comparatively infrequent occurrence of tuberculosis in Indian cattle. They appear to show beyond all possibility of doubt that the strains of tubercle bacilli infecting cattle in India possess a distinctly lower degree of virulence than tubercle bacilli isolated from cattle in Europe.

A point which would appear to support the view that the lower virulence of the organism is the more important factor is that in practically every instance the natural lesions which have come under observation have been restricted to a few glands and have been to a very large extent calcified.

NOTES ON THICK FILM METHOD OF STAINING PIROPLASMS AND ANAPLASMS. IN ROUTINE VETERINARY DIAGNOSTIC WORK.

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SOME time ago Mr O. F. Gibbs, Bacteriological Assistant of the Public Health Department (Union of South Africa), drew my attention to the thick film method he was employing in the diagnosis of malaria in the human subject, and advised me to give it a trial in the detection of the various protozoa so constantly encountered in the blood of animals in South Africa. Owing to pressure of work and paucity of material a favourable opportunity did not present itself until just recently, and this short note is written with the view of recording the results obtained.

Technique.—A fairly large drop of blood is allowed to fall on the centre of a clean slide, spread out to the size of a threepenny piece or sixpence with the corner of another slide, and allowed to dry at room temperature protected from dust. An endeavour should be made to make the film of a uniform thickness and not too thick, as otherwise coagulation will distort the blood elements. When thoroughly dry the film can be stained, or it can be kept unstained like an ordinary blood smear, provided the drop is not rubbed off.

For field work the films can be conveniently prepared in an ordinary slide carton provided with a lid, and when dry transported in this carton to the place where they are to be examined.

Staining.—No fixation is necessary, the films being merely plunged into a bath of the following solution and allowed to stain for thirty minutes: 2 cc. of a 1 per cent. watery solution of eosin, 4 cc. of a 1 per cent. watery solution of azur II., 220 cc. distilled water.

This solution, or whatever quantity is required, is made up fresh every day, but it can be used as often as necessary during the day for fresh lots of slides. The slides should be numbered with a diamond for identification purposes, as grease pencil marks come off in the bath. After staining for thirty minutes the films are washed with distilled water and allowed to dry at room temperature. During the process of staining the hæmoglobin is dissolved out,

and if parasites are present one gets a field of them together with leucocytes and lymphocytes, which, with the exception of a varying quantity of *débris*, is practically all that is seen.

Staining Reaction.—The chromatin and plasma elements of the parasite come out very distinctly, with practically the same colouring as is obtained when they are stained intracellularly by Giemsa's method, but with the great advantage that one generally obtains a field of several parasites in various stages, which can be studied together without shifting the field.

Bovine Piroplasmosis (*P. bigeminum*).—Two films were obtained from a clinical case of redwater at the same time. One was made into an ordinary thin film and the other a thick film. The former was stained by Giemsa's method and the latter by the method described above. The thin film showed one or two parasites in about every six fields, but the thick film showed fields of twenty or more parasites in varying stages in every field examined. For this particular disease I am convinced that the thick film method is superior for diagnostic purposes to any other, as in thin films one very rarely encounters the parasites in large numbers. It is often a very tedious job searching for them, and unless one has the time to make a very thorough search they could easily be missed. This remark, although I have not had an opportunity of testing it, would, I imagine, apply with even greater force to canine piroplasmosis, and *Nutallia equi* and *P. caballi*, which are often extremely difficult to demonstrate in even well-advanced clinical cases.

Anaplasmosis.—The parasite can be easily demonstrated by this method although it is extremely small, being practically only a dot of chromatin without any definite structure. The varieties of *marginalis* and *centrale* cannot, of course, be demonstrated, but I have no hesitation in saying that it is very useful in cases where a rapid diagnosis is required.

Parasites of East Coast Fever.—I have not as yet had an opportunity of testing the method in this disease, but, judging by the results obtained with other parasites of a very similar nature and structure, I do not see why it should not answer. If Koch bodies can be demonstrated, it would probably be of immense value to the veterinarian in making a diagnosis from a gland or spleen smear in a doubtful case. No one who has not had practical experience can properly understand what a tedious and unsatisfactory process the search for Koch bodies often is in the ordinary thin film preparation, and how easy it is to make a mistaken diagnosis unless one has ample time at one's disposal.

Diagnosis of Anthrax.—Since starting to use the method, my colleague, Mr Keppel, Government Veterinary Officer, has tried it in the case of thick "blobs" of blood sent in under the guise of a blood "film" by laymen. In these preparations staining by ordinary methods is often extremely difficult owing to the enormous quantity of blood on the slide. By the thick film method, if anthrax bacilli are present, one gets a very beautiful picture of what appears almost a pure culture, owing to the hæmoglobin having been dissolved out in the process of staining. Two specimens of the same blood stained by any ordinary method and the thick film method give a much better picture in the latter case, when the blood is plastered

on the slide, as is so often the case in specimens obtained from animal owners.

Advantages of Thick Film Method.—(a) The film can be very easily made even by a layman in the field. After many years experience, I am fast coming to the conclusion that it is only by good luck that one ever obtains a satisfactory thin film from the average layman.

(b) As a routine method the fact that large numbers of films can be stained by one process at one time without fixation is a distinct advantage, as is the fact that the same solution when once made up can be used several times in one day.

(c) For field work the elimination of many accessories is a tremendous advantage. Two small bottles containing a few cc. of each of the stains, a bottle of distilled water, an enamel pie-dish, and a measure glass or pipette would be all that one requires.

(d) Owing to the comparatively small size of the film to be examined and the "concentration" of the parasites, a much more rapid and accurate examination can be made than in the film method. With a little practice the whole of the preparation can be thoroughly searched over in ten minutes or less.

(e) The presence of considerable numbers of parasites in one field makes the study of the different forms present, and their comparative numbers, a fairly easy matter.

(f) Owing to the obliteration of the erythrocytes the study of the actual structure of the parasites is facilitated, and the distortion so often encountered in badly prepared thin films eliminated. The piling of erythrocytes one on the other, crenation, etc., are factors which often render the detection of parasites practically impossible in unsatisfactory thin films.

I would desire to state that the above notes are written only after a very limited trial of the method, but the results obtained so far, from the point of view of the practical veterinarian working with very unsatisfactory material, have been so satisfactory that I shall certainly, when an opportunity offers, make an extended use of it and hope to publish properly tabulated figures and photographs of the different parasites.

I can only express the hope that other observers will give the method a trial and publish their results.

I must tender my thanks to Mr Gibbs for drawing my attention to the method and giving me practical demonstrations as to how to employ it.

ST. JOHN'S WORT AND ITS ACTION ON LIVE STOCK.

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ST. JOHN'S WORT (*Hypericum perforatum*) is a common perennial weed in many countries, and once it has established itself in a pasture is difficult to eradicate, being a very hardy plant. Cutting