the microscope, while in other cases the milk capillaries were crowded with bacilli.

In view of the great variety of bacilli found in tuberculous udders, as seen in my investigations in this field, I venture to express a doubt whether the method (first recommended by Nocard) of harpooning does always furnish us with a sure means of judging whether in a doubtful case we are dealing with udder tuberculosis or not. Besides this, the harpooning method has been pronounced recently by Kühnau not quite without danger to the animal.

So far we have seen that the clinical examination of milch cows bearing on the question of the detection of tubercle bacilli in the milk is, without the aid of auxiliary methods of research, both insufficient and unreliable. On the other hand, the method of demonstrating the presence of tubercle bacilli by means of experiments on animals may prove very tedious. There remains only the tuberculin test, which is the sole quick and sure method. The tuberculin test is recommended by most of the distinguished authors, along with the clinical examination, as the best means of diagnosing and thus aiding the extermination of tuberculosis among cattle. It is, on the other hand, not difficult to understand that, in view of the enormous spread of bovine tuberculosis, the farmers try to discredit tuberculin. But the great diagnostic value of tuberculin can scarcely be diminished by the fact that the failures in the diagnoses only amount to 2.9 per cent. (as Schütz did prove) of all the cases subjected to this test.

In cases where the diagnosis of tuberculosis has been made with great certainty by means of clinical examination, the tuberculin test becomes naturally superfluous. This does away with the argument that about 10 per cent. of the cattle which fail to react to tuberculin nevertheless are tuberculous. These 10 per cent., as is well known, consist of highly tuberculous animals, which have themselves produced and hold in their organism so much tuberculin that they are unable to respond any longer to the tuberculin test; nevertheless this argument is brought forward again and again by the adversaries of our method. The tuberculin test should only be applied to animals which are suspected of tuberculosis; and, when thus used, failures in the diagnosis never occur, as I am also informed by Nocard, who based his opinion on a prolonged experience in France. Bang, in his last

year's treatise, also appears as a champion of tuberculin ; for diagnostic purposes he still considers it to be the finest and safest means for diagnosing tuberculosis in animals, in spite of the many opinions to the contrary.

We must point out the fact that the present scientific data with regard to the extermination of bovine tuberculosis clearly show this to be practically impossible without the aid of tuberculin. Naturally we must, in the first place, make a point of exterminating the cows affected with udder tuberculosis and general tuberculosis, since their milk is, more than all other, to be regarded as infectious. In the second place come the animals in which the clinical diagnosis can only be established by the help of tuberculin. These cows must first of all be separated from the healthy ones. The question whether they should be killed, and the proper time for doing so, will depend on the quicker or slower progress of the tuberculous disease. That is to say that, of course, not all the animals which react to tuberculin need be immediately slaughtered. Therefore we may say that, combined with the clinical and bacteriological examinations, the tuberculin test furnishes us with the safest means of obtaining milk free from tubercle bacilli as well as of rearing cattle free from tuberculosis.

GLANDERS.¹

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TO an assembly of veterinary practitioners it would be a waste of time to attempt an exhaustive essay on this disease. We are all well acquainted with its nature, its methods of spread, and with the heavy losses it has caused.

The object I have in view to-day is to demonstrate that the loss of human and equine life due to glanders will continue so long as the obsolete regulations of the Board of Agriculture are unaltered ; and to indicate how the disease may be economically and certainly stamped out by Orders based upon common sense and modern veterinary knowledge.

It has been said by the President of the Board of Agriculture that the information possessed by veterinarians is insufficient to warrant more stringent measures, based upon their advice, being enforced. Mr Hanbury's excuse for this statement is a paragraph in the Report of the Departmental Committee of 1899: "We feel that further research is needed on some of the most elementary facts of the disease before our prevention or treatment can be formulated with scientific exactness." It is perhaps unfortunate that an abstract opinion of this sort should have appeared in the report, but I venture to think that not more than one member of the Committee ever imagined that such a truism could have been used as an excuse for delay. The same words might be applied to every disease of animals that has been stamped out by State regulations, and to every disease of man that has been scheduled for control by the Local Government Board. If the Committee had believed that their pious opinion was a bar to

¹ Read at the Annual Meeting of the National Veterinary Association, London, 1902.