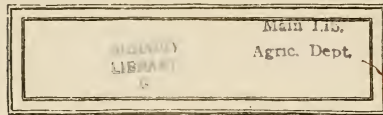
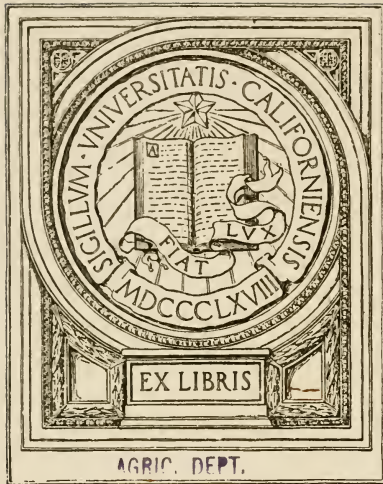


UC-NRLF



B 3 109 339









Digitized by the Internet Archive  
in 2008 with funding from  
Microsoft Corporation









DEPARTMENT OF AGRICULTURE

OTTAWA

---

HEALTH OF ANIMALS BRANCH

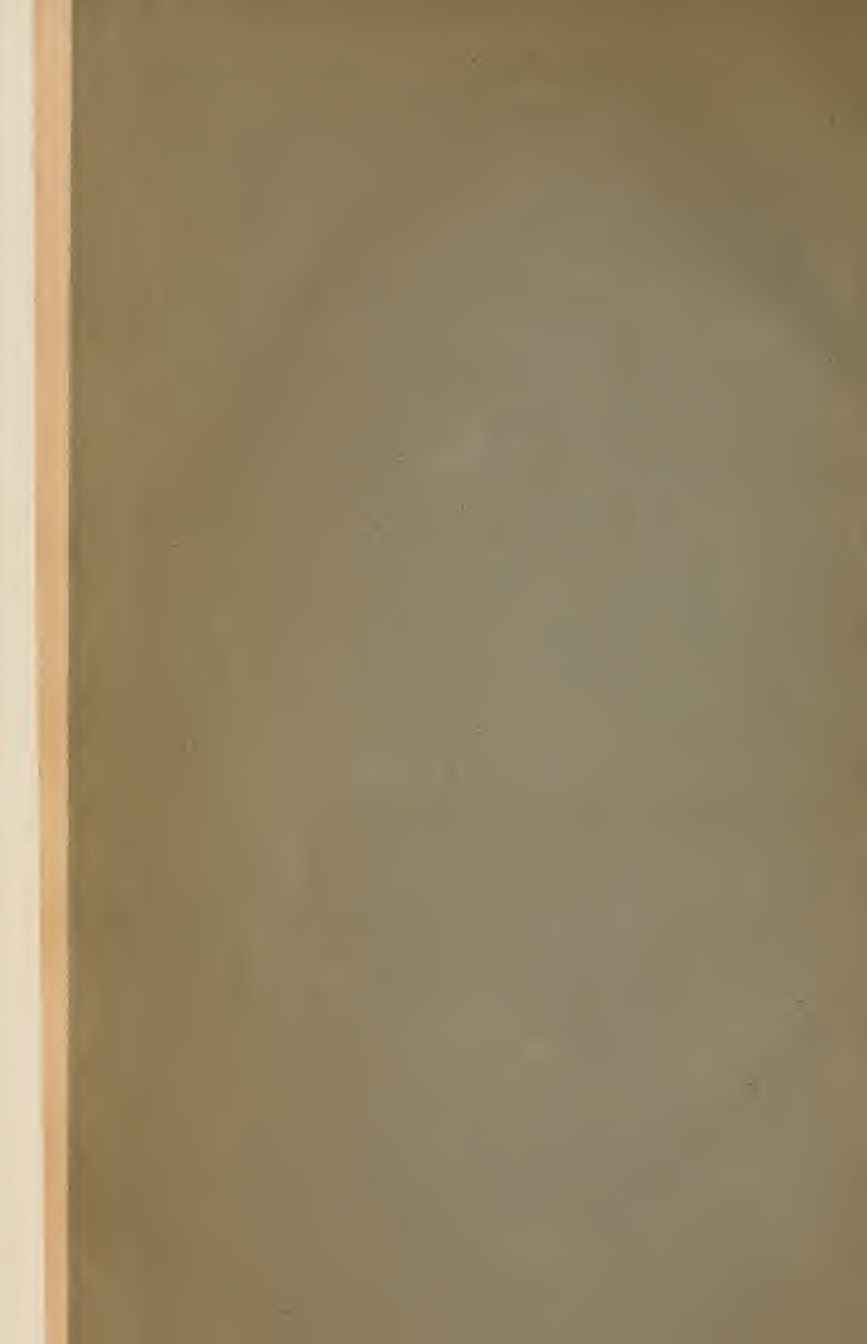
SPECIAL REPORT

ON

MALADIE DU COÏT OR DOURINE

---

NOVEMBER, 1907



Canada

DEPARTMENT OF AGRICULTURE

OTTAWA

---

---

# HEALTH OF ANIMALS BRANCH

## SPECIAL REPORT

ON

# MALADIE DU COÏT OR DOURINE

---

NOVEMBER, 1907

LIBRARY OF  
AGRICULTURE

SF 959  
D6C3

BIOLOGY  
LIBRARY  
G

1900 12  
Dept

AGRIC DEPT.

70 VINU  
AMSONLIAO

## MALADIE DU COIT OR DOURINE

OTTAWA, November 15, 1907.

The Honourable  
The Minister of Agriculture,  
Ottawa.

SIR,—I have the honour to present herewith a brief special report on the disease known as Dourine or Maladie du Coit, which has, for some years, existed to a limited degree among horses in certain districts in Southern Alberta and in one locality in Southwestern Saskatchewan.

The most interesting features of this report will be found in the accounts furnished by Dr. Higgins, pathologist to this branch of your Department, and his assistants, Dr. Watson and Dr. Hadwen, of the work done by them in investigating and determining the true nature of the disease as also of the efforts made by them to discover some more reliable and practical method of diagnosis than is at present available and, at the same time some reliable curative or, if possible prophylactic agent.

As has been already announced, the identity of the disease found in America with that seen and recognized in various countries of the old world has now been established beyond question, through the successful identification by Dr. Watson in February last, of the *Trypanosoma Equiperdum* or *Rougeti*, the specific causal organism of Dourine.

As the detailed reports of Drs. Higgins and Watson are in themselves very full and complete, I leave to these gentlemen the task of presenting the results of their scientific labours, and confine myself to a short historical review of our experience with this disease since the first discovery of its existence in Canada.

In this review there will, naturally, be but little new information, most of the facts having been already submitted in previous annual reports, but, for the sake of convenience, I have thought it best to summarize them, together with some intervening data as an explanatory introduction to the more strictly technical contributions which follow.

The presence of dourine in Canada was discovered in 1904 when Inspector Burnett, Chief Veterinary Officer of the Royal Northwest Mounted Police, and at that time also an official of this branch, reported its existence in a stallion and several mares owned by a rancher near Lethbridge.

As soon as possible after receiving this information, I made a personal investigation and although quite satisfied as to the correctness of Inspector Burnett's diagnosis, determined, in consideration of the critical nature of the matter, and of its grave importance to the horsebreeding industry of Canada and to that of Alberta in particular, to ask Dr. Salmon, then Chief of the Bureau of Animal Industry at Washington, to detail an inspector familiar with dourine to visit and examine the affected animals. My principal reason for adopting this course was that, while the disease had never before been seen in Canada, it had made its appearance from time to time, in widely separated localities, in the United States ever since its first introduction to Illinois by a French horse in the year 1882. These outbreaks, due to the fact that the true nature of the malady had not been recognized until 1887 and that, in the intervening five years, many contact horses had left the area originally infected and become widely scattered, had given the American inspectors a fund of experience altogether lacking among our own veterinarians.

Dr. Salmon, with great kindness and promptitude, complied with my request by at once sending to Lethbridge Dr. E. T. Davison, of Rushville, Nebraska, to whom had been entrusted the work of dealing with dourine in Nebraska, South Dakota and the other western states.

Dr. Davison, after making a careful examination of the affected animals, had no hesitation in confirming my diagnosis of dourine, and so reported to Dr. Salmon, under date of May 14, 1904.\*

I may here explain that, while Dr. Davison, like myself, entertained no doubt as to the identity of the disease, his opinion, like my own, was based solely on the clinical evidences presented by the affected animals, for although dourine had, as above stated, existed in the United States for upwards of twenty years, all efforts to determine the specific causal organism had been entirely unsuccessful. In fact it was not until the following year that the question of the identity even of the disease as found in Europe with that seen in Africa and Asia was definitely settled, for although the presence of the *Trypanosoma Equiperdum* in French cases has been successfully demonstrated by Professors Nocard and Leclainche, other investigators had utterly failed to corroborate their findings.

In 1905, however, Messrs. Buffard and Schneider, two French army veterinarians who had closely studied the disease in Algeria, were able to demonstrate to the satisfaction of the French authorities that the disease in France was due to the same causal organism as they had found in Africa.

Almost immediately afterwards, the contention of those who maintained that dourine was invariably due to the presence of the *Trypanosoma Equiperdum* was still further strengthened by its discovery in Hungarian cases by Professor Marek who, with his compatriots Messrs. Kern and Hutyra, had previously been of the opinion that the parasite did not exist in Europe and that the malady seen there was, although clinically closely resembling it, of an entirely different nature from that observed in Algeria by Buffard and Schneider, and in India by Pease, Baldrey and Lingard.\*\*

It is, however, generally admitted that the detection of the trypanosoma is much more difficult in Europe than in Asia and Africa, in both of which continents dourine is apparently indigenous. This being the case, it is not surprising that, until the discovery of Dr. Watson last February, the organism had not been found in America, especially when due consideration is given to the further fact that the lack of definite clinical manifestations, often observable in Europe, is even more frequently characteristic of the disease as seen on this continent.

In Asia and Northern Africa, dourine seems almost invariably to reach a fatal termination, after running a definite and fairly uniform course, lasting from about three months to three years.

In Europe, however, and to an even greater degree in America, its manifestations are far less regular and certain, a feature which not only renders clinical diagnosis much more difficult, but may also be fairly taken as an indication that, in many cases, the parasites are few in number and less active and aggressive than they are in the natural habitat of the disease. This last hypothesis is borne out, as will be seen from Dr. Watson's report, by his repeated failure to detect the trypanosoma, even in fairly well marked cases, and after he had thoroughly familiarized himself with its appearance and, as far as possible, with its life history and habits.

These two features, which characterize the disease in America, together tend to complicate and render much more serious the task of dealing with it in a prompt and effective manner. The irregularity of the clinical manifestations and their frequent lack of specific character render diagnosis uncertain and precarious, while the difficulty attending the detection of the causal organism deprives us to a large extent of the assistance afforded in the case of so many other diseases by the skilled bacteriologist.

---

\* See Davison's Report. Page

\*\* See Report of Buffard and Schneider. Page

Under our present policy of slaughter and compensation which, taking into consideration the loathsome, insidious and frequently fatal nature of the disease, is the only one possible, certainty of diagnosis is all important. Hitherto the utmost caution in condemnation has been observed, but many cases are met with, especially in mares, where the symptoms shown are so slight, intermittent or even evanescent, that it is practically impossible to reach an intelligent decision as to their disposal.

It is not, at first sight advisable, to order the slaughter of valuable animals merely on suspicion, or on circumstantial evidence alone. On the other hand, our experience has shown that many cases slight at first and subsequently apparently recovered have, under adverse or unfavourable conditions, broken down and developed the disease in an aggravated form.

Prolonged quarantine for observation purposes is a very serious matter to owners who are frequently far from rich, and whose principal source of income is, not uncommonly, the progeny of the very animals held under suspicion, and the breeding of which is forbidden.

Further, in spite of all precautions, such animals, especially mares, kept under range conditions are liable to escape temporarily from surveillance and to become a source of danger to neighbouring stallions and colts, and through them to the mares of other breeders which, unsuspected, may in turn convey the disease to distant studs.

I at one time entertained a hope that, by the removal of the ovaries of such mares as were apparently but slightly affected and on the road to recovery, the problem of their safe disposal might be solved. I thought it possible that such spayed mares, if clearly and heavily branded as unfit for breeding, might find a market as work animals only, and although, as all horsebreeders know, the failure of mares to come in season will not always protect them from the cruelty of an ignorant owner, under the influence of an unscrupulous stallion groom, I decided to operate on a few of those held for experimental purposes. The results were anything but encouraging. One mare died immediately after the operation, this unfortunate sequel being, according to the operator, a veterinarian of long experience and surgical ability, due to a well marked degeneration and consequent tenuity of the arterial walls. A second mare has since developed nymphomania and become a chronic rutter, while in her case also the local symptoms became somewhat aggravated.

These results were not of such a nature as to encourage further experimentation in this direction, especially as, even if uniformly successful, the operation could not, for reasons already given, be looked upon as a satisfactory solution of the difficulty confronting the Department.

The only other course not involving slaughter, viz.—permitting to go free such mares as seem but slightly affected and subsequently appear to recover is not, in my opinion, to be recommended, the risk of spreading the infection, not only to the breeding stock of the individual owner interested, but through shipments of horses to other districts, being altogether too great.

The policy at present followed in dealing with animals so slightly affected as to be doubtful, or in regard to which reasonable suspicion of infection may be entertained, but the slaughter of which is scarcely justifiable, is to hold them under close supervision, at the same time forbidding their use for breeding purposes. This method is, as above stated, satisfactory neither to the owner nor to the Department, but without some more reliable means of accurate diagnosis, it is not easy to devise a better.

The discovery of an accurate diagnostic agent, or of any constant pathological condition on which an accurate diagnosis could be based, would be of the greatest possible value, and it is with this as the principal object that our present research work is being carried on. It would also, needless to say, be very gratifying if our efforts in this direction were to result in the discovery of any method of successful treatment, or better still, prevention.

The subjoined reports of the various breeding and other experiments conducted by our officers will perhaps be of greater interest to the specialist than to the ordinary reader. The subject is, however, one of so much importance to the horse-breeding industry that I have deemed it advisable to embody our findings in this special report.

I have the honour to be, sir,  
Your obedient servant,

J. G. RUTHERFORD,  
*Veterinary Director General.*



# MALADIE DU COIT OR DOURINE

By CHAS. H. HIGGINS, B.S., D.V.S.,

*Pathologist, Health of Animals Branch, Department of Agriculture.*

OTTAWA, November 15, 1907.

While I was familiar with the literature of maladie du coit or dourine prior to the reported outbreak in Southern Alberta, it was only natural that the occurrence of the disease in Canada interested me in its clinical and pathological features to a far greater extent than would have otherwise been the case had this disease not appear within Canadian territory.

Although the disease was reported early in 1904, it was not until the spring of 1905 (May), that I had an opportunity of observing cases of the affection at an inspection made by the Veterinary Director General, during which I was present. While I was prepared to do my utmost, in an endeavour to demonstrate the causative parasite of this affection, I embraced the opportunity of familiarizing myself with the pathological changes to be noted at the autopsies. Further, I endeavoured to assist the careful study of the clinical manifestations then made in an attempt to more accurately diagnose suspected cases coming to the notice of officers of the Department.

At this inspection it was deemed advisable to destroy many of the animals which presented marked clinical evidence of the disease, twenty selected cases, however, were reserved for further experimental observation. There were destroyed at this time, one hundred head, including the stallion 'Brucefield Prince,' and the autopsy lesions in every instance confirmed the ante-mortem diagnosis.

During the same month some cases of the disease were seen in the Porcupine Hills and Medicine Hat districts, a number of which were destroyed.

While it was impossible to examine material taken from each individual case coming under our observation at that time, particular attention was given to preparations taken from cases of the disease, which, in the judgment of all present, were the most likely to give favourable results, and these smears of the body fluids and tissues revealed no bodies simulating trypanosomata when carefully and systematically examined. Specimens were also brought to the biological laboratory, but their examination for trypanosomata proved fruitless.

In September, 1905, Dr. S. Hawden was transferred from Nelson, B.C., and placed in charge of the experimental station at Lethbridge, where his work, both clinical and experimental has given us many details connected with the disease, not previously recorded.

In November, 1905, three mares, two from those reserved at the spring inspection (588 and 589) and one (590)\* obtained in the Medicine Hat district, were received at the biological laboratory, Ottawa, for the purposes of experimental observation, breeding experiments, and to supply us with the necessary material for animal inoculations, in the attempt to recover the causative parasite. One of these mares (590) died shortly after arrival, she having manifested during the short period of observation, marked inco-ordination in the movements of both the fore and hind limbs. As a result of this partial paralysis, she fell in turning, never again to regain her feet,

---

\* These numbers refer to the accession numbers given at the laboratory, and not to cases of dourine.

death ensuing a few days later from a terminal infection. From the time of her arrival to the date of her falling (barely a month), she was never seen in a recumbent position, and there was no evidence of her having lain down.

Many inoculations were made into smaller animals with the body fluids and tissues, including emulsions from various portions of the brain and cord, but in none were we favoured with any data indicating a dourine infection.

The other two mares (588 and 589) have been continually under close observation since their arrival. During the first year much attention was given to the systematic examination of the blood taken from the general and peripheral circulation. These examinations consisted, not only in the careful search for the causative parasite of the disease, but also, the enumeration of the various forms of white blood corpuscles. Certain alterations from the normal were found, and to more clearly demonstrate these variations charts and tables have been prepared. These charts and tables appear in connection with our remarks on the 'Blood Examinations in Dourine,' at the end of this report.

On rare occasions only was there any deviation in the temperature from the normal, and the temperature chart of either animal would add little to this report.

Many small animals have been inoculated with blood from these mares, using as high as 150 cc. for rabbits and puppies, and 50cc. for guinea-pigs. In none of these trial animals have we observed any evidence of an infection, nor have they been inconvenienced through the reception of these relatively large amounts of blood under the skin or into the abdominal cavity. There has been noted, however, a slight rise in temperature, in certain of the inoculated animals, appearing from three to ten days after inoculation, but careful and repeated examinations of the blood revealed no parasites, nor has a prolonged period of observation indicated an infective inoculation. The animals which presented the rise in temperature were those receiving the largest amounts of blood, intra-abdominally, and the manifestation was then and is still considered to have been caused by the inoculated blood acting as a foreign body, the rise in temperature being attributed to a general disturbance connected with the absorption of the inoculated material.

Experiments have been conducted to determine whether there was any possibility of stimulating the trypanosomata to increased numbers, for purposes of detection, by bleeding and by keeping blood diluted with citrate and saline solutions at various temperatures from  $+20^{\circ}$  C., to  $+37^{\circ}$  C., but this work did not enable us to detect the parasite.

In the spring of 1906, two healthy stallions, (650 and 660), natives of eastern Canada, were procured for the purpose of conducting breeding experiments, neither, however, subsequent to covering the mares in question, has given any evidence of infection. One of the mares (589, 'Puss,') was with foal to the stallion 650, which subsequent to the services with this mare was bred to a native mare 670. No evidence of an infection has presented itself in this latter mare. During the summer of 1906 she was purposely bred late in the period of oestrus that impregnation might be avoided and she did not become pregnant. During the season of 1907, however, the services were performed early in the oestral period and the mare has not yet shown evidence of pregnancy.

In October, 1906, arrangements were made to work the affected mares (588 'Sleepy,' and 589 'Puss') and they were early in the spring of 1907, apparently in better physical condition than at any time subsequent to their arrival in Ottawa. Evidence of inco-ordination was slight in each case, and in the absence of a direct history pointing to dourine, would have escaped the observation of even experienced observers. They were fat, their coats were sleek and they worked well in harness. The differential blood counts revealed in both cases a condition which could safely be considered normal in our present acceptance of that term, but I do not consider that they had made a full recovery as one 'Puss' (589), was destroyed September 30, 1907, after becoming paralyzed to such a degree that she could no longer stand. The

autopsy lesions in this case were those usually found in advanced cases of dourine. Full data concerning these individual mares will be found later in this report.

In September, 1906, for the second time I visited the Lethbridge quarantine station. While there, a large number of small animals were inoculated by Dr. Hadwen and myself with fluids and emulsions from living and recently killed horses that had naturally contracted the disease. The period of observation with all of the inoculated animals was sufficient to determine the presence or absence of infection, and they were continually under the close observation of Dr. Hadwen, then in charge of the quarantine station, and later of Dr. Watson, who was transferred to this station in November of that year. In no instance has there been any evidence that any of these inoculations carried with it an infective agent, a feature determined by repeated examinations of the blood from the general and peripheral circulation, by examination of other body fluids and tissues, and by general clinical appearances.

Thus, although we had conscientiously and systematically endeavoured to demonstrate the *Trypanosoma equiperdum* by various devices and methods, we were unable to do so, nor had it been observed by any individual working with this disease in Canada, or in fact with naturally infected cases of dourine or maladie du coït on the North American continent, prior to the finding of trypanosomata by Drs. E. A. Watson and M. V. Gallivan, on February 11, 1907. The subject furnishing the trypanosomata was a mare found to be clinically affected with dourine on the premises of her owner, Mr. R. Tiffin, near Lethbridge, Alberta. The material was taken from a well defined vesicle on the inner border of the right labium, the base of which was about one centimeter in diameter, slightly raised, firmer and more œdematous than the surrounding tissues. Three smear preparations were made after the scarification of the vesicle in question and its base. In two of these smears trypanosomata were found; the organisms, however, were rare, sixteen only being observed after examining the entire area of three smears.

Subsequently, I was favoured with an opportunity of observing the parasites at the Lethbridge quarantine station, in fresh and stained preparations which I took from the Tiffin mare on the 21st, 23rd and 25th of March. Still later (June 12), on another visit to the Lethbridge quarantine station, I was able to again demonstrate the parasite in material taken from the vagina of this mare.

Since the demonstration of the parasite in material taken from the Tiffin mare, the disease has been transmitted to two fillies, from one of which it has been recovered.

Parasites have also been observed by Dr. Hargrave of Medicine Hat, in smears taken by him from the vagina of a naturally affected mare near Olds, Alberta. In this instance the smears were made by Dr. Hargrave on July 9, and examined on the 13th of that same month, and I was able to verify his finding of the parasite in the smears on the 16th.

The diagnosis made some three years ago, based entirely upon clinical evidence is, therefore, fully substantiated and confirms the statement made in my annual report of 1905 that the disease, undoubtedly identical with the dourine of other countries, is caused by the same parasite (*Trypanosoma equiperdum*), and that the demonstration of this parasite is largely a matter of obtaining suitable cases, coupled with careful, systematic experiments and close observation.

Further, the possibility of diagnosing this affection by purely clinical means is fully established, a fact, strange to say, still questioned by some well known authorities, who appear to have forgotten that dourine and many other diseases caused by specific organisms were regularly diagnosed by practical men, many years before the discovery of their causal agents.

The details connected with the first demonstration of the parasite, together with an explanation of the improved technique which he has devised can be studied in the article of Dr. Watson which constitutes a portion of this report.

While the causative parasite has been demonstrated in a number of naturally affected animals, and also, in two artificially infected cases, it is impossible to give a

reason why the parasite cannot be observed in all clinical cases. This difficulty is experienced in all affections caused by trypanosomata and it is my belief that we still have much to learn concerning the life cycle of this class of parasites.

The advanced changes which take place in the different organs of the body, particularly the degeneration of the bones, their increased brittleness, joint ulceration, &c., are beyond our present knowledge and do not, for the present at least, admit of an explanation. That a few trypanosomata located in the vaginal mucous membrane can of themselves create such a disturbance, it is unreasonable to presume, and future studies must be directed to this feature, with a view of determining the cause of these marked changes. There is a possibility of this whole process being due to defective nerve supply and located in those centres having direct control over the nutrition of the animal. The extensive histological studies of Marek, however, have failed to reveal such lesions and we will, therefore, have to defer any remarks in this connection until our knowledge in this direction is more complete.

We have found in working with the *Trypanosoma gambiense* (sleeping sickness) that there is a toxine present in the blood serum which creates a disturbance when injected into guinea-pigs. With sleeping sickness, however, there is little or no trouble in the demonstration of the parasite, and we are led to conclude that the toxine of the *Trypanosoma equiperdum* is much more disastrous to the growth of the organism than is the case with the parasite of sleeping sickness. Further, we are led to believe that it is the toxine, in cases of dourine, that to a large extent is responsible for the lesions produced.

Certain bodies have been observed in some of the preparations taken by Watson and later studied by both of us, which may be trypanosomata in an involution stage.

I cannot agree with Thiroux and Teppaz,\* however, that the red granules seen in the large mononuclear lymphocytes of horses (when stained with eosin and methylene blue in any of its combinations), represent the remains of trypanosomata, for we have observed these granules almost without exception in smears taken from the general and peripheral circulation of healthy horses. (See tables I to IX given under 'Blood examinations in cases of Dourine.') Another criticism of their work is, that their diagrams do not indicate the evolution spoken of in the text of their article, as the trypanosoma shown within the large mononuclear cell (fig. 16, their illustrations) has lost its centrosome and its undulating membrane, while the next figure presents what are described as the centrosomes of a number of trypanosomata, together with a portion of an undulating membrane.

That the organisms of dourine may be taken up by the large mononuclear lymphocytes, I cannot refute or substantiate, as none of our observations have included such phenomena. It would appear, however, that the lymphocytes exert some action inimical to the parasites, otherwise these cells would not appear in such large quantities in the circulating blood of animals in a passive stage of the disease as indicated by the tables appended to this report.

## PATHOLOGICAL ANATOMY OF DOURINE.

With the pathological anatomy of dourine I have endeavoured to make myself familiar, and, therefore, will indicate the lesions usually found at autopsy.

All of the cases upon which it has been my privilege to hold autopsies, have been destroyed on account of their presenting clinical evidence of the disease, and, with one or two exceptions they were of long standing.

As a rule the general condition of an affected animal is much below normal, there is little evidence of fat, the ribs are prominent and the hip bones are easily distinguished. Incision of the skin reveals the connective tissue to be of a yellowish

\* Les Trypanosomiases animales au Senegal. M. Thiroux et M. Teppaz. Annales de l'Institut Pasteur, March, 1907.

tinge with but very little fat. The muscles are somewhat pale, but otherwise appear to be normal. Where emaciation is marked the muscles present evidences of gelatinous degeneration with yellow serous infiltration. Removal of the hind legs at the coxo-femoral joint reveals considerable oedema about the joint and the ligamentum teres. The ligamentum teres is not of its normal size in cases of long standing. The articulatory surfaces of this joint, particularly that of the acetabulum, in a majority of instances, present a distinct ulceration, usually somewhat triangular in shape and in one instance each side of this irregular triangle was one inch in length. (Plate II.) The synovial fluid presents no alteration either in colour or consistency.

The bones when split open were found to be very brittle, spongy, and infiltrated with gelatinous material. (Plate VII.) The articular cartilages may be eroded and this erosion is particularly to be noted in those cases where the characteristic lameness has been a clinical symptom during the course of the affection.

On opening the abdominal cavity, fluid was invariably present, in which were seen numerous nematodes (*filaria papillosa*). These, however, are usually met with in horses kept under range conditions, and therefore bear no special relationship to the disease under consideration. The connective tissue of the abdominal cavity was of the same yellowish tinge noticed in connection with the subcutaneous connective tissue. The mesentery also exhibits this peculiar colouration. The intestines are pale, and on their serous surface evidence of previous inflammatory changes are usually present.

The spleen is of a greyish colour and the surface presents many petechial spots. In consistency the spleen is soft and flabby, pitting on pressure, the pit not resuming its natural position on the removal of the finger. In size it is about normal or somewhat enlarged, and on its surface may be seen scars, which from a careful histological study, appear to be the result of hæmorrhage beneath the capsule. (See plate III.)

The liver appears to be much shrunken in size, in some cases appearing to be but half that of the normal organ, and it is of a much darker colour than is the case in horses not suffering from this disease. The organ is very firm, with no evidence of cirrhosis. The capsule of the liver usually presents evidence of an old inflammatory process.

Dr. Hadwen, while working with this disease at Lethbridge found that the maximum weight of the spleen in thirty-six cases was 2,976 grammes, while the minimum was 992 grammes, the average being 1,587.2 grammes. He also found that the liver presented a maximum weight of 9,672 grammes, a minimum weight of 6,944 grammes, while the average was 5,952 grammes. In the most advanced and emaciated cases he also found that there was no increase in the size of the liver, but that it was decreased in size, due to the fact that all available fat and nutriment had been absorbed in an endeavour to sustain life. The horses upon which these observations were made were condemned as soon as the symptoms warranted such action, and therefore were in different stages of the disease. The weight of the animals in question varied from 900 to 1,300 pounds.

A gelatinous deposit is usually found at the hilum of the kidney, and this deposit responds to the tests for mucin. An enlargement of the kidney is also frequently noted, while histologically the cells are degenerated and largely replaced by strings of mucin.

Dr. Hadwen found ammonium urate in advanced cases, but was unable to demonstrate albumen or sugar. We have been unable to find evidence of sugar in urine taken from the affected mares with which we were experimenting here, nor in urine forwarded to this laboratory by Dr. Hargrave, of Medicine Hat, Alberta, taken from affected horses in various stages of the disease at the time of their slaughter. Calcium carbonate was found by Dr. Hadwen in the urine of two well marked cases of dourine, in which there was paralysis of the hind limbs. In both instances, the hair of the tail and legs became matted with the urine, giving off a very offensive odour.

In both of these animals the walls of the bladder were found at the autopsy to be one centimetre in thickness. This increase of the calcium salts in the urine seems to be responsible for the great irritation following urination manifested by switching of the tail and eversion of the clitoris. In two of the cases which we have had here the urine has contained a great quantity of mucin.

In the seminal fluid of two diseased stallions collected after the covering of mares, Dr. Hawden found very few spermatozoa, and even these ceased their movements very quickly in coverslip preparations. The fluid ejected from these stallions was plentiful in supply, and from its general appearance was considered to be largely of prostatic origin.

The vaginal mucous membrane is pale and of a leaden hue with some scars. The mucous membrane is in folds and there is an œdematous condition of the os, but otherwise nothing abnormal is noted. The mucous membrane of the uterus is usually of a leaden colour and œdematous. In some cases the œdematous folds were very pronounced, increasing the size of the organ considerably. The ovaries were invariably cystic, though I am not prepared to assert that this was due to the infectious process under discussion. None of the mares upon which I held autopsies had recently shown a profuse vaginal discharge, indicating that all were more or less chronic.

In addition to the general lesions described above, the testicles of two stallions were atrophied, in one instance the process had progressed to an almost complete absence of testicular tissue. In one of these stallions 'Brucefield Prince,' (Plate IV and V) there was a diffuse œdematous swelling of the sheath. No marked changes were noted about the penis save a slight roughness of the mucous membrane of the urethra.

#### SLEEPY 588.

This mare. (bay. aged, weighing about 1,600 pounds) was quarantined in the spring of 1904, condemned in May, 1905, held for experimental observation and transferred to the Biological Laboratory, Ottawa, in the fall of that year arriving on November 22. On arrival she was in excellent physical condition and apart from the evidences of the disease which were to be noted at a careful examination of the mucous membrane of the vagina, she appeared to be perfectly healthy, although she would at times manifest a slight knuckling of the hind fetlocks, but this manifestation was by no means well marked, nor would it, under ordinary conditions, admit of detection.\*

She was stabled during the winter and at various times furnished material for animal inoculations. As the major portion of our work was conducted with the other mare coming from this infected herd (Puss 589); I will not detail the experiments conducted with material from this individual.

In the spring of 1906 she was bred to a stallion (660) purchased for that purpose, in an endeavour to obtain a recently infected animal that a suitable case for the detection of the parasite might be available. This course was pursued as a careful study of the literature indicated that a recently infected stallion was more likely to give positive results in the detection of the parasite, than would follow repeated examinations of material from animals in other stages of the disease. Our efforts in this direction, however, were productive of no data indicating an infection.

A detailed record of the blood examinations of this mare is to be found in connection with the remarks upon 'Blood Examinations in Dourine,' appended to this report. A number of examinations of vaginal smears were made but the temper of the mare rendered our efforts in this direction particularly difficult and dangerous. In none of these vaginal smears were we able to detect evidence of parasites nor of forms simulating them.

---

\*Prior to the transfer of this mare to Ottawa marked clinical manifestations of the disease under consideration, at one time presented themselves.

As already stated this mare was put to work in October, 1906, and has continually worked since that time in the division of the agriculturist on the experimental farm. At the outset she was worked with her mate (Puss 589) and was very green, having been but partially broken before leaving Alberta. She was soon able, however, under the experienced guidance of the farm teamsters to perform her share of the work required from time to time. At present (since the relief of her mate Puss 589 from work), she is working single or double with another horse and even in a three-horse hitch. In the spring of 1907 she was bred to the stallion (650) but apparently without results.

She at present shows a slight inco-ordination in the movements of her hind limbs, her general condition, however, is good; she is fat, her coat is glossy, she feeds well and there is nothing further which would indicate a dourine infection save the yellowish tinge of the mucous membrane of the vagina and the entire absence of pigment from the clitoris.

#### Puss 589.

The history of this mare is similar to that of the one immediately preceding (Sleepy 588) and she was received at the laboratory on the same date. She was an aged brown mare weighing about 1,450 pounds. On arrival her general condition was good but there was at times a marked inco-ordination in the movements of the hind limbs with considerable knuckling of the fetlocks. The genitals were normal in their exterior appearances but the mucous membrane of the vagina was pale, had a marked yellow tinge and was in folds, and there was a complete absence of pigment in the clitoris. She was stabled at the laboratory during the winter and furnished the major portion of the material with which we conducted our animal inoculations and other experiments. All our experiments with animals were negative and for the greater number blood was the inoculated material.

During the period from November, 1905, to April, 1906, relatively small amounts of blood were used (up to 25 cubic centimetres) in guinea-pigs, rabbits and puppies), (the latter having been used as young as five weeks of age), in an endeavour to obtain an infective inoculation. In none of these experimental animals did we observe a swelling at the point of inoculation nor in fact, any indication of there having been an infective disturbance in the inoculated animals. No parasites were found in material taken from or near the site of inoculation, nor from the general or peripheral circulation, nor was there a subsequent development of the paralytic or eye lesions observed by other authors. The period of experimental observation was from one to three months, therefore, we were forced to conclude that the parasite was not in the material inoculated.

During the month of January, 1906, in an effort to demonstrate the causative parasite the method of Rogers\* was followed. Blood was drawn into citrate solutions of various strengths and also into the combined salt and citrate solutions. These mixtures were subsequently kept at various temperatures ranging from 20°C. to 37°C. and examined at stated intervals to determine the presence or absence of trypanosomata. The receptacles used in this work were large test tubes two inches in diameter and fifteen inches long. These tubes were sterilized in the autoclave, the top being plugged with cotton wool through which a glass tube was placed for the purpose of attaching a rubber hose when withdrawing the blood from the animal. Before sterilizing, however, the solution which was to act as the diluent was accurately measured and placed in the tubes which were graduated at points to which it was desired to fill with blood. The tubes were filled with blood by the use of a trocar in the jugular vein connected by means of a rubber hose, with the glass tube in a test

---

\*Leonard Rogers, M.B., etc., Capt. I.M.S., Preliminary Note on the Development of Trypanosoma in Cultures of the Cunningham-Leishman-Donovan Bodies of Cachexial Fever and Kala-Azar. London *Lancet*, July, 1904, page 215.

tube. Microscopic examinations were made daily of smears taken at various depths in the mixtures. Smears were prepared from the sediment after centrifuging one cubic centimetre of saline solution. In none of the examinations made from this diluted blood were we able to detect any trypanosomata or what we understand as their involution forms.

In March this mare was in an exceedingly poor physical condition, she was thin, her coat had an unthrifty look and there was marked inco-ordination in the movement of both the fore and the hind limbs. The knuckling of the fetlocks was particularly noticeable even to the inexperienced clinician. When at rest she would raise the near hind foot as shown in an accompanying photograph (plate VI) taken May 5, 1906.

Immediately after the advent of warmer weather and the appearance of green grass in the inclosure in which the experimental animals were held, there was a marked improvement in her condition with a lessening of the clinical evidence of the disease. The inco-ordination of the fore and the hind limbs gradually became less and could scarcely be detected during the latter part of the summer. She was bred to the stallion (650) at various times after his purchase for reasons similar to those mentioned under (Sleepy 588), and was later found to be in foal.

In October, 1906, this mare was worked with (588) and worked up to the time of foaling (June 20, 1907), when she was relieved and, although several efforts were made to subsequently use her at some light work her condition rendered it necessary to abandon all efforts in this direction. The foal which presented no evidence of dourine died of a septic infection (navel ill) eleven days after birth.

Shortly after being placed at work in the fall of 1906 (November), she was noted to be lame in the near foreleg. A careful examination was made without locating the exact seat of the difficulty but it was considered to be above the fetlock. This lameness was of an intermittent character and we were therefore forced to conclude that it was but another clinical manifestation of the disease from which she was suffering and not due to an injury.

On January 1, 1907, a large number of animal inoculations were made, using untreated and defibrinated blood. In these inoculations large amounts were used, 75 and 150ccm. in guineapigs and rabbits and 300ccm. in a young puppy. The guineapigs and rabbits were inoculated subcutaneously and intra-abdominally with the untreated blood and with that which was defibrinated. The puppy (weighing about ten pounds and about two months old) received 150ccm. of the untreated blood subcutaneously and 150ccm. of the defibrinated blood intra-abdominally. No reaction followed the inoculations of these relatively large amounts of blood, save a slight rise in temperature in the cases of a few of the inoculated animals on the third and fourth days. This rise in temperature was attributed to the blood acting as a foreign body, the rise in temperature being occasioned by the general metabolic disturbance during the absorption of the inoculated material. Careful and systematic examinations of the blood of these experimental animals were conducted subsequent to their inoculation, but in none of the preparations taken were we able to discern any evidence of trypanosomata.

On the date of making the inoculations about 3,000 cubic centimetres of blood were removed from the mare, and there was little or no difference to be noted in her condition following this procedure.

After foaling (June 20, 1907), the condition of the mare rapidly grew worse, and although she appeared to eat well it was impossible to put any flesh on her. Inco-ordination of the hind limbs was very marked and there was a distinct swaying of the quarters. Early in August, on account of the rapid failure of her condition she was brought to the laboratory stable that she might be available at such times as it was deemed advisable to make clinical or other examinations. Within a few days of her return to the laboratory she was picketed and allowed to eat all the green grass that she desired. This was followed by a temporary improvement in her condition; there was a partial subsidence of the inco-ordination, her coat



looked sleeker, and she put on some flesh. The improvement, however, was only of a temporary character, and it was seen that she was retrograding rather than improving. On the morning of September 21 she was found down. Efforts to raise her were fruitless, there being little or no control over the hind limbs. There was even then a keen desire for food, but this could only be partially satiated owing to a marked inco-ordination of the muscles of the jaws. This condition of these muscles had probably been present in a minor degree for some time, but was not observed, as mastication ceased at irregular intervals only.

There having been no improvement in her condition up to the afternoon of September 23, it was decided to destroy her. At this time she was very much emaciated, and there was little or no control exercised over any of the limbs.

At the autopsy it was noted that the œdematous infiltration in the inter-muscular connective tissue was a more marked feature than had previously been observed, although this was not apparent during the last weeks of life, nor even up to the time of her destruction.

The cut surface of the muscular structures revealed large amounts of œdematous fluid and the tissue was of a much darker colour than is normally seen. The heart muscle was very flabby and there was considerable œdema about this organ. The valves were normal, and the pericardial sac contained an amount of fluid slightly in excess of that normally present. The lungs were normal, with a few indications of old adhesions along their borders. The bronchial lymph glands were swollen and œdematous. The liver was shrunken in size, very firm and dark in colour. Its surface presented the usual adhesions upon the capsule.

The spleen presented the characteristic appearance usually seen in cases of this affection, having many petechial spots upon its surface, which was pale grey in colour and very flabby in its consistency. The lymph glands of the abdominal cavity presented an increased œdematous infiltration and were swollen. The kidneys were pale, very friable, and there was considerable œdema about the pelvis in each. The walls of the urinary bladder were somewhat thickened and the urine within it contained a large amount of mucus. The ovaries presented but slight alterations from the normal. The uterus did not contain a fœtus, its mucous surface presented many folds, and these folds were œdematous.

The coxo-femoral joint of the near hind leg presented quite a large ulceration, but this ulceration was covered with freshly formed cartilaginous material. The elbow joint of the near foreleg presented an anticipated ulceration, as has already been mentioned the mare was at one time lame in this limb, and the seat of the trouble was now easily established. This ulceration was in an active state, apparently at a small point only, the remainder of the lesion being covered with fresh cartilage.

The bones were spongy and very brittle. The cavities of the long bones contained a large amount of gelatinous material (plate VII.), and ulcers were found on their articulating surfaces.

During the later stages of the disease in this animal the history is comparable to that of the mare illustrated in plate I., with the exception that the mare referred to suffered from a more acute form of the disease.

There is but little doubt that the severe work to which this animal was subjected was partially responsible for her death through an aggravation of the lesions from the fatigue sustained, and it would further appear from the autopsy lesions that she could never have made a full recovery. That severe work aggravated the symptoms was first observed by Dr. Warnock of Pincher Creek, Alberta, and he has made use of this fact in dealing with suspects by having them broken to harness and worked. If the animal is diseased marked evidence is apparent in a very short period, death invariably following.

## NIGGER 590.

This mare was received at the laboratory with the two preceding and originated at the ranch of the Medicine Hat Ranch Company, having been selected by Dr. Hargrave as a recently infected case of dourine. She was a young mare (6 years) weighing 1,100 pounds and was in fair condition. There was a marked inco-ordination of both the fore and hind limbs which was particularly noticeable when she was forced to step over a small obstruction. The movements of the forefeet gave the impression that she was feeling for the ground, the nervous involvement evidently affecting the flexor and extensor muscles. The hindquarters swayed considerably and a distinct knuckling was to be observed even when walking. Eye lesions were looked for but it was impossible to detect any abnormality of the external surface of these organs or of their muscles, and the retina presented a normal appearance. There was no distortion of the genitals although the mucous membrane of the vagina was found to be in the folds common to all cases of *maladie du coït* or dourine.

During the period of her life at the laboratory, no evidence was obtainable of her having lain down (from November 21 to December 14), until the 14th day of December when she was led out to water. On this date the ground was very slippery, ice having formed over the surface of the ground as the result of a rain and sleet storm, this ice being subsequently covered with about three inches of snow making it impossible for one to discern the spots which were particularly treacherous. She slipped on such a spot and fell never again to regain her feet. After falling she was carried to the stable where a careful examination was made to determine whether there was any broken bones. Being unable to find any abnormalities she was made comfortable with a plentiful supply of bedding and allowed to remain till the following morning when an effort was made to place her in slings. She was quite easily raised and could support some weight on her fore feet but was unable to exercise any control over her hind limbs, even when they were placed directly under her. It being evident that she was more comfortable in a recumbent position she was unslung. She ate very little, although no paralysis of the jaws was noted and death resulted on the 18th from a terminal infection.

The autopsy revealed the usual findings, yellow colouration of the subcutaneous connective tissue, œdematous infiltration, swollen lymph glands, flabby heart muscle, firm liver with adhesions, pale grey flabby spleen with petechiæ, joint ulcerations, etc. Ulcerations were found in the coxo-femoral, the hock, the stifle and the fetlock joints of the hind limbs and in the shoulder, elbow and knee joints of the fore limbs and in some instances the ulceration was intense and active. A few animal inoculations were made from the fluid surrounding the spinal cord and brain, and with emulsions from the cord itself. These animals, however, died of septicæmia, a result accounted for by the presence of the terminal infection in the mare.

During the entire stay of this mare at the laboratory there was a distinct leucocytosis with but little deviation from the normal proportion of the various cells as instanced by the counts recorded in connection with the remarks on 'Blood Examinations in Dourine,' (table IV).

## RIDEAU, 650.

This is an aged stallion purchased May 15, 1906, for experimental breeding in connection with *maladie du coït*. This stallion was raised in this immediate vicinity, and has never been used at a point remote from the Rideau and Ottawa rivers. During the season of 1906 he was bred to (Puss, 589), she being the case which seemed more likely to prove infective to the stallion, and subsequently he was bred to the healthy mare (Boulger, 670).

The result of these breeding experiments was that the mare (Puss, 589) was impregnated and gave birth to a filly foal on June 20, 1907, which, as has already been stated, died of navel ill on July 1. The breeding of the stallion to the mare

(Boulger, 670) did not result in impregnation. This latter mare was purposely bred late in the period of oestrus that impregnation might be avoided, the desire being to infect her from 589 through the stallion 650.

This stallion has at no time presented any evidence that the covering of the mare (589) resulted in an infection.

During the breeding season of 1907 he has been used on the mares (588, 589 and 670), and up to the present writing, November 15, there is no clinical evidence of a dourine infection in the stallion or in the mare (670).

From the time of his purchase till August 8, 1907, he was kept at the laboratory stable, having the use of the paddock for exercise. On that date, however, he was taken by the agricultural division of the Experimental Farm for work where he has maintained his excellent physical condition.

By working him it was anticipated that in the event of an infection, clinical manifestations would be present much earlier than would be the case with only the exercise obtainable in a small inclosure.

A record of blood examination made can be found in connection with our remarks on 'Blood Examinations in Dourine.' (Table V.)

#### MANOTICK, 660.

This stallion was purchased on June 9, 1906, for the purpose of conducting breeding experiments with *maladie du coït*. He was an aged stallion, but had a history indicating that he had never been further than fifty miles from Ottawa. He provided a cheap experimental subject for our purposes and was bred to the mare (588 Sleepy) only. Services with this mare were somewhat difficult owing to the stallion's small size, but we were able to effect them on June 20 and 21 and on July 13. As will be seen from the tables of blood counts of this horse (Table VI.), there was never a marked variation in the polynuclear elements, and but slight variations in the lymphocytes, and these could be accounted for by the increased number of eosinophile cells, the presence of which in the blood was due to the parasites which were found at autopsy. The existence of this parasitism was recognized from the first, but it was considered that it would interfere but little with our dourine experiments.

The death of the stallion occurred on October 16, 1906, as a result of the nematode parasitic invasion, and there had never been any evidence during the entire period he was under observation that he had become infected with the trypanosoma for which we were searching.

The autopsy revealed the following lesions:—

Some subcutaneous and intermuscular œdema was noted, particularly at the hips, withers, and in the sternal regions. The œdema, however, did not exhibit the characteristics noted in dourine-infected animals.

The majority of the joints of both the fore and hind limbs were opened without finding any evidence of articular lesions. The heart was enlarged and its muscle was firm. The lungs were normal. The spleen was normal weighing 780 grammes. The liver was normal save the presence of small nodules about the size of bird shot which were found to be parasitic in their origin. The stomach contained a large number of larvæ of *Oestrus equi*. The small intestine was free from parasitic invasion. The cæcum and anterior portion of the colon contained large numbers of sclerostomata and there were many small tumors in the mucous membrane, the largest of these being about the size of a hickory nut, containing bloody, purulent matter. This bloody, purulent material proved on microscopic examination to consist of degenerated leucocytes many of which were eosinophiles. No other parasites than those above mentioned were noted in the intestinal tract. The left kidney weighed 570 grammes and presented evidence of cloudy swelling. The right kidney weighed 420 grammes, was inflamed and œdematous. The afferent and efferent blood vessels were

involved in a mass of diseased tissue containing a nodule about one inch in diameter and this nodule was composed of very hard fibrous tissue with calcareous deposits. There was also contained in this mass an aneurism of the renal artery and a dilation of the ureter. Nematode parasites (*Sclerastoma Armatum*) were found in the aneurism and in the dilation of the ureter. The walls of each cavity were from one-half to three-quarters of an inch in thickness. The bladder contained very dark coloured urine in which there was much mucus. The ascitic fluid was scanty, contained a few nematode worms (*Filaria papilosa*) and one of these was found in the scrotal sac.

There was in none of the organs a condition simulating that commonly found in cases of dourine.

#### Boulger, 670.

This is an aged mare which was purchased on June 9, 1906, in order that a healthy mare might be available for breeding with the stallion (650) after he had covered the dourined mare (589 Puss). Boulger (670) was bred a number of times during the season of 1906, but we have failed to find any evidence of her having contracted the disease. During the season of 1907 she had been bred to the same stallion (650) he having previously covered the mares (588 and 589).

No evidence of an infection in this mare has presented itself, the mare being in first class condition and there has never been any swelling or distortion of the external genitals nor has there been any vaginal discharge or abnormal appearance of the mucous membrane

### BLOOD EXAMINATIONS IN DOURINE.

Shortly after the receipt at the Biological Laboratory, of the mares affected with dourine for experimental purposes, some blood studies were undertaken with a view of determining the pathological changes which were taking place.

We were, from the very inception of this work greatly interested in the changes which manifested themselves and have since embraced every opportunity afforded for increasing our knowledge of the subject, with the result, that we are now able to present some interesting data.

In detailing this work it is first necessary to indicate the method of differentiating the various cells that our tables and general remarks may be more fully understood.

We have considered the enumeration of the blood cells under four general varieties, one of which has been subdivided into four divisions.

- 1 Poly-morpho-nuclear Neutrophiles,
- 2 Eosinophiles,
- 3 Mast cells,
- 4 Total lymphocytes:
  - a. Lymphocytes (large and small),
  - b. Large mononuclear,
  - c. Granular mononuclear,
  - d. Transitional basophiles.

For a description of the general characteristics of the varieties mentioned, their source and functions, the reader is referred to the text books dealing with the normal and pathological histology of the blood.

In addition to the cells mentioned we have also enumerated those cells which could not be classed in any of the above subdivisions but which from their peculiar staining characteristics seemed to identify themselves as transitional neutrophiles.

A much better conception of the details of our classification can be obtained by referring to the coloured plate (Plate VIII.) than can be given by any description which we may offer.

For the differential staining of the white blood cells we have used stains prepared by following the directions of Jenner, Leishman, Nocht-Jenner, Hastings-Nocht-Jenner, Wright, &c. The essential dyes in each of the foregoing, while being the same—eosin and methylene blue—possess certain advantages in particular combinations, although unavoidable difficulties are encountered with the above-mentioned preparations, and these difficulties are familiar to those who have used these dyes in the enumeration of the various blood cells.

As uniformity is an essential feature in connection with a staining process where examinations are to continue for weeks, months, or even years, it is essential that a stain possessing the same characteristics should be available at all times. To this end, Watson and I, during the winter of 1906, endeavoured to obtain a more uniform staining material. Our experiments were very encouraging and we were able to obtain a staining material which we could modify almost at will to suit our special requirements. This staining material was made in the following manner—

a. polychrome methylene blue solution is prepared, using the formula of Romanowsky:

Methylene blue (med. pur.), 10 G.

Sodium carbonate C. P., 5.

Distilled water, 1000.

This mixture is steamed over a water bath with occasional stirring and sufficient water should be added from time to time to cover the loss by evaporation. When a rich purple coloration is noted, which is usually after two or three hours, the solution is evaporated to dryness. After cooling the residue is dissolved in pure methylic alcohol, making a saturated solution. To this alcoholic solution of the polychromed methylene blue, is added a solution of eosin in methylic alcohol of the following strength:—

Grubler's yellow eosin, 1 gramme.

Methylic alcohol, C.P., 1,000 grammes.

After the preparation of these two solutions the stain is made by mixing them in the following proportions, which, after much experimenting, has been found to give the best results:—

Polychrome methylene blue solution, 70 parts.

Eosin solution, 30 parts.

After this has been allowed to stand for an hour the staining of a blood film will reveal any error in the stain, which can be remedied according to its cause, provided, however, that the methylene blue has been prepared in a proper manner. If it is found that the solution is too alkaline, the addition of a few drops of acetic acid (0.5—1.0 per cent of acetic acid in methylic alcohol) will remedy the difficulty. In the addition of this acid great care should be exercised for the reason that if the solution is rendered too acid it is very difficult and may be impossible to fully restore its characteristic properties. If it is only very slightly acid it may be brought back to the proper degree by the addition of an alkaline solution such as 1.0 per cent caustic potash in methylic alcohol.

By preparing the stock solutions in large quantities and working out the details for mixing in small quantities only (100 cc.) as required, the stain gives excellent results. Many of the tiresome details connected with the preparation of blood stains are overcome by following the above technique and it is not necessary to have such a large amount of the staining fluid to manipulate during the process of preparation, a serious consideration with many of the other differential stains.

In staining the blood film, the slide or coverslip preparation of blood is completely flooded with the undiluted stain for about one minute, when two or three times the volume of water is added and the film remaining immersed in this for from three to five minutes for purposes of differentiation as indicated by Leishman or until a greenish yellow scum appears on the surface. If this does not appear some error in preparation has been made. After this differential staining, rinse in water for about thirty

seconds and carefully dry between blotting paper. The addition of the water to the stain on the slide is a very important matter, for, if it is not quickly and evenly performed one portion may be lightly and another deeply stained. The examination of the stained blood smear may be made with a dry or oil immersion lens. Personally a 4.0mm Zeiss objective with a No. 4 compensating eyepiece is preferred.

Through the use of the foregoing staining material we have been able to secure more uniform results than were possible prior to its adoption. Further, we are able to modify the stain to suit the requirements of any special work in hand and modifications have been found necessary when the stain is used on smears taken from the vagina, especially after an irrigation with citrate solution in a search for trypanosomata.

To those familiar with the ordinary materials and their use in connection with films of human, guineapig or rabbit blood, the need for a material which will give equal results when horses' blood is to be stained is not appreciated. We have found horses' blood much more difficult to stain than the blood of any of the other animals either wild or domestic, which we have had occasion to examine. Watson has been able to secure some remarkably uniform results with this stain in his studies of the *Trypanosoma equiperdum* at the Lethbridge quarantine station.

Apart from the difficulties of staining we have also found other problems which need further elucidation in connection with blood work. One of the most important is the number of cells it is necessary to count that a fair average result may be obtained. To indicate our efforts in this direction I append hereto a table (table I) which gives the counts made on three different smears taken from the same animal at the same time. A careful study of this table reveals that the smaller the number of cells counted, the greater is the possibility of error, and while four hundred cells may indicate a percentage very near to the average, five hundred may give a percentage which is far from the average. As a rule, however, the counting of one thousand cells on a single smear will give an average very similar to the average of a larger number. Whenever circumstances have permitted, we have counted one thousand cells, curtailing the number enumerated when the smear and the laboratory conditions did not admit of a longer search.

Tables II., III. and IV., give the results of counts made of the blood of horses affected with dourine. The two former tables are made up from data obtained in connection with the chronic affection (see also Chart I. and II.), while the latter (Table IV.) refers to data obtained from an acute case of dourine.

Table V. gives the counts which we obtained from the blood of a stallion (650), already referred to as being used in our breeding experiments. (See also Chart III.) This stallion has presented nothing out of the ordinary in his general clinical appearance, although he was used on the mare (589) during the season of 1906, on various occasions. There has never been any clinical manifestation indicating a dourine infection, and the knowledge of systematic examination of horses' blood is so limited that we are unable in this particular instance to explain the marked variations which occurred in the differential counts on various occasions.

The stallion 660, a record of whose blood-counts, appears in Table VI., was, as has already been mentioned, known to be suffering from a parasitic invasion of the blood, merely from the results of the differential blood-counts made at the time of his purchase. This marked eosinophilia was very interesting. This horse was used for service with the mare 588, but did not develop any symptoms indicative of a dourine infection, nor did this mare subsequently present any evidence of being with foal. A reference to the autopsy findings in this case reveals the correctness of the diagnosis made months before his death.

Table VII. presents a few counts made of the blood of the mare (670), used in our breeding experiments with the stallion (650). This mare has not given any evidence of a dourine infection up to the present time, nor has it been possible to get her with foal.

As an indication of the counts which may be obtained from healthy horses, the reader is referred to Table VIII. In this table the average findings of Moore, Fischer, Cozette and P. Mier are given, as well as some findings which are a portion of the records of this laboratory. The first three numbers referring to the biological laboratory records are of healthy horses kept under the ordinary conditions of stabling accompanied by work. No. 1 was a mare used for light driving around Ottawa, and her blood was taken while in harness on a trip to the laboratory. No. 2 refers to the stallion No. 30 (Dick) at the Lethbridge quarantine station, and it is this stallion that has been used in connection with the practical breeding experiments conducted at that station. No. 3 is a gelding attached to 'K' division of the Royal Northwest Mounted Police, whose blood was taken three hours after arrival at the Lethbridge quarantine station. From the results obtained in case of the horses Nos. 2 and 3, it would appear that the differential blood-count of a horse, even in a range country and feeding on prairie hay, provided it is stabled, will be very similar to that found by the authors above mentioned, which are without doubt records obtained from horses kept under the usual conditions common to a well settled community.

The counts given as our findings (Nos. 4 to 13 inclusive) are of blood taken from healthy horses kept under range conditions, and indicate that the blood of such animals, even though in perfect health as far as clinical evidence can indicate, presents a marked variation from that which is usually considered normal. These horses were in a district which is and has been free from dourine, and further, were inclosed within a pasture, and were considered as being suitable to supply us with blood smears for purposes of comparison with our many counts of the blood from horses affected with dourine, as given in Table IX. The age of these animals did not seem to occasion any marked variation in the differential counts.

In Table IX. the first two counts are from blood smears which Dr. Lingard of India was kind enough to supply us; the remainder, however, are of smears taken by myself at the Lethbridge quarantine station and at other points. All of the animals mentioned in this table from which I took smears, save No. 13, were under range conditions at the time of making the blood preparation, having been put through a chute or squeeze for purposes of clinical examination. In a few instances a slight variation in the count from the normal of a particular individual may have been occasioned by the excitement attending their handling.

A careful study will, however, indicate that there is a marked variation in the differential counts as here recorded. In this connection we have found that the variations noted in cases of dourine are in a large measure due to the stage of the disease. In all cases where the disease is advanced and clinical symptoms are in evidence, the differential blood-count is almost invariably that of the normal animal. In cases of dourine where a strong suspicion of the disease exists, and there is insufficient evidence to warrant the destruction of the animal, the differential blood-count will usually reveal a marked decrease in the poly-morpho-nuclear elements and a decided increase in the lymphocyte groups of cells. While this has been true with our counts there is yet a large amount of work necessary to positively determine the value of this method as an aid to the diagnosis of dourine.

TABLE I.

Smears from tail of 588 May 26, 1906.	Polymorpho- nuclear.	Eosinophiles.	Mast cells.	Total Lymphocytes.	Total cells counted.
Slide I—Transverse.....	49·5	4·8	1·4	44·3	1,000
.....	49·4	3·9	2·5	44·2	1,000
.....	51·0	4·8	1·4	42·7	1,600
Slide II.....	Trips across Smear				
10	46·7	5·4	2·3	45·5	779
14	52·5	3·4	1·3	43·0	1,078
10	54·6	3·7	0·2	45·4	510
13	51·0	4·0	1·6	43·3	600
17	50·5	3·6	2·1	43·6	515
18	51·9	3·3	2·4	42·3	539
18	50·8	3·8	0·6	44·8	500
19	56·2	4·6	0·6	38·6	500
17	49·4	3·0	3·0	44·4	821
Slide III—1 Upper edge of smears 1 trip	52·0	3·3	1·2	43·5	400
2 Middle of smears.... 3 trips	53·0	4·2	0·7	42·0	400
3 Lower third of smears 3 trips	48·3	5·7	1·7	44·2	400
4 Lower edge of smears. 1 trip	50·7	6·0	2·2	41·0	400
Average.....	51·3	4·2	1·6	43·5	690

Note Variation.

Total cells counted, 11,040.



TABLE II.\*

588 (Sleepy.)												
Date.	Polymorpho- clear neutro- philes.	Eosinophiles.	Mast Cells.	Total Lympho- cytes including large Mononu- clear.	Lymphocytes.	Large Mononuclear.	Granular Mononuclear.	Transitional Basophiles.	Transitional Neutrophiles.	Venous or Peri- pheral Blood.	Cells Counted.	
Nov. 22, 1905.....	50.7	6.0	2.3	41.0	30.1	5.9	.....	4.9	.....	V	345	
Dec. 7, ".....	49.1	8.0	3.4	39.3	32.8	2.3	.....	.....	.....	.....	350	
Feb. 16, 1906.....	41.6	4.3	0.8	54.2	46.2	3.6	.....	3.3	.....	.....	714	
" 24, ".....	43.9	3.3	3.3	49.5	36.2	10.0	.....	3.3	.....	.....	455	
Mar. 5, ".....	50.0	4.3	2.1	43.3	33.4	7.8	.....	2.1	.....	.....	350	
" 13, ".....	43.2	6.8	3.5	46.5	38.3	5.7	.....	2.5	.....	.....	560	
" 21, ".....	38.7	7.0	2.0	52.2	34.6	7.0	7.1	3.5	.....	.....	958	
" 28, ".....	40.8	6.8	2.3	50.2	41.7	1.2	4.6	2.7	.....	.....	857	
April 6, ".....	46.0	5.7	2.8	45.4	34.2	3.0	3.7	4.5	.....	.....	(1) 700	
" 18, ".....	48.1	3.5	1.7	46.6	37.5	4.5	1.1	3.5	.....	.....	337	
" 25, ".....	47.5	6.5	2.2	43.8	34.4	4.1	1.6	3.8	.....	.....	1,000	
May 3, ".....	47.5	6.6	2.1	43.8	.....	.....	.....	1.8	.....	.....	2,000	
" 11, ".....	41.3	5.6	1.5	51.6	.....	.....	.....	.....	.....	V	11,040	
" 26, ".....	51.3	4.2	1.6	43.5	.....	.....	.....	.....	.....	P. Tail.	2,000	
June 7, ".....	45.0	4.2	1.6	49.1	.....	.....	.....	.....	.....	P. Vulva	1,200	
" 14, ".....	51.7	3.5	1.6	41.2	.....	.....	.....	1.3	0.6	P. Tail.	2,000	
" 22, ".....	50.0	8.6	2.2	38.2	31.7	1.7	1.4	3.4	1.6	P "	1,000	
" 30, ".....	38.6	4.9	1.1	54.2	49.6	1.2	1.5	2.8	0.2	P "	730	
July 7, ".....	44.1	10.5	1.4	42.5	38.7	0.7	1.7	1.4	1.3	.....	1,100	
" 14, ".....	46.6	17.3	2.0	32.0	28.7	1.1	0.6	1.6	2.0	.....	801	
" 21, ".....	47.7	18.3	1.3	29.6	24.4	1.3	0.6	3.3	2.5	.....	901	
" 28, ".....	43.4	15.5	1.0	37.5	27.3	1.0	6.0	3.2	1.8	.....	1,000	
Aug. 4, ".....	50.0	9.5	1.0	38.5	32.0	2.4	0.6	3.5	1.0	.....	1,000	
" 11, ".....	56.6	9.0	0.6	32.3	30.0	1.0	0.3	1.0	1.0	.....	600	
" 18, ".....	51.4	9.8	0.7	37.1	31.4	2.1	1.3	2.3	1.0	.....	700	
Sept. 29, ".....	51.6	8.3	1.2	37.8	33.3	1.7	1.0	1.8	1.0	.....	600	
Oct. 10, ".....	43.3	14.2	2.5	36.7	28.3	1.6	3.3	2.5	2.2	.....	660	
Nov. 29, ".....	51.5	8.8	0.4	39.2	38.0	.....	0.4	0.8	.....	.....	510	
Jan. 28, 1907.....	57.1	6.1	1.4	33.0	28.7	3.4	0.9	1.6	0.8	.....	1,000	
Feb. 16, ".....	60.2	8.4	1.8	27.9	24.2	3.2	0.5	1.2	0.5	.....	1,000	
April 16, ".....	63.1	4.1	.....	32.3	31.8	0.2	0.3	0.5	.....	.....	1,000	

(1). 12,000 whites per cubic Millimeter.

(2). Aug. 4, '06. 11,000 whites and 8,584,000 reds per cubic Millimeter.

\* Unless otherwise stated all figures refer to percentage of cells found based on the total number counted.

TABLE III.

Date.	Time.	Cells per cubic millimeter.		Polymorphonuclear neutrophils.	Eosinophiles.	Mast cells.	Total lymphocytes including large mononuclear.	Lymphocytes.	Large mononuclear.	Granular mononuclear.	Transitional basophiles.	Transitional neutrophils.	Venous or peripheral blood.	Cells counted.
		Reds in millions.	Whites in thousands.											
Nov. 22, 1905				53.0	9.1	1.0	36.1	28.1	0.6		0.2		V.J.	430
Feb. 2, 1906				31.0	4.8	.6	62.8	50.0	9.6		3.8		P.	310
Mar. 24, 1906				53.2	4.3	1.6	58.6	49.4	7.6		1.6		V.J.	417
" 5, 1906				27.0	7.7	1.0	61.1	57.9	5.2		1.0		P.	518
" 13, 1906				22.5	8.3	1.2	68.0	62.0	3.3		2.7		P.	846
" 17, 1906				31.0	7.3	1.0	69.7	46.6	2.0	9.4	2.7		P.	1,286
" 21, 1906				57.5	4.0	1.0	37.5	24.4	1.5	8.5	3.1		P.	1,446
" 28, 1906				48.6	10.0	2.0	38.5	27.7	2.7	5.1	3.0		V	1,085
April 4, 1906			18	50.2	8.7	1.3	39.8	30.7	2.1	4.0	3.0		V	916
" 11, 1906				85.6	4.5	1.1	58.8	51.7	1.6	3.0	2.5		P.	600
" 18, 1906				39.5	7.2	2.1	51.2	46.7	(?)	2.8	3.7		P.	1,900
" 25, 1906	a.m.			45.0	8.2	1.9	44.9	33.4	5.0	3.9	2.9		V.J.	1,000
" 25, 1906	p.m.			41.5	11.0	1.0	46.5	35.4	4.3	3.9	2.9		V.J.	1,000
May 3, 1906				41.7	6.1	1.0	50.7	?	?	?	2.0		V	5,685
" 8, 1906				50.2	4.3	1.0	42.5	?	?	?	?		V	2,787
" 10, 1906				47.8	6.8	0.8	44.5	?	?	?	?		V	4,140
" 22, 1906				43.5	5.5	0.7	50.3	?	?	?	?		V	1,000
" 30, 1906				45.8	7.0	1.4	45.8	?	?	?	?		V	1,000
June 7, 1906				43.0	7.5	1.5	48.0	?	?	?	?		V	2,650
" 14, 1906				41.9	7.0	1.0	50.1	?	?	?	?		P. tail.	1,000
" 18, 1906	9 a.m.	8.78	14.0	43.8	9.4	0.7	46.5	42.0	0.7	0.7	0.7		P.	1,004
" 19, 1906	9 a.m.	8.20	12.6	42.1	8.0	0.5	48.4	40.2	6.5	4.2	1.7		P.	1,400
" 20, 1906	9 a.m.	9.57	12.4	42.7	8.3	1.2	46.3	44.5	0.7	1.1	0.7		P.	1,500
" 21, 1906	9 a.m.	8.76	12.0	42.0	7.6	1.2	48.3	44.6	1.2	1.5	0.2		P.	1,600
" 22, 1906	9 a.m.	9.44	13.4	45.9	12.0	0.8	41.2	37.0	0.8	0.8	2.1		P.	2,517
" 23, 1906	9 a.m.	9.80	11.6	47.4	9.6	0.7	41.6	38.5	1.1	1.1	1.0		P.	2,210
" 30, 1906				43.2	10.1	0.8	44.2	34.0	3.5	3.6	3.3		P.	1,000
July 7, 1906				31.8	9.3	1.1	57.4	47.5	6.1	0.1	3.7		P.	1,000
" 10, 1906				40.5	16.0	1.1	41.2	33.5	2.2	3.3	2.2		P.	1,800
" 11, 1906				43.7	12.6	1.1	40.0	25.2	2.4	5.6	0.8		P.	1,300
" 14, 1906				53.8	13.1	1.3	31.0	34.5	0.5	1.0	2.0		P.	1,100
" 17, 1906				38.6	13.0	0.7	44.5	38.0	3.4	6.1	1.0		P.	800
" 21, 1906				47.1	12.5	1.1	37.8	28.7	0.6	5.4	3.1		P.	1,000
" 24, 1906				74.0	1.7	0.4	17.9	12.6	1.8	1.5	2.0		P.	1,000
" 25, 1906				54.0	7.5	1.1	36.4	28.4	1.4	2.0	4.0		P.	1,000
" 25, 1906				44.3	13.4	1.0	39.6	33.0	1.2	2.0	3.4		P.	900

Aug. 4, 1906	.....	.....	49.0	8.0	1.3	40.7	35.5	1.5	1.5	2.2	1.0	1,000
" 11, 1906	.....	.....	46.4	11.0	1.3	40.4	32.0	2.4	4.0	2.0	0.8	900
" 14, 1906	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	606
" 18, 1906	.....	10 a.m.	.....	11.0	1.0	41.6	29.3	4.0	5.0	3.3	1.3	400
Sept. 29, 1906	.....	3 a.m.	52.5	9.0	1.2	36.7	32.5	1.5	1.2	1.5	0.2	400
Oct. 10, 1906	.....	3 p.m.	53.0	11.0	1.0	34.0	24.0	2.0	6.0	2.0	0.4	500
Nov. 29, 1906	.....	.....	50.9	7.7	0.7	41.6	21.2	5.2	2.5	0.1	.....	528
Jan. 28, 1907	.....	.....	46.4	12.2	0.8	40.6	37.0	3.4	.....	.....	.....	500
Feb. 16, 1907	.....	.....	58.6	7.7	1.2	31.4	30.0	1.0	.....	0.3	.....	2,000
April 16, 1907	.....	.....	75.0	4.6	1.0	34.0	19.6	0.2	4.0	2.0	.....	1,000

<sup>1</sup>All leucocytes in smear counted.  
The myelocytes observed are recorded on the accompanying chart.

TABLE IV.

590 "Nigger."

## ACUTE DOURINE.

DATE.	Time.	Reds in Millions.	Whites in Thousands.	Polymorpho nuclear neutrophiles.	Eosinophiles.	Mast Cells.	Total lymphocytes including large mononuclear.	Lymphocytes.	Large mononuclear.	Transitional basophilus.	Cells counted.
1905.											
November 21.....				70·	3·4	·6	26·	23·8*	1·	1·2	500
December 1.....		4·952		73·2	2·7	1·7	22·2	20·7	.....	1·5	580
" 12.....				60·4	8·1	1·2	30·1	25·3	3·2	1·6	493
" 16.....				84·5	.....	.....	15·5	12·5	.....	3·	600

\*The Lymphocytes contain granules in the majority of instances and the protoplasm is very darkly stained.

TABLE V.

650.		Polynuclear neutrophiles.	Eosinophiles.	Mast cells.	Total lymphocytes including large mononuclear cells.	Large mononuclear.	Large granular mononuclear cells.	Transitional basophiles.	Transitional neutrophiles.	Total cells counted.	
1906.											
May,	25	59.2	6.4	1.7	32.5					2,000	
	28	55.6	6.1	0.9	37.4					12,500	
	31	61.0	5.7	1.3	33.0					1,000	
June	4	60.0	6.4	1.1	32.5					1,000	
	7	65.7	4.5	1.3	28.5					1,000	
	11	71.6	5.0	1.1	22.3					1,000	
	16	62.0	3.3	0.7	32.8	2.1		0.9	0.7	1,000	
	30	41.0	4.9	0.9	54.2	1.4	1.3	1.2	0.7	1,000	
July	7	37.1	7.0	0.2	55.4	1.3	0.7	2.1	0.2	1,100	
	10	58.0	4.5	0.3	37.9	0.6	0.6	0.1	0.7	500	
	11	65.1	11.0	0.9	25.0	0.7	3.1	1.6	0.3	1,000	
	12	55.0	11.0	0.5	33.5	2.0	1.1	4.0		400	
	13	60.0	12.0	0.5	27.0	1.5	2.0	0.5		400	
	14	56.6	12.5	0.7	28.3	2.3	1.0	1.0	1.2	600	4 myelocytes
	16	55.0	8.0	1.2	33.8	1.0	1.8	2.0	1.0	400	4 "
	17	58.0	7.3	1.5	31.1	1.5	5.0	0.8	1.3	1,000	8 "
	17	61.4	9.6	1.3	26.3	0.4	3.4	2.0	0.1	700	4 "
	18	63.0	9.0	1.8	25.3	2.0	2.3	2.0	0.4	500	1 "
	19	52.6	14.0	1.0	32.0	1.0	1.0	2.0	0.4	500	
	20	55.0	12.0	1.0	30.6	2.0	1.4	1.2	0.4	500	
	21	57.8	13.3	1.2	26.2	1.0	1.0	2.2	1.5	1,000	
	23	48.3	7.0	0.6	33.0	1.0	1.0	2.0	1.0	300	
	25	53.3	11.0	1.0	33.2	2.6	2.3	3.3	1.0	600	2 "
	28	45.0	17.0	0.3	37.2	1.0	1.0	2.0	0.3	300	
Aug.	2	56.7	11.8	0.7	29.3	2.0	3.1	0.4	1.4	700	
	7	67.2	7.0	0.8	25.0	1.0	2.0	2.0		500	
	14	55.0	8.0	0.5	36.0	2.0	2.0	1.0	0.5	200	
	21	50.0	14.0	2.2	33.2	0.8	1.0	0.8	0.8	500	
	28	43.0	15.2	0.4	41.2	2.8	2.2	3.0	0.2	500	
	29	55.0	8.8	1.0	36.0	1.0	2.0	2.0	0.6	500	
Oct.	11	55.5	8.5	1.0	35.0	1.0	1.5	1.5		400	

<sup>1</sup>Fifteen mulberry bodies noted similar to those mentioned by Kern.

<sup>2</sup>Smear taken at eleven in the morning.

<sup>3</sup>Smear taken at seven-thirty in the evening, three hours after service.

<sup>4</sup>All subsequent smears taken at nine in the morning.

TABLE VI.

Date.	Time.	Cells per cubic millimeter.		Eosinophiles.	Total lymphocytes including large mononuclear.	Small lymphocytes.	Large mononuclear.	Granular mononuclear.	Transitional basophilic.	Transitional neutrophilic.	Venous or peripheral blood.	Cells counted.
		Reds in millions.	Whites in thousands.									
June 9												
" 24	5 p.m.	9.4	15.0	76.3	1.2	17.0	13		9.7		V	700
" 25	1 p.m.	8.3	18.4	65.7	1.2	17.0			3.1		V	1,000
" 25	5 p.m.			67.1	1.4	12.5	1.1		2.2		V	1,200
" 25	8 p.m.			70.0	1.7	11.1	1.2	0.6	2.0		V	1,300
" 26	7 a.m.	7.3	18.0	72.0	1.0	10.3	1.0	1.0	2.5		V	1,000
" 26	3.30 p.m.	7.0	14.0	64.8	1.6	15.0	3.7		3.1		V	1,100
" 26	8 p.m.			54.0	2.1	20.0	1.0		2.7		V	1,000
" 27	9.30 a.m.	7.4	15.4	52.0	3.4	18.1	2.0	0.4	3.5		V	700
" 27	8 p.m.			56.0	2.0	22.0	1.1		4.9		V	1,100
" 28	9 a.m.			53.0	2.5	16.1	0.6	0.4	0.2		V	1,000
" 28	4.30 p.m.	7.5	16.0	57.0	2.1	18.2	0.8	2.1	3.0	0.7	V	1,000
" 29	9 a.m.			54.0	3.3	19.5	1.0	1.2	1.8	0.2	V	1,000
July 3	9 a.m.			57.6	2.0	18.4	11.7	0.5	3.0	0.5	V	1,040
" 5	9 a.m.			61.2	1.4	19.2	13.1	1.0	3.2	0.2	V	700
" 7	9 a.m.			55.6	2.4	25.2	22.0		2.0	0.8	V	500
" 7	5 p.m.			59.7	1.4	24.9	20.0	0.7	2.4	0.7	V	700
" 11	10 a.m.			59.4	13.4	24.8	30.3	1.1	1.7	1.7	V	700
" 16	10 a.m.			60.7	14.5	21.1	14.3	3.0	1.7	1.7	3d	700
" 20	2 p.m.			60.6	8.5	26.1	18.5	1.5	2.1	2.1	4V	800
" 23	9 a.m.			60.0	12.0	23.3	18.0	3.5	2.7	2.0	6V	701
" 26	3 p.m.			56.5	8.7	31.5	25.0	1.1	2.7	1.1	6V	800
Aug. 7	9 a.m.			62.5	11.6	23.3	17.0	1.2	3.1	1.0	V	400
" 11	9 a.m.			65.5	9.5	20.3	20.3	0.7	1.7	1.2	V	600
" 18	9 a.m.			60.0	11.3	25.0	15.6	4.2	2.5	2.7	V	600
" 21	9 a.m.			46.6	14.0	20.0	50.0	1.0	1.0	1.0	7V	600
Oct. 10	9 a.m.			70.0	5.0	22.6	19.0	1.0	1.6	1.0	8V	600

<sup>1</sup>One dose mallein at 6.30 a.m. <sup>2</sup>Four myelocytes noted. <sup>3</sup>Five myelocytes noted. <sup>4</sup>Nine myelocytes noted. <sup>5</sup>Two myelocytes noted.

<sup>6</sup>Four myelocytes noted. <sup>7</sup>Three very large lymphocytes noted which were stained a much deeper blue than is ordinarily the case. <sup>8</sup>One myelocyte.

TABLE VII.

670. - HEALTHY MARE.

Date.	Time.	Polymorpho nuclear neutrophiles.	Eosinophiles	Mast cells.	Total lymphocytes including large mononuclear.	Lymphocytes.	Large mononuclear.	Granular mononuclear.	Transitional basophiles.	Transitional neutrophiles.	Cells counted.	
June 11, 1906.	.....	70.0	6.6	1.0	23.0	.....	.....	.....	.....	.....	2000	
July 10, "	9 a.m.	41.6	2.7	0.2	54.1	39.0	3.2	8.6	2.3	1.5	1100	
" " "	4 p.m.	51.6	5.0	0.6	33.9	26.3	2.0	5.6	2.1	0.7	600	
" 11, "	9 a.m.	61.0	4.1	1.1	32.8	18.5	4.5	7.7	2.2	2.2	700	
" 14, "	"	58.6	4.3	1.1	33.0	28.5	0.6	1.7	1.8	0.8	1200	
" 16, "	"	61.8	3.0	0.3	34.0	30.0	2.3	0.9	1.8	0.8	890	
Aug. 11, "	"	57.0	5.6	1.4	34.8	22.0	2.8	8.8	1.2	0.6	500	
" 28, "	"	59.0	7.5	2.3	30.6	20.0	3.3	3.3	4.0	0.5	600	
Sept. 27, "	"	37.4	7.0	3.4	51.2	36.4	2.6	1.6	10.6	1.0	500	Seven myelocytes noted.

TABLE VIII.  
HEALTHY HORSES.

Authority.	Number.	Name.	Age.	Sex.	Cells per cubic Millimeter.		Polymorpho nuclear neutrophils.	Eosinophiles.	Mast cells.	Total lymphocytes including Large mononuclear.	Lymphocytes.	Large mononuclear.	Granular mononuclear.	Transitional basophiles.	Transitional neutrophils.	Source of blood.	Cells counted.	Hæmoglobin.	Specific gravity.
					Reds in millions.	Whites in thousands.													
V. A. Moore, 1907	1	Average.					59.0	4.0	1.0	36.0	30.0	6.0	a	a	a	d	a		
	2	"	10	F	10.42	60.0	61.5	5.1	2.0	31.4	21.4	2.5	2.5	5.0	a	d	700		
	3	"	M	C	10.66	60.0	61.4	6.7	0.4	32.9	b	b	b	b	b	d	1000		
	4	"	N. P.	M		61.4	61.4	3.0		35.6	b	b	b	b	b	d	500		
Biological Laboratory Records.	4	Nancy.	14	F	b	52.6	52.6	12.2	0.6	33.8	30.0	1.8	0.4	1.6		V	500		
	5	Jessie II.	6	F	b	50.9	50.9	4.4	0.3	43.8	41.7	1.0	0.1	1.0		V	1000		
	6	Pedro	7	G	b	51.3	51.3	8.6	0.6	40.0	41.2	0.6		0.6		V	522		
	7	Baby	10	F	b	55.4	55.4	10.0	0.6	34.0	33.8					V	500		
	8	Elephant.	8	G	b	44.3	44.3	12.4	1.0	40.8	37.6			0.2		V	500		
	9	Togo.	1	M	b	41.2	41.2	7.0	0.6	50.6	44.1	2.2	1.0	1.4	0.6	V	1000		
	10	Jessie I.	17	F	b	51.8	51.8	9.3	1.1	36.5	25.3	4.3	0.8	2.6	1.3	V	1000		
	11	Syd.	9	F	b	46.6	46.6	0.9	6.8	45.1	25.0	6.6	2.0	1.9	8.1	V	1000		
	12	Jennie.	22	F	b	38.5	38.5	0.9	7.1	53.5	46.9	4.2	0.6	0.6	1.8	V	1000		
	13	Steel.	3	F	b	51.2	51.2	0.7	2.1	46.0	27.0	17.0	0.9	0.9	1.1	V	1000		

a Not given. b Not enumerated.



TABLE IX.—Cases of Dourine in Various Stages.

Authority.	Number.	Name.	Age.	Sex.	Cells per cubic millimeter.		Polymorphonuclear neutrophils.	Eosinophiles.	Mast cells.	Total lymphocytes, including large mononuclear.	Lymphocytes.	Large mononuclear.	Granular mononuclear.	Transitional basophiles.	Transitional neutrophils.	Source of blood.	Cells counted.	
					Reds in millions.	Whites in thousands.												
Lingard	1	*Galbar	.....	M	.....	.....	54.6	5.7	1.4	40.6	39.6	0.4	.....	0.6	.....	b	1000	September 12, 1906.
"	2	Mare VIII.	.....	F	.....	60.6	6.2	0.8	33.0	33.0	b	b	b	b	b	b	1000	"
"	3	Perry.	.....	F	.....	45.0	6.2	0.8	38.0	38.0	b	b	b	b	b	b	1000	"
"	4	Whitney.	.....	F	.....	71.1	1.2	0.2	27.3	27.3	25.9	0.7	0.2	0.5	0.2	V	1000	"
"	5	No. 5.	.....	F	.....	41.7	8.6	.....	47.5	46.2	46.2	1.0	0.4	.....	2.1	V	1000	"
"	6	No. 6.	.....	F	.....	33.4	7.1	.....	58.7	49.0	49.0	9.3	0.4	.....	.....	V	1000	"
"	7	Moonshine.	.....	M	.....	47.8	2.6	1.6	46.0	41.6	41.6	2.8	.....	1.6	.....	V	500	"
"	8	H3.	.....	F	.....	30.2	14.0	0.2	54.6	50.8	50.8	2.8	.....	1.0	.....	V	500	"
"	9	Bay HI.	.....	F	.....	33.8	7.2	0.4	59.6	57.4	57.4	2.0	.....	0.2	.....	V	500	"
"	10	No. 7.	.....	F	.....	43.0	4.0	3.8	48.8	48.8	38.6	8.6	0.8	0.8	.....	P	500	"
"	11	No. 2.	.....	F	.....	31.2	4.0	0.4	64.4	64.4	59.2	1.6	28.0	0.8	.....	P	513	"
"	12	No. 9.	.....	F	.....	19.1	3.5	0.6	76.4	76.4	b	b	b	b	b	V	522	"
"	13	McKenzie No. 33	.....	M	.....	61.0	7.1	0.1	30.7	30.7	b	b	b	b	b	V	489	"
"	13	"	.....	M	.....	8.144	4.9	0.9	13.9	13.9	b	b	b	b	b	V	1000	"
"	13	"	.....	M	.....	7.38	5.0	0.3	17.9	17.9	b	b	b	b	b	V	1008	"

\* Arab stallion.  
b Not enumerated.

## REPORT ON A CASE OF DOURINE WITH EXPERIMENTAL INOCULATIONS AND MISCELLANEOUS NOTES ON ITS SYMPTOMATOLOGY AND DIAGNOSIS.

By E. A. WATSON, V.S.

*Assistant Pathologist in charge Lethbridge Quarantine Station, Lethbridge, Alberta.*

NOVEMBER 15, 1907.

### DOURINE (NATURALLY ACQUIRED).

No. 36. Mare "Tiffin" (plate XI) 9-10 years of age. Clydesdale stock (Ontario).

*Date and source of infection.*—Not definitely determined. The mare was covered several times by different stallions in the summers of 1904-5 and 6. Fertilization did not occur. A Clydesdale stallion, a few weeks after the covering of this mare in August last, exhibited a slight swelling of the sheath and scrotum. This swelling was somewhat increased in November and assumed an intermittent type. In February, 1907, the swelling had not reappeared. A grey stallion that covered the mare in September has remained apparently healthy up to the end of the year 1906.

*The earliest sign of disease.*—First noted by the owner of the animal in the latter part of November, 1906, as a tenderness over the loins followed by a peculiar stumbling and erratic gait, these symptoms rapidly becoming so severe that the mare could only be taken out of her stable to water with great difficulty.

The mare had been steadily worked during the previous summer and fall and up to the time that the disease was manifested by the above symptoms. It is of interest to note that this is not a range mare, but one that has been more or less continuously under the immediate observation of the owner who states that he never observed any abnormal condition of the genital organs save that the mare will not breed.

On December 19, the animal came under my personal observation and her general appearance and condition was good. With the exception of the loss of control described later under 'Nervous Symptoms.'

Further investigation has left little doubt that this mare (36) was infected by the Clydesdale stallion No. 35, already mentioned as the probable source. Other mares covered by the same stallion previous to the covering of mare 36, have been found to be diseased. Furthermore, the stallion after a period of nearly one year subsequent to the covering, a period in which only slight and occasional manifestations of the disease were noted, has completely broken down, presenting characteristic and intensely severe symptoms of dourine. (See details case No. 35.)

### THE GENITAL ORGANS.

December 19, 1906. A slight orange tinge to the mucous membrane of the vulva, less so of the vagina, was observed. Secretion very scanty, clitoris very pale.

Microscopical examinations of the scrapings of the genital mucous membranes were made upon the following dates:—December 19, 23, January 1, 4, 15 and 29, at intervals of 4, 9, 3, 11 and 14 days respectively. Bacteria were usually very plentiful and in great variety. Spirochæte were numerous, and rarely a few cell-like irregular forms without flagella which were suggestive of immature or amœboid forms of Trypanosomata.

The first demonstration of the *Trypanosoma Equiperdum* was on February 11. On this date the mucous membrane of the vulva, extending to the vagina, was distinctly orange tinged, slightly corrugated and œdematous. A well defined vesicle was visible on the inner border of the right labium, and the base of the vesicle was a little more than two centimetres in diameter, being raised, much firmer and more œdematous than the surrounding tissues.

This small œdematous area, including the vesicle, was slightly scarified. Three smear preparations were made of the sero-sanguineous fluid, in two of which the trypanosomata were found. The organisms were very rare, 16 only being counted after searching the whole of the smears occupying many hours.

Trypanosomata have since been demonstrated in the vaginal mucus or in traces of blood from scarified œdematous points of the mucous membrane, more or less constantly up to this date, March 16. (For detailed findings see table XI).

The contour and general appearance of the vulva is slowly changing. (March 31, 1907.) A few small depigmented spots recently appeared at the edges of the labia, gradually increasing in size until there is now a distinctly leucodermic area on either side of the vulva. The mucous membrane is slightly everted, the clitoris totally depigmented and the vulva tumefied.

The mucus is very scanty, the inner borders of the labia and the clitoris being pale and dry. The œdematous 'pinheads' of the mucous membrane have disappeared but on the floor of the vagina are two well marked œdematous areas, about 2½ centimetres in diameter, straw coloured and semi-transparent.

The temperature has assumed a more normal and regular curve.

The appetite has never failed and the general condition has improved slightly, though inco-ordination of the limbs is still apparent and the hind quarters sway from side to side.

Trypanosomata have been demonstrated in the sero-sanguineous fluid of the vaginal mucous membrane, upon the following additional dates: March 20, 21, 23, 24 and 25. A few of the organisms appear to be assuming an amœboid form, the body of the parasite being much less elongated than usual, the nucleus large and the flagellum absent or unstained.

Sexual excitement was manifested for a period of 5-6 months commencing four months after infection, and was accompanied by pruritis. From March to June, 1907, the condition was that of nymphomania save during this latter period, the mucous secretion has at all times been scanty. The external genitals under surface of tail and inner sides of thighs have never shown evidence of a vaginal discharge. In June and July, 1907, the depigmented spots and patches spreading from the muco-cutaneous margins of the vulva had partially and at this date, (November 15), totally regained their colouring matter, and a normal appearance. Neither an ulcer nor a cicatrix has ever been observed. Tumefaction of the labia has disappeared. The vaginal mucous membrane remains swollen, tough and elastic. In small areas or patches there are clusters of lymph follicles giving the membrane a finely nodular or puckered appearance.

## CUTANEOUS MANIFESTATIONS.

### THE SO-CALLED DOURINE 'PLAQUES.'

No. 1. Appeared December 22, 1906. A small swelling about one centimetre in diameter, oval surface, hardened skin, not pitting on pressure. Situation, posterior left ribs. The swelling persisted for two days. Trypanosomata not found in smear preparations from small incision.

No. 2.—Appeared December 31. A small swelling similar to No. 1. Situated on the lower portion of the right breast. Trypanosomata were not found in a blood

smear from this swelling. Disappearance slow and gradual the skin remaining hard for several weeks.

*No. 3.*—Appeared February 11, 1907. Size about four centimetres in diameter. The outer border was harder than the centre. February 13, swelling decreased. February 14, the fourth day, the swelling was harder and flatter. On the 16th the sixth day, only a circular area of hardened skin remained. The swelling was punctured on February 11 and 14, there was no exudation of sanguineous fluid, but just sufficient blood for three small smears on each date, all of which were negative.

*No. 4.*—Appeared on the same date as *No. 3* and was slightly larger circular shaped not œdematous, situated just below point of left hip, very much diminished on February 16. Skin remained hard for three weeks. Blood smears taken February 11 and 12, negative.

*Nos. 5 and 6.*—Appeared August 25, 1907, in the form of a double plaque over the middle of the right ribs. The swellings adjoined one another, each being five to six centimetres in diameter and raised one-half to one centimetre above the surface of the surrounding skin. The swellings were quite firm, flat and not more raised at the centre than at their circumference. Six microscopic preparations of the sero-sanguineous contents were obtained from a puncture. Trypanosomata were found in two of these six specimens, the parasites being very few, vacuolated, swollen degenerated or involution forms.

The plaques had entirely disappeared on the third day after eruption.

*No. 7.*—Appeared September 2, over the middle left ribs and was about the size of a silver dollar. Parasites could not be found in the fluid from puncture.

September 4 only a trace of plaque *No. 7* remained.

September 7. Reappearance of the plaque in the same position and of four smear preparations made, Trypanosomata were found in one. The parasites were very rare.

November 15. No further cutaneous lesions have appeared to date.

None of these swellings could by any means be called a typical plaque, being neither the distinctly œdematous, nor the disc shaped tumour as described by Lingard and others. It is possible, however, that they appeared before the dates given above, and were not observed until in the disappearing stage, which would account for the absence of the trypanosomata from the blood smears.

One peculiar characteristic of the swellings is in the persistence of a hardened area of skin for a very considerable time after the swelling has been absorbed.

### NERVOUS SYMPTOMS.

On December 19th, 1906, there was a great tenderness and weakness of the loins. The animal could be forced to the ground by slight pressure on the lumbo-sacral nerves, on either side.

Paraplegic symptoms of the hind limbs were pronounced. The hind-quarters appeared 'dropped' and the loss of control was most evident. Both fore and hind limbs were placed in most erratic and singular positions. The attitude was crouching. The hind-quarters swung to the left, often remaining in that position when the animal was at rest. Knuckling of the hind-fetlocks was extreme, the pasterns being at times in contact with the ground. On being turned loose in the corral, locomotion seemed almost entirely mechanical, the animal appearing to wish to travel in a different direction to that which her limbs were taking her.

On December 29th, 1906, the animal was profusely bled from the jugular vein until there were evinced signs of distress and weakness. During the next week locomotion and the condition of the loins and limbs steadily improved. The knuckling

was greatly reduced, nervous and muscular control was resumed and the tenderness over the loins finally disappeared. During this time ( the month of January) the animal was kept under severe conditions in a pasture where the grass was scanty, the snow deep and the cold intense. Since February 11th, when trypanosomata were demonstrated in the vaginal mucus, the nervous symptoms have re-appeared, slowly and gradually increasing in intensity, but have not yet reached the same degree as before the blood letting.

On June 1st there was great improvement in her condition (see photo plate XI). The knuckling and nervous inability can scarcely be observed. Trypanosomata at this time were occasionally in vaginal blood.

Throughout June, July and August, 1907, a great moderation was noticed in the symptoms of neuro-muscular inco-ordination. The gait had become much steadier and the knuckling of the fetlock joints was scarcely perceptible. Occasionally, for brief periods, there has been seen an intermittent 'tripping' gait with swaying of the hind-quarters from side to side, but on the whole, a steady, slow improvement has been maintained up to the present date, November 15, 1907.

### THE BODY TEMPERATURE.

Previous to February 15 the temperature was only occasionally recorded, and was found to be between 100° and 101° F. Morning and evening temperatures have been taken daily since February 16. From the fifth day after demonstration of trypanosomata in vaginal mucus, the temperature remained constantly between 101° and 101:4° F., a variation of but half a degree, with almost an entire absence of a normal regular daily rise and fall. On the 14th day there was a sudden elevation to 103.6° F, the respirations being shortened and pulsations increased to 60-65 per minute. The nervous symptoms of the hindquarters and loins became more apparent. The temperature gradually fell again to the neighbourhood of 101° F., the lowest being 98° and the highest 102° F.

### THE LEUCOCYTE COUNT. (TABLES XII AND XIII).

Seven differential counts of the leucocytes of the blood of this animal have been made during the three months of observation at intervals of 4, 8, 2, 40, 6 and 7 days. Upon every occasion the relative percentages were found to be about normal, but at the same time there was a decided absolute leucocytosis, all classes of cells being increased. This could easily be determined by a short examination of the stained smear, and by the ratio of the red to the white cells, without actually making a quantitative count. The bone-marrow type of cell, the polymorphonuclear neutrophyle, always predominated over the lymphoid cell, and this blood-picture has been found in the other cases of Dourine at this station, in which the blood has been studied, as evinced by nervous symptoms or intermittent œdematous swellings. An opposite condition, namely, an absolute leucocytosis with a predomination of the Lymphocyte type of cell, has been observed in those cases of Dourine in which the disease is in a latent stage, where the *materies morbi* is lying dormant, or where the animal is making a recovery, apparent or real. The Hæmoglobin scale (Talquist) registered 80-85 normal.

For details of leucocyte counts and comparisons, see Table No. XII.

THE DEMONSTRATIONS OF THE TRYPANOSOMA EQUIPERDUM. (See Plate XVI. and XVII.)

*Summary of Table No. XII.*

In vaginal mucus, trypanosomata present in	14	preparations.	
		35	examined.
“ blood “ “		27	preparations.
		72	examined.
In blood from the plaques, trypanosomata were <i>not</i> present in		19	examined.
“ tail “ “		10	“
“ jugular vein “ “		9	“

Thus, the organisms were never found in blood drawn from the general circulation or cutaneous swellings, but it is probable only in the early eruptive stage of the latter that the organisms are ever observed.

In the vaginal mucus and vaginal blood the organisms were found in nearly the same proportion of preparations, namely, 40 and 38.8 per cent respectively.

In the mucus they usually appear small, contracted and often vacuolated, or very thin and elongated, are stained with difficulty and not always easy to differentiate from the cellular debris, &c. To secure a good preparation, the mucus must be very thinly spread and without pressure, free from dirt and dried instantly. On several occasions preparations from the blood and mucus were made before and after a thorough irrigation of the vagina with a solution of sodium chloride and sodium citrate (sodium chloride, 5 grammes, sodium citrate, 5 grammes, boiled water 1000 c. cm.), and where but one or two trypanosomata were found in several smears before irrigation, they were more numerous in those taken immediately after. The preparations of February 20, in which the organisms were the most numerous during the whole of this series of examinations, were taken immediately after an irrigation.

Trypanosomata were found in the mucus only in the absence of bacteria, or when the latter were very scanty. The irrigation of the vaginal canal with a citrate solution is favourable to the detection of trypanosomata, not only removing the bacteria and toxic mucus, but causing a fresh and increased secretion from the vaginal membranes, in which, especially after a slight scarification, the trypanosomata are more likely to be found. It is important to scarify the membrane very lightly so that only a small trace of blood exudes, and at those minute points that present an œdematous or vesicular appearance.

In preparations made from drops of blood flowing from a deep puncture through the mucous membrane and into the underlying tissues, I have not been able to find a single trypanosoma.

Several conjugating parasites were observed in the vaginal blood and multiplication forms were not uncommon. The trypanosomata were extremely rare in a great majority of the preparations, two to three organisms only being observed in each, and it has frequently been necessary to search a slide for one to two hours before finding a single parasite.

In the series of examinations given in Table No. XI., the time occupied in the search of each slide averaged about 40 minutes.

As already detailed, trypanosomata could be found more or less constantly in the vaginal blood and mucus, between February 11 and the end of March, provided a sufficient number of preparations were carefully searched on each day of examination. In April, the parasites had almost wholly disappeared and were found on only one occasion, April 9. The last observation of trypanosomata in the vaginal blood was made on June 29, but on August 25th and September 7 the organisms were found in the contents of the cutaneous plaques.

NOTE.—I would again call attention to the value of irrigating the vaginal mucous membrane with a warm solution of sodium citrate and chloride, previous to the pre-

paration of blood smears or the collection of blood for examination. This procedure has in my hands greatly facilitated the search for the parasites and has now become habitual with me. I have repeatedly secured preparations before and after irrigation, finding the parasites only in those taken after.

*Some points of interest.*—Three months elapsed after the covering by the Clydesdale stallion, (No. 35), and the first manifestation of the disease. Notable features of the case are—the absence during these three months, according to the animal's owner, of any lesions of the genital organs and the fact that the disease instead of being primarily localized to these organs, as is usual, became rapidly generalized.

The nervous phenomena were the first noticeable manifestations of the disease, by which the diagnosis was made, being confirmed 78 days later by the finding of the trypanosomata. A marked sexual excitement has at all times been evident on bringing the mare in proximity to a stallion.

To the profuse blood-letting, which was pushed to the physiological limit, I attribute the disappearance of the nervous symptoms, by the removal with the blood of a great amount of toxine of the disease.

## EXPERIMENTAL INOCULATIONS WITH DOURINE.

### EQUINES.

ANIMAL No. 26.—Two year old filly. 'The vaginal discharges of two dourined mares were injected into the vagina and uterus, last injection made July 18, 1906. No positive reaction obtained.'—(Dr. S. Hadwen.) Blood inoculations.—November 21, 1906. Intraperitoneal inoculation of 30 c.c. of citrated blood from stallion 33 (jugular blood).

November 27. Intramuscular inoculation of 30 cc citrated blood, (jugular vein), stallion 33.

December 3rd. Intramuscular inoculation 30 cc of citrated blood, (jugular vein), stallion 33.

No secondary swellings occurred at the points of inoculation. January 15th. Microscopic examination of peripheral blood and vaginal mucus, negative. Feb. 1st. The animal is in a very poor unthrifty condition, rough staring coat stiff gait.

March 12. Animal has improved in condition. Vaginal mucus is abundant and milky in appearance. Microscopic examination—The leucocytes and macrophages are degenerated and vacuolated, and there is a very active phagocytosis.

November 15, 1907. Results of inoculation still doubtful. Trypanosomata have never been detected. In April and July a stiffness of hind limbs was apparent with just a trace of knuckling of fetlocks. There has ever been noted a general air of ill-health and a poorly nourished condition. The genitalia appear normal.

ANIMAL No. 27.—Two year old filly. 'October 29, 1906, vaginal discharge from mare 28 injected into vagina.'—(Dr. S. Hadwen.)

Inoculations:—

- { Nov. 14 No signs of disease.
- { Nov. 19 Intravenous inoculation 30 cc blood of mare 28.
- { Nov. 21 Intraperitoneal inoculation 25 cc blood citrated of mare 28.
- { Nov. 24 Intraperitoneal inoculation 10 cc blood citrated of mare 28.
- { Dec. 3 Intramuscular inoculation 30 cc blood citrated of mare 28.

Jan. 15.—Microscopic examination, vaginal preparations—negative.

Jan. 28.—Microscopic examination peripheral blood—negative.

Condition of animal has not altered, no sign of disease, 4,000 cc of blood withdrawn.

Jan. 30.—Knees swollen, also fetlock joints.

Jan. 31.—Knees and fetlocks greatly swollen, lameness. Left knee punctured and a quantity of serous fluid slightly tinged with blood collected. Microscopic examination—negative.

Feb. 6.—Sero-sanguineous fluid drawn from right knee microscopic examination—negative.

Feb. 20.—Swellings diminished. The gait remains very stiff, more particularly in hind-quarters. Vagina irrigated with citrate solution and mucous membrane scarified, smear preparations—negative.

Mar. 1.—Swellings have disappeared. Gait still stiff and awkward.

Mar. 12.—Vaginal blood—negative.

Nov. 15, 1907. Very similar conditions to the preceding case, (No. 26). On July 27 however in the fluid obtained from a secondary swelling following an experimental injection of a dourine serum, a few developmental trypanosomata were found. While the animals from which the inoculated blood was obtained were undoubtedly affected with dourine, the parasites of dourine were never found in any of these animals. It is possible that the 'contagium' present may have lost part of its virulence or again that these native bred fillies may have possessed a certain degree of natural resistance to infection.

ANIMAL No. 39.—A foal about 9 months old, weaned from mare 25 (Seeley mare) showing characteristic symptoms of dourine in the last stages:—emaciation and loss of control, hind limbs soiled with vaginal discharges and urine.

The foal had always appeared in good health.

Feb. 11th, 1906, Inoculation No. 1 intramuscular, side of left ribs, 5 c.c. of citrate solution containing a trace of sanguineous fluid from scarified mucous membrane from the vagina of Mare 36. Trypanosomata present but rare.

Feb. 17. Inoculation No. 2, intramuscular, flank, 10 c.c. of undiluted blood from peripheral circulation of Mare 36, in which trypanosomata were not demonstrated.

Feb. 21st. Circular plaque or swelling at seat of inoculation No. 1. Swelling harder at outer border than in centre about five centimetres in diameter, slightly œdematous, painless and a little more heated than the surrounding tissues.

Trypanosomata were demonstrated in both fresh and stained preparations of the sanguineous fluid drawn from this plaque. Immature and mature organisms recognized, also swollen vacuolated forms undergoing disintegration and staining feebly. On Feb. 22nd, the eleventh day after inoculation, the swelling had slightly increased in size was more oval and œdematous. On the thirteenth day, the third day of the swelling, it became flatter and broader; microscopic preparations were negative on this date, also on Feb 25th and March 1st, when the swelling had disappeared. March 16th:—no further cutaneous lesions or visible signs of systemic disturbance have been observed to date.

April 23.—A small cutaneous plaque over the last right rib. Anterior to angle of ileum; the swelling persisting for two days. May 4.—Sub-maxillary lymphatics much enlarged and softened. May 13.—Paralysis of brachial and subscapular nerves, the limb hangs pendulous from the shoulder or slightly supported on the toe.

May 20.—Progressing paralysis, knuckling, staggering gait, losing flesh, fever.

May 27.—Muscular tremors, genitals swollen.

Frequent attempts at micturition. Urine scanty, mucous membranes anæmic, lymphatics enlarged and softened, emaciation.

May 29.—1000 c.c. of blood withdrawn and replaced with normal saline solution.

June 12.—See photograph Plate XII.

June 20.—A better appetite and improved muscular control since the operation of May 29.

June 26.—Condition very grave. The animal is down and helpless. Suppurative processes involving hip and shoulder joints. Genitalia swollen and distorted. The operation of blood letting and saline infusion repeated.

June 27-28.—The animal was able to stand again and appetite returned.



June 29.—General paralysis and death.

*Autopsy.*—Body greatly emaciated. Muscles soft, anæmic. Distortion and contraction of tendons of limbs. Pus in shoulder and hip joints. Ascites. Pericarditis. Heart enlarged, muscle soft. Suprarenal bodies very large, soft, and of an orange colour. Surface of spleen profusely scattered with bright red hæmorrhagic spots and patches. Liver large and soft. Estimated weight of animal at time of death, 400 lbs.; heart, 4 lbs.; spleen, 1 lb.; liver, 8 lbs.; kidneys, 1½ lbs. each.

Trypanosomata were found only on one occasion and in the secondary swelling or plaque, 10 days after inoculation. Numerous subsequent examinations of gland juice from enlarged lymphatics, blood from tip of ear, tail and genitalia, and post-mortem smears of the internal organs, failed to reveal any parasites.

ANIMAL No. 29.—Foal (filly) about 9 months old, weaned from mare No. 28 on January 1, 1907 (the mare being in the final stages of dourine, viz.:—emaciation, loss of nervous and muscular control, &c.)

*Inoculation No. 1.*—February 17, 1907. Intramuscular, flank 10 c.c. citrated blood from general circulation of mare No. 36.

*Inoculation No. 2.*—February 22. Subcutaneous, middle right ribs, a trace of vaginal blood of mare No. 36, diluted in 5 c.c. of citrate solution, active trypanosomata being demonstrated in this preparation.

March 4.—Skin at seat of second inoculation appears raised and hardened. Microscopic examinations negative.

March 13.—No further swellings observed, but the animal does not look healthy, being dull, and her movements are sluggish.

*Inoculation No. 3.*—March 13. Subcutaneous (2 inches, 5 centimetres anterior to seat of inoculation No. 1), 5 c.c. of vaginal blood from mare No. 36, collected from scarified mucous membrane after a thorough irrigation of vagina with citrate solution; (trypanosomata being demonstrated in the diluted blood).

Eight hours later a marked local reaction occurred, the swelling being four inches in length, oval shaped and soft, with little heat and no pain.

March 14.—Swelling flatter and circumscribed; a trace of serous fluid tinged with blood aspirated; microscopic examination negative.

March 15.—Swelling has persisted. Trypanosomata not detected.

*Inoculation No. 4.*—March 23. Scarified mucous membrane of the vagina rubbing in blood containing trypanosomata from the vagina of mare 36. Trypanosomata were first detected in the vaginal mucus, May 13. The parasites were numerous and were again observed on the following dates, May 27, June 24, July 13, August 26, and October 4. The parasites were never observed in blood taken from a region other than the vaginal mucous membrane. June 17.—Enlargement and softening of submaxillary lymphatics. The gland juice did not contain trypanosomata.

November 15.—In very good health and condition. A slight tumefaction of vulva and a swollen, anæmic vaginal mucous membrane have been the only other signs of the disease visible during the nine months following infection. Complete absence of depigmentation, vesication, ulceration and nervous phenomena.

ANIMAL No. 41.—Aged mare not diseased.

Feb. 17th, 1907. Inoculation, subcutaneous, left ribs 5 c.c. diluted vaginal blood from mare No. 36.

March 16th. No reaction has been noted either local or general.

July 7th, 1907. A slight nasal discharge from the right nostril tinged with blood; vaginal secretion abundant and the mucous membrane is swollen.

Trypanosomata first obtained from the vagina on this date, and subsequently, July 19th. Submaxillary lymphatics much enlarged and softened.

Aug. 27th. Purulent conjunctivitis of the left eye.

Sept. 17-20. Bloody nasal discharge and hæmorrhagic mucous membranes (following an injection of serum from a dourined stallion—these serum experiments will form the subject of a later report).

Nov. 15th. Present condition:—rough, staring coat, poorly nourished body, and general appearance of ill health: but this was also the condition before the experimental infection.

ANIMAL No. 70.—Healthy 2 year old filly.

Oct. 4th. Inoculation No. 1. Subcutaneous, a trace of blood from vaginal mucous membrane of experimental filly No. 29.

Oct. 8th. Inoculation No. 2 per vaginal mucous membrane, a trace of blood from vaginal mucus of experimental filly No. 29.

Oct. 21. Nov. 15. Trypanosomata in vaginal blood and mucus, often very numerous. The mucous membrane is swollen and anaemic.

#### EXPERIMENTAL INOCULATION OF A GELDING.

ANIMAL No. 43.—A two year old gelding. April 24.—1,000 c.c. of blood withdrawn from jugular vein, followed by a direct transfusion of blood from mare 36, the transfusion lasting 5 minutes.

July 22. Intermaxillary lymphatics, especially the sublingual are much enlarged and softened.

Sept. 2. Neuro-muscular inco-ordination, knuckling of hind fetlocks, crepitation in hock joints.

Nov. 15. The foregoing symptoms have appeared in paroxysms and intermissions to date. The body is well nourished and health seems good

#### ATTEMPTS AT REINFECTION OF MARES AFFECTED WITH DOURINE.

ANIMAL No. 9. 'DOT.'—An experimental mare, the subject of latent dourine. On the floor of the vagina there is a well marked ulcer which has been present for many months without alteration, either in size or appearance. On December 11th, 1906, 500 c.c. of blood was drawn from the jugular.

Feb. 17th, 1907. Intramuscular inoculation, left shoulder, with 10 c.c. of blood from the general circulation of Mare 36, a few drops were also inoculated beneath the vaginal mucous membrane at the same time.

March 16. No swelling has occurred since inoculation. The vaginal mucous membrane appears slightly œdematous and congested. Microscopic examinations have been made as follows:—

November 14.—Peripheral blood—Negative.

December 11.—Jugular blood; vaginal mucus—Negative.

January 15.—Vaginal mucus—Negative.

February 27.—Vaginal blood—Negative.

March 7.—Vaginal blood—Negative.

March 12.—Vaginal blood—Negative.

May 13.—The whole of the vaginal mucous membrane is very much swollen and œdematous, translucent, of an orange tint, with a few small hæmorrhagic spots and papules. Trypanosomata observed in preparations from the mucous membrane on this date, and, subsequently, on May 27 and October 11.

May 27.—Mucous membrane still more œdematous with prominent infiltrated patches. Depigmented spots appear along muco-cutaneous margin of vulva, their surfaces rough and 'frosted' or suggestive of a ground-glass surface. In micro-preparations from these latter lesions numerous spirochætæ were observed.

July 16.—Depigmentation spreading to perinæum. Several ill-defined cicatrices or scars in vaginal mucous membrane. Submaxillary lymphatics enlarged.

July 27.—Vaginal mucus anæmic, almost white, tough and thickened.

September 16.—Experimental injection of a dourine serum. A remarkable reaction following:—

Temperature rose to 103° F., loss of appetite, unwillingness to move, neuromuscular inco-ordination. Large œdematous swellings at site of serum injection, the flanks, lower surface of abdomen and mammary glands.

September 24.—Commencing absorption of swellings, return of appetite and muscular control.

October 10.—Large œdematous swelling under sternum.

November 15.—Intermittent swellings have continued to date. Paralysis of muscles of left eye. An old single ulcer on the floor of the vagina, that has persisted for 2 years, still remains.

ANIMAL No. 7. 'DEVIL.'—Experimental mare in latent dourine served by healthy stallion (No. 30), October 10, 1906. No signs of ill health. Right labium of vulva is greatly atrophied.

February 22.—Inoculations, intramuscular, 5 c.c. citrated vaginal blood from mare No. 36, trypanosomata present in the preparation. On the twelfth day following, the skin appeared slightly hardened and raised at the seat of inoculation. Microscopic examination of the blood smears from this region were all negative.

March 16.—No cutaneous lesions or systemic disturbances have been noted to date.

In July and August the submaxillary lymphatics were found to be enlarged and softened. No further change has been noted in this animal. Gland juice and blood has been searched in vain for the dourine parasites. Present condition is healthy.

ANIMAL No. 17. 'BELLS.'—May 27th. Apparently in the best of health. Inoculated per vaginal mucous membrane with a few drops of blood containing trypanosomata of experimental animal No. 29.

June 27.—Ophthalmitis of right eye, a slight milkiness of cornea of left.

November 15th.—Genitalia and lymphatics normal. Left cornea clear, spreading opacity of right cornea. General condition healthy.

#### INOCULATIONS OF DOGS.

The fact that dogs have proven so satisfactory in attempts to recover the causative parasite of this disease, with other observers led to their being used in an attempt toward this end by Dr. Higgins at Ottawa, and Dr. Hadwen at this station during the period he was in charge.

Following in the footsteps of the various authorities, and of Drs. Higgins and Hadwen, I have used a large number of dogs with a similar end in view. Unfortunately, however, no observer on this continent has succeeded in positively transmitting the naturally acquired disease to dogs of any age, nor have my experiments been attended with more satisfactory results.

I append the data secured which show that, although an infection sufficiently severe to kill the animal or to allow the recovery of the parasite has not occurred, certain symptoms have been noted in connection with these inoculated animals which are indicated in the table. (Table X.)

TABLE No. X.—INOCULATIONS INTO DOGS.

Dog No.	Date.	Material Inoculated.	Subsequent Signs of Disease.	Microscopic Examination.	Present Condition—Autopsy.
2	Sept. 14, '06	70c.c. Peritoneal fluid	None	Blood—Negative	Destroyed Jan. 22, '07. Autopsy—Negative.
3	" 14, '06	40cc. Cord emulsion	"	"	"
4	" 25, '06	50cc. " "	"	"	"
6	Oct. 3, '06	50cc. Marrow	"	"	"
7	" 23, '06	60cc. " "	Inguinal glands enlarged.	"	Destroyed Mar. 20, 5 months after final inoculation. Autopsy—negative.
	Jan. 6, '07	10cc. Peritoneal fluid mare 28	None	"	"
8	Oct. 24, '06	40cc. Edematous fluid from stallion 33.	"	"	Destroyed March 20, '07. Autopsy—negative.
	Jan. 6, '07	20cc. Peritoneal fluid mare 28	"	"	"
9	Oct. 24, '06	40cc. Edematous fluid stallion 33.	"	"	In good health, Apr. 30, '07. Destroyed. Autopsy—negative.
	Jan. 6, '07	10cc. Peritoneal fluid mare 28	"	"	"
	Feb. 11, '07	5cc. Diluted vaginal blood of mare 36.	"	"	"
11	Nov. 19, '06	25cc. Blood No. 28.	"	"	"
	" 21, '06	20cc. " "	"	"	"
	" 24, '06	15cc. " "	"	"	"
	" 27, '06	16cc. " "	"	"	"
	Dec. 3, '06	15cc. " "	"	"	In fair health " " "
12	Nov. 21, '06	20cc. Blood No. 33.	"	"	"
	" 24, '06	15cc. " "	"	"	"
	" 27, '06	20cc. " "	"	"	"
	Dec. 3, '06	25cc. " "	"	"	"
	Jan. 10, '07	30cc. Edematous fluid of No. 33.	"	"	Found dead on morning of March 5, '07. Death very unexpected; no signs of disease ever noted. Autopsy—negative.
	" 28, '07	" "	"	"	"
13	Dec. 3, '06	25cc. Blood No. 23.	"	Blood—Anemic.	In good health. Destroyed Apr. 30, '07. Autopsy—negative.
	Jan. 6, '07	10cc. Cerebro-spinal fluid of No. 28.	"	Blood—Negative	"
14	Dec. 18, '06	15cc. Sanguineous fluid from vulva No. 3.	"	"	"
	Jan. 6, '07	10cc. Cerebro-spinal fluid of mare 28	"	"	"
	Feb. 11, '07	5cc. Vaginal blood 36.	Emaciation	"	In poor health. " " "
	Mar. 16, '07	" "	"	"	"
15	..... 12, '06	5cc. Blood of 36	None	"	"
	..... 12, '06	5cc. " "	"	"	"

16	Feb. 11, '07 10cc. Diluted blood 36 " 14, '07 15cc.	" "	" "	.. ..	In good health. "	" "
	Jan. 6, '07 10cc. Cerebro-spinal fluid of No. 28. Feb. 14, '07 15cc. Diluted blood of 36	" "	" "	.. ..	" "	Aug. 24, '07. "
17	Jan. 6, '07 10cc. Ascitic fluid 28 Feb. 14, '07 15cc. Blood 36	" "	" "	.. ..	" "	Apr. 15, '07. "
18	Feb. 16, '07 Trace of vag. blood 36 " 17, '07 10cc. Jugular blood 36 Mar. 10, '07 10cc. "	Severe conjunctivitis Of right eye Of both eyes	" " "	.. .. ..	March 16—The puppy is sickly, eye symptoms have been very severe. Nov. 15, '07, still alive; held for further observation.	" "
19	Feb. 17, '07 Trace of vag. blood 36	Large abscess	"	..	Escaped from captivity Apr. 15, '07.	"
20	" 19, '07 " 20, '07 4cc. Trace of vag. diluted blood 36	Severe conjunctivitis	" "	.. ..	March 16—The symptoms shown have exactly tallied with dog No. 18. Nov. 15, '07, still alive; held for further observation.	" "
21	" 20, '07 10cc. Venous blood 36	Severe conjunctivitis	"	..	In good health. Destroyed Apr. 30, '07. Autopsy—negative.	"

Time has not permitted a daily examination of the blood of these dogs; however, numerous microscopic preparations of vaginal blood, blood smears from the tip of the ear, from the tip of the tail and the juice of enlarged glands, have been made at frequent intervals. At the autopsies on these animals, smear and fresh preparations from the spleens, livers and lymphatics have been examined. Trypanosomata have never been observed.

Dogs 11, 15, 18 and 20 have been subjected to a severe bleeding, in the hope that lessened resistance to the development of infection might result, as has been the case in some of the equines. Blood-letting in the dogs has not resulted in any visible manifestation of the disease.

Dog No. 18 has been inoculated several times per vaginam, with blood containing trypanosomata. Certain ocular phenomena have been witnessed in dogs Nos. 18 and 20. At this date (Nov. 15th) the vulva of No. 18 appears slightly swollen, and the mucous secretion is rather abundant. In No. 20 the penis is swollen and inflamed. There has been a complete absence of œdema in all the dogs. A few have shown enlarged lymphatics, others conjunctivitis. The animals have all been kept on the chain during the period of captivity, a circumstance that may be responsible for some of these indefinite abnormal conditions.

### RABBIT INOCULATIONS.

Inoculations, corresponding to those of the dogs have been made with 14 rabbits.

Two of these animals died within 24 hours of septicæmia and two were accidentally killed. The remainder are alive and apparently in the best of health. No signs of disease have ever been noted. Numerous blood examinations negative.

The only suspicious indications of a dourine- infection lie in a few abnormal conditions of the eyes. Conjunctivitis, with, or without a slight discharge. A slight milkiness of the cornea, fugitive in character, with one exception, a rabbit which has become blind in one eye, with ex-ophthalmia. Three rabbits have been destroyed, the remainder survive, and appear healthy. Oedema never noted.

### MICE INOCULATIONS.

Thirty field mice have been inoculated, five of which died within a few hours of septicæmia.

March 31, 1907. Two mice inoculated with sediment of œdematous fluid of stallion 33 are still living, two and a half months after inoculation.

Of three mice inoculated with the sediment of serous fluid from the knee swellings of filly No. 27, one died on the 4th day and the other two are still living, six weeks after inoculation. Curiously, at intervals of 10-15 days, they were found in an extremely lethargic condition, the eyes were closed and breathing was rapid. After several hours in this condition they recovered and soon became as active as ever.

Of two mice inoculated with sero-sanguineous fluid containing the *Trypanosoma equiperdum* from the inoculation plaque of animal No. 39, one died on the twelfth day in the lethargic condition described above, while its mate, after being in the same condition for eight hours, recovered and is still alive. Of those inoculated from mare 36 (*Trypanosoma equiperdum* demonstrated in the preparation), three died within three days, one on the tenth day and the others are still living, but the most of those surviving have had one or more attacks of the coma or paralysis described above. Of six control mice, one has died and none have ever been found in the comatose condition. One mouse inoculated with the centrifugalized sediment of the ascitic fluid of mare 28, died on the seventh day, and in a blood smear from this mouse an hour before death, six trypanosomata were observed, which possessed some of the characteristics of the *T. equiperdum*.

The blood of all these mice was examined before inoculation and in none of them were found the trypanosomata with which a small percentage of these field mice are naturally infected. Numerous blood preparations have been made subsequent to the inoculation, trypanosomata being found in but one preparation taken just before death.

November 15, 1907. In all, sixty native 'white posted' mice have been subjected to inoculations. In my foregoing observations on mice, I have mentioned in detail the case of one mouse dying on the seventh day after inoculation and the finding of six trypanosomata in a blood preparation taken an hour before death. In view of all subsequent failures to infect these animals, I think it probable that the parasites were of the non-pathogenic variety found in about 15 per cent of these native mice. The lethargic condition noted in a few of the inoculated mice I have since rarely observed in control mice, in the first few days of their captivity.

TABLE XI.—Showing the presence or absence of Trypanosomata.

9. Mare No. 36.	Vaginal.				Peripheral Blood.				General Circulation from Jugular Vein.		Remarks.
	Mucus.		Blood.		From the Tail.		From Plaques.				
	+	-	+	-	+	-	+	-	+	-	
Dec. 19, '06..		2				2			3		Tryp. absent.
" 22, '06..											
" 23, '06..						2					
" 29, '06..									6		
" 31, '06..								2			
Jan. 1, '07..		2				2					
" 2, '07..								2			
" 4, '07..		2									
" 15, '07..		2									
" 29, '07..		2									
Feb. 11, '07..			2	4				6		Tryp. very rare; 16 counted in all smears.	
" 12, '07..				5				6		Tryp. fairly numerous in the mucus.	
" 13, '07..	3	1	3								" quite numerous in the mucus; rare in vag. blood.
" 14, '07..	2		1	1		1				" rare in mucus; a few in vag. blood.	
" 15, '07..	1		2							" absent in mucus; rare in vag. blood.	
" 16, '07..		2	2	2						" rare in mucus; absent in vag. blood.	
" 17, '07..	2		2			1				" rare in mucus; rare in vag. blood.	
" 18, '07..	2	1	1	1						T. before irrigation of vagina with citrate solution very rare; after irrigation fairly numerous.	
" 19, '07..		1		4							
" 20, '07..	4	2	4						2	T. rare (preparations made after irrigation).	
" 21, '07..				4							
" 22, '07..			2							Very rare (3-4 T. in each smear after irrigation). Very rare; only 1 T. found. Very rare; 2 T. found.	
" 24, '07..		2		2		2					
" 25, '07..				2							
" 26, '07..			3								
" 27, '07..			1	5						Rare; 5 T. counted in the two <sup>2</sup> smears. Very rare.	
Mar. 1, '07..			1	2							
" 4, '07..				4							
" 6, '07..				3							
" 9, '07..		1		3							
" 10, '07..		1		1							
" 12, '07..			2								
" 13, '07..			3								
	14	21	27	45		10		19		9	Tryp. found in 41 preparations out of 147 examined.

Explanation.—The numbers in the columns represent the number of preparations, fresh or stained, that have been examined. Trypanosomata being present in those under the positive sign, and absent in those under the negative sign.

TABLE XII.

DIFFERENTIAL LEUCOCYTES COUNTS.—SUB-DIVISION.

Animal Number.	Date.	MARROW CELLS.		Mast cells.	Total of Mononuclear group.	MONONUCLEAR GROUP.				ATYPICAL L.		
		Polymorpho-nuclear neutrophils.	Eosinophils.			Large and small lymphocytes, large and small.	Large hyaline Mononuclears.	Large granular Mononuclears.	Transitional Mono. Types.	Transitional Neutrophils.	Myelocytes.	
36 Mare.....	19-12, '06..	63.0	4.0	0.6	29.4	22.0	4.6	2.0	0.8	2.4	0.6	Mitoses, rare A few nitotic mono and myelocytes.
Acute—	23-12, '06..	52.0	3.2	1.2	42.0	38.0	1.0	1.0	1.4	1.0	0.6	" " " "
Generalized.	31-12, '06..	55.0	4.0	1.0	38.0	25.0	11.0	1.5	5.0	1.5	0.5	Trypanosomata present in vaginal blood.
Doitrine, .....	2-1, '07..	56.5	3.0	1.0	37.5	32.5	3.0	0.5	1.5	1.5	0.5	" " " "
	11-2, '07..	62.0	1.0	0.4	36.2	34.6	1.0	.....	0.6	0.4	.....	" " " "
	17-2, '07..	48.0	2.4	2.0	47.2	40.4	5.0	.....	1.0	0.8	0.4	Always an absolute leucocytosis (a great increase of all varieties.)
	24-2, '07..	65.0	1.3	0.8	32.3	30.0	2.0	.....	3.0	0.6	.....	
CHRONIC (AND INTERMITTENT) DOITRINE.												
33 Stallion.....	14-11, '06..	59.3	8.0	2.0	30.4	28.0	1.2	0.4	0.8	0.3	.....	Absolute leucocytosis.
	21-11, '06..	52.4	13.2	1.8	31.0	23.6	5.0	0.8	1.6	1.0	0.6	Mitotic myelocytes, eosinophilia.
	27-11, '06..	58.2	9.5	2.5	28.0	22.0	3.0	1.5	1.5	1.2	0.5	White cells 68,000 per c. m. m.
	13-1, '07..	63.7	6.0	1.2	27.3	23.7	3.0	0.1	0.5	1.0	0.6	Immense numbers of leucocytes.
35 Stallion....	19-12, '06..	55.7	4.0	0.1	37.5	35.4	8.0	0.3	0.1	2.0	0.6	Absolute leucocytosis.
25 Mare.....	14-11, '06..	52.5	3.0	0.5	42.8	39.0	2.0	2.0	0.8	0.7	0.5	" " " "
23 " .....	29-11, '06..	51.0	7.5	2.0	39.0	32.0	3.0	2.0	2.0	0.5	.....	White cells 20,000 per c. m. m.
28 " .....	14-11, '06..	50.0	4.5	1.0	44.2	41.0	1.0	1.0	1.2	1.1	0.2	Absolute leucocytosis.
28 " .....	21-11, '06..	47.2	0.8	2.0	36.8	32.1	1.7	2.0	1.0	4.0	2.0	2 days following venesections.



LATENT DOURINE. (TOTAL ABSENCE OF SYMPTOMS.)

3 Mare..	29-11, '06..	25.4	8.0	2.6	62.0	55.0	0.4	6.0	0.6	1.4	0.6	Absolute leucocytosis.
	18-12, '06..	35.0	6.0	4.0	52.0	46.0	3.5	1.0	1.5	3.0	.....	"
8 Mare.....	14-11, '06..	27.0	8.0	1.0	63.6	53.0	3.4	3.2	2.0	0.4	.....	"
	29-11, '06..	29.6	6.0	1.2	60.4	58.0	1.0	0.8	0.6	2.4	0.4	"
19 Mare.....	26-1, '07..	27.5	2.5	3.0	66.5	60.0	5.0	0.5	1.0	0.5	.....	mitotic myelocytes.
21 ".....	26-1, '07..	38.0	4.5	1.0	56.0	52.5	2.0	0.5	1.0	0.5	.....	A spayed mare 6 months after operation.
22 ".....	.....	35.0	4.6	1.0	59.0	55.2	2.0	0.6	1.2	0.4	.....	"
10 Healthy Horses.....		54.2	5.9	1.7	37.9	30.7	2.04	2.07	2.1	1.0	0.04	Normal average of 10 healthy horses.



## The Symptomatology and Diagnosis of Dourine.

### MISCELLANEOUS NOTES.

In previous reports on Canadian dourine, Dr. Rutherford has laid stress on the great difficulty of diagnosing this disease with any certainty during its earlier stages and has drawn attention to the apparent mildness of the infection in many cases, especially in mares. In these respects, my own observations are in complete accordance with those of Dr. Rutherford.

A careful study of the cases detailed in this report will give much more information than any attempt of mine at a general description of the symptoms and course of dourine (such may be found in the text-books and clinical literature upon the subject, but this literature I have found very misleading, not stating with sufficient clearness the fugitive character, the frequency or otherwise of the symptoms).

Many authors commence their discourses on the symptoms by saying that they may be divided into three stages. Baldrey (*Journal of Comparative Pathology*, March, 1905) states that these stages are distinct, and may, if the case is carefully watched, be recognized with comparative ease. This has been the exception rather than the rule in my experience with Canadian dourine.

Lingard, in his 'Report on Dourine,' 1905, states that what really is observed in practice is an overlapping, or a partly concurrent exhibition of certain symptoms referable to the respective periods, and goes on to say that the arrangement is purely an arbitrary one, simply utilized for the sake of description.

It is well, I think, to remember this reservation. The following notes may help to put the diagnostician on his guard.

In the case of mare No. 36, a stabled animal, and, therefore, under daily observation, the first visible signs of disease were symptoms originating from the central nervous system, which belong, according to the arbitrary division, to the 'third' stage. These indications were followed by the 'first' stage, namely, tumefaction of the genitalia, sexual excitement, &c. The first appearance of symptoms belonging to the 'second' stage, namely, patchy infiltrations of the skin, the so-called plaques, were concurrent with the nervous manifestations, and have later appeared when only a trace of the 'first' and 'third' stage remains.

In the case of experimental animal No. 39, the disease ran an acute course, terminating fatally 139 days after infection. Nervous symptoms predominated throughout the infection.

In the case of experimental animal No. 29, dourine parasites were present in the vaginal mucus at irregular intervals from the 85th to 229th day after infection, and yet only at the end of this period have there appeared any visible signs of disease, these being more or less indefinite and limited to a slight tumefaction of the vulva and a somewhat swollen, anaemic, vaginal mucous membrane.

These may be extreme cases, but others can be cited, both in naturally and experimentally infected equines. It is only necessary, I think, to emphasize the fact that symptoms may appear shortly after, or not for a very long period following infection, or that they may abate or disappear for equally long periods at any stage of the disease, and lastly, that loss of co-ordinate locomotion or other signs of nervous derangement may be the first and only signs of the disease visible to the naked eye.

*Oedema.*—Most authors argue that the only truly pathognomonic sign of disease is the eruption of the cutaneous plaques. These patchy infiltrations of the skin, however, have been found so rarely, in cases that have come under my observation, that some other sign has had to be searched for.

It is well established that in the various trypanosomiasis of animals, including dourine, œdema in some form is the most constant feature. In dourined mares I have found that the most frequent and constant sign of infection is the tumefied or infiltrated mucous membrane of the vagina. This condition has been observed in 70 per cent of my cases. In the absence of plaques, it is only in blood or mucus from this lesion that I have succeeded in finding the dourine parasites. In stallions, the œdema usually commences in the mucous membrane of the urethra and meatus urinarius. This condition, however, is sometimes difficult to ascertain or may escape notice, the first sign of disease being noted in a tumefied penile sheath, intermittent in character.

*Vesicles and ulcers.*—As far as I am aware, trypanosomata have never been proven to be the actual cause of vesicles and ulcers. These lesions do occur in a small percentage of dourined mares, and in a large percentage of dourined stallions (see Table of Symptoms), but they are not, in my opinion, caused by the trypanosomata. In the case of mare 36, I have recorded the finding of trypanosomata in fluid from a vaginal vesicle. This was not a typical vesicle, and it would have been more correct, I think, to describe the lesion as small, patchy or nodular infiltration of the mucous membrane. From typical vesicles and ulcers I have never succeeded in isolating a trypanosome, and I am not aware that other investigators have succeeded in doing so. I may state, however, the frequent occurrence of spirochætæ in these lesions, in some micro-preparations these organisms appearing as in a pure culture, in nests and colonies. This is of interest in view of the discovery of spirochætæ (now called the *Treponema pallidum*) in lesions of human syphilis. Jewett, in his publication, 'Notes on Blood—serum Therapy,' describes spirochætæ in cases of canker and grease in equines. The appearance of the genitals in dourine when ulceration and vesication are extensive, is not unlike the condition occurring in 'grease.' Professor Schaudin's interesting hypothesis on the transition of trypanosomata into spirachætæ has provoked severe criticism, and is not generally accepted.

*Depigmentation.*—In several cases of dourine I have observed depigmentation independently of pre-existing vesicles or ulcers, and, further, that certain tissues, especially the muco-cutaneous margins of the vulva, which had become depigmented during a genital tumefaction, regained their colouring matter when these swellings subsided. This phenomenon is unusual, I think, depigmentation generally persisting for a lengthy period, if not permanently and often associated with vesicles and ulcers.

*Conception and abortion.*—'Infected mares do not, as a rule, conceive, but if they do, almost invariably abort at about six months.'—(Pease.)

'It has been noticed in India that from this time—(the primary symptoms)—although the stallion is capable of efficiently covering the mare, he is entirely sterile.'—(Baldrey.)

Three mares in the 'third' stage of dourine have given birth to apparently healthy offspring at this station. Three other mares, found to be affected with chronic dourine, and in each of which the diagnosis has been confirmed by the finding of the trypanosomata, had young foals at foot. At autopsies on slaughtered mares I have several times found them to be pregnant. This is probably the exception. Nevertheless, abortion in my experience is a rare event, occurring only in those advanced cases where there is emaciation and loss of co-ordination. In examining the semen of dourined stallions I have found numbers of dead spermatozoa and rarely a motile form.

*Methods of diagnosis.*—It was hoped that in the absence of trypanosomata in the blood and vaginal mucus, puncture of the enlarged lymphatics would yield good results, similar to the success of that method in the diagnosis of sleeping sickness. I have punctured the glands in a number of cases and in various stages of the disease but without ever finding a trypanosoma. Neither have I ever been able to find the parasite in the cerebro-spinal fluid.

Blood-letting has proved an important aid in some cases. Serum-tests, after further elaboration, will, I trust, yield better results. In the meantime, owing to the absence of parasites, the diagnostician must depend on clinical symptoms and consider the history of the suspected animal.

With the assistance of Dr. Gallivan, I have prepared a table of symptoms. The percentages are based on an analysis of 64 cases of dourine, (56 mares and eight stallions), that have come under our observation within the past twelve months. This may be of some value to those called upon to diagnose the disease, who have not had the opportunity of familiarizing themselves with the frequency of certain conditions.

*Acknowledgments.*—To the Veterinary Director General, for the privilege of carrying on this investigation and for advice and interest in the progress of the work. To Dr. M. V. Gallivan, of Lethbridge, in securing valuable cases and for assistance rendered on numerous occasions. Lastly, to the numerous capable investigators who in India and elsewhere have furnished me with a foundation and a safe guide for my own studies, greatly assisting in establishing the identity of dourine on this continent.

TABLE OF SYMPTOMS—Showing the frequency (percentages) of Symptoms in Stallions and Mares, respectively.

Signs, Symptoms, Lesions.	% in Stallions.	% in Mares
<i>Genitalia :</i>		
1. Tumefaction of vulva.....		34
2. " " penile sheath.....	75	
3. Genital discharge.....		20
4. Abnormal sexual excitement. Nymphomania.....		15
5. Distinctly hæmorrhagic mucous membrane.....	12	5
6. Swollen œdematous " ".....		70
7. Protrusion of mucous membrane of vulva.....		5
8. Eversion " " " Meatus urinarius.....	65	
9. Phymosis, paraphymosis.....	37	
10. Depigmented spots or leucodermic patches (vulva, perinaeum, penis and sheath).....	62	34
11. Vesication.....	25	10
12. Ulceration.....	37	6
13. Cicatrization.....	12	9
14. Distortion, atrophy.....		15
<i>Nervous System :</i>		
15. Muscular and nervous inco-ordination—		
(a) Front limbs and quarters.....		10
(b) Hind " ".....	25	30
16. Facial paralysis and distortion.....		5
<i>Cutaneous Lesions :</i>		
17. Patchy infiltrations or plaques.....		6
18. Localized œdema.....	75	10
<i>Ocular Phenomena :</i>		
19. Conjunctivitis.....	25	5
20. Keratitis, corneal opacity.....	25	4
<i>Suppurative Processes :</i>		
(a) Limbs and joints.....	12	6
(b) Genitalia.....	12	
22. Enlarged Lymphatics.....	25	43
23. Nasal Discharge.....		4

## APPENDIX A.

## NATURALLY ACQUIRED DOURINE.

CASE NO. 21. MARE.—Ovaries removed, September 15, 1906, one to two years after infection. Absence of signs of disease during winter of 1906-7. July 27, 28, sexual excitement; covered by healthy stallion on each date. Genital examination revealed a marked excoriation of mucous surfaces and external genitals, scars, loss of pigment of the under surface of the tail, perinæum and L. pudendi.

August 2.—Ulceration involving mucous membrane of vulva, and portions of the vagina, these areas presenting numerous small vesicles and ulcers.

August 10.—Lesions are disappearing.

August 15.—Genitalia had resumed an almost normal condition. The mare again appeared 'in heat,' and was covered by the healthy stallion. Submaxillary and sublingual glands thickened and prominent.

November 15.—No further manifestation of disease. Genitalia appear normal. General health and condition very good.

CASE NO. 25 MARE.—'A REMARKABLE RECOVERY(?)'—Admitted to Quarantine Station February, 1906, in advanced stage of dourine.—Dr. S. Hadwen.

December, 1906.—Great emaciation, dragging, paralytic gait. Complete loss of co-ordinate locomotion.

January-March, 1907.—The condition extremely severe. Death expected daily. The animal was scarcely able to move and would stay for days at a time in a corner of the pasture, during intensely cold and blizzardy weather.

April.—Slow, steady improvement.

June-September.—Symptoms gradually disappearing. A great general improvement.

November 15.—The back is rather hollow. The quarters somewhat crouched. Normal co-ordination. Mare is very active and in very fair health and condition.

NOTE.—Other examples of apparent recovery could be cited from milder cases, but not from such a severe condition as above described.

CASE NO. 73. NATURALLY ACQUIRED DOURINE.—Probably infected by stallion 33 in 1905. A range mare, with young foal at foot.

August 2, 1907.—Emaciation; genital discharge, scars and cicatrices. Crepitation of joints. Loss of co-ordinate locomotion.

August 4.—Profuse genital discharge.

August 13.—Severe genital exacerbation. Papules, small vesicles and ulcers. Raw mucous surfaces. Submaxillary lymphatics are enlarged. A secondary swelling rapidly followed the subcutaneous injection of a test-serum. In the bloody serosity of this swelling, developmental or involutive forms of trypanosomata were discovered.

November 15.—Increasing emaciation, diarrhœa and loss of co-ordination.

CASE NO. 74. NATURALLY ACQUIRED DOURINE. (Plate XV.)—Gelding rising 4 years. It is doubtful whether this animal was castrated as a yearling or a 2-year-old. The animal has been running with the above mare, No. 73, and the pair are always found together.

July 29.—A complete loss of co-ordinate locomotion. The animal frequently falls to the ground when suddenly startled and is unable to stand without a voluntary effort. (See photographs.) Trypanosomata have not been found in the blood.

November 15.—Paralysis of tail.

CASE No. 75.—NATURALLY ACQUIRED DOURINE.—A range mare, with young sucking foal at foot.

September 25, 1907.—The whole of the visible genital mucous membrane is scattered or mottled with copper-coloured hæmorrhages. Trypanosomata present in fair numbers.

October 16.—Labia greatly tumefied, œdematous, pitting on pressure of the fingers. The perinæum in a similar condition. Hæmorrhagic patches have totally disappeared. The mucous membrane is markedly œdematous with isolated infiltrated patches. Trypanosomata are still present.

November 25.—The whole of the genital mucous membrane is infiltrated. The animal is in rather poor flesh, but not emaciated. Absence of nervous symptoms.

CASE No. 82.—NATURALLY ACQUIRED DOURINE.—Range mare, with young foal at foot.

September 25.—Infiltrated patches on vaginal mucosæ. Tumefied vulva.

October 26.—Similar condition. Trypanosomata present.

November 15.—Localized œdema on lower surface of abdomen. Poorly nourished condition but not emaciated. Absence of nervous symptoms.

CASE No. 33. NATURALLY ACQUIRED DOURINE.—Clydesdale stallion. Admitted to quarantine station, July 21, 1906, with swollen sheath and testicles.—(Dr. S. Hadwen).

December, 1906, and January, 1907.—Paroxysmal and intermittent œdematous swellings of sheath, scrotum and lower surface of abdomen. Swellings would increase for three or four days and then subside, with intermissions of 10-12 days. Numerous examinations of the fluid of these swellings have been made. An immense number of leucocytes were observed, 99 per cent of which were of the lymphocyte or mononuclear variety, many intro and extra-cellular, spore-like bodies, and crescentic forms, the significance or identity of which I am at present unable to determine. An indisputable stage or form of trypanosome was not observed.

February, 1907.—Continuous and moderate swelling of penis, sheath and scrotum not extending to lower abdomen.

April and May.—Steady increase in swellings, without intermissions, extending on either side and beneath lower abdomen and thorax, involving the lower dependent regions of the neck.

June.—Enormous swellings with organization. Emaciation. Rupture of scrotum and continuous discharge of thin pus.

July—Apart from the weak, dragging gait, due to the swellings and emaciation, neuro-muscular inco-ordination has not appeared.

July 24.—Death, preceded by general paralysis.

July 24.—Autopsy. Extensive serous infiltrations of subcutaneous connective and muscular tissues. Anæmia. Flesh pale and yellow. Clear yellow fluid exudes on section of muscles. The enormous swellings organized in a dense, white fibrous structure, on section, honeycombed with small serous cavities.

The position of testicles could not be located by hand manipulation; on section they were found embedded in a fibrous mass 12-15 inches in thickness. After being dissected out of this mass they were found to weigh three pounds each. Scarcely any recognizable testicular tissue remained. The under surface and sides of abdomen and thorax presented a covering of the same dense fibrous character, 3-4 inches in thickness.

Kidneys.—5 lbs. each, dark, brown, speckled, infiltrated.

Spleen.—2½ lbs. mealy, doughy, fibrous, petechiæ.

Liver.—20 lbs. pale, firm, absence of fibrous adhesions to capsule or diaphragm.

Heart.—10½ lbs., pale, firm, absence of endo-carditis.

Lungs.—Slightly altered.

Lymphatics.—Enlarged and softened.

Pleural and pericardial sacs contained transudate fluid. The abdomen contained an abundance of ascitic fluid.

The subject was a heavy Clydesdale stallion, which in normal condition would have weighed about 1,800 lbs.

CASE No. 35. NATURALLY ACQUIRED DOURINE. (Plate XIV.)—Heavy Clydesdale stallion (which covered the infected mare, No. 36).

The first suspicions of the disease appeared during the autumn of 1906, and according to the owner of the stallion consisted of a slight intermittent swelling of the sheath. During the following winter and the spring and summer of 1907 intermittent swellings of the sheath were occasionally observed. By October the animal had lost in flesh and developed a corneal opacity of the right eye, and a very considerably swollen sheath.

Intermittent swellings have continued to date, November 15, 1907, increasing in extent. There is now œdema of lower surface of abdomen and some emaciation. The hind legs are swollen and pus is escaping from the right hock.



## APPENDIX B.

REPORT OF DR. DAVISON.

RUSHVILLE, NEB., May 14, 1904.

Chief of Bureau of Animal Industry,  
Washington, D.C.

SIR,—In accordance with instructions received, I went to Lethbridge, Alberta, for the purpose of conferring with J. G. Rutherford, Veterinary Director General of the Dominion of Canada, in regard to suspected outbreak of *maladie du coit* among horses in the vicinity of Lethbridge. I examined such suspects as were available, and have no hesitation in confirming the diagnosis of Dr. Rutherford.

I found no good cases. However, considering in the aggregate the symptoms manifested by different individuals does not leave room for a reasonable doubt as to the character of the disease. At the ranch of W. T. McCaugherty, eight miles west of Lethbridge, I examined a herd of about fifty mares and one stallion. The stallion presented a general unthrifty appearance, was quite emaciated, eyes and nostrils weeping, scrotum thickened and of a doughy consistency, two plain cicatrices on under surface of penis just below inferior border of prepuce. No plaques showing, but owner gives history that would indicate that they have been frequently in evidence. Meatus highly inflamed, constant dripping of mucus from urethra, voiding of urine frequent and attended with considerable discomfort.

Of the fifty mares, about fifteen are quite suspicious. Several show vaginal discharge and defective muscular co-ordination. Several had small white spots on vulva and cicatrices on vaginal mucous membrane. One mare showed two well defined plaques. In addition to the symptoms which were in evidence, we have the history of a large percentage of abortions. Also the owner gave in detail the history of mare which had died two weeks previous, a case which, I judge from his description, had all the characteristic symptoms of an animal in the advanced stage of *maladie du coit*.

I examined a stallion at Macleod, the property of one Wm. Damon. This stallion's sheath was badly swollen, and had been so for about six months. This was the only suspicious symptom in evidence, and considered alone would hardly justify one in regarding the stallion as a suspect. However, I learned that the stallion had covered a mare afflicted with a venereal disease of some kind, and that another stallion, which had previously covered the mare died with some kind of a venereal affection.

The history of various suspects and current rumours would seem to indicate that the affection had been prevalent in Alberta for two or three years at least. The origin of the difficulty is at present, of course, only a matter of conjecture.

Very respectfully,

(Sgd.) E. T. DAVISON,  
*Inspector.*

## APPENDIX C.

## THE IDENTITY OF DOURINE.

(*Annales de l'Institut Pasteur.*)

*Buffard and Schneider.*

Even until recently some doubt appeared to exist regarding the presence of a specific trypanosome in the dourine of Europe. We have successively seen Thanhoffer, Lidemann and Marek in Hungary, and Tchernogorow in Russia declare that they have not been able to reveal the presence of trypanosomes in subjects infected with authentic dourine. Prof. Marek was even led by his negative evidences to admit the existence of two dourines, one due to a trypanosome being seen in Algiers, the other, of which the causal agent was still to be determined, constituting the European affection. A certain tendency to consider this double theory plausible has since been manifested in some scientific circles, and in some medical literature.

We have, from the first, held firmly that the Algerian trypanosomiasis was actually the true dourine or 'mal du coit,' that which was studied by Signol, Saint Cyr, Trasbat, Laquerriere, Blaise, Nocard, Rouget, ourselves, and, as supporting our conclusions, Nocard, Martinet and Bremond, who carried on an experimental dourine farm after examining some animals which served as our proofs that dourine was of trypanosomian origin. In all that concerned the possibility of a dourine or of a European pseudo-dourine, we maintained the greatest reserve, having regard to the difficulty of the bacteriological diagnosis of dourine on the one hand, and to the small number of inoculations or their entire absence in the apparently negative cases. The facts have since, as it appears to us, solved the question both in France and Hungary. In France dourine makes its appearance nearly every year upon the Spanish frontier, in the department of the Lower Pyrenees. The mares of the districts near the frontier are sent during the summer into pastures common to France and Spain, where they are served by stallions which are often affected with dourine. Many owners, however, act as do the Arabs, that is to say, that they first have a mare served by a jackass, then if she does not hold they send her to a stallion. The jackasses perform service on both sides of the frontier and most frequently infect the mares, which in their turn infect the stallions of the national breeding studs, or those owned by private parties. Nay, more, through purchases made in Spain, mares probably infected are frequently introduced into France; the enzootic outbreak of 1903, was thus caused by Spanish mares brought into France.

In 1886, 34 mares and 4 stallions died in the canton of Accous; in 1890 some cases were observed in the valley of Aspe; in 1898 the stallion Kars of the national stud at Pau infected 37 mares. In 1903 many mares had, in fact, already succumbed to mal du coit when the sanitary service was advised of the situation; it was only possible to find two private stallions affected, which succumbed shortly afterwards. In 1904 the national stallion 'Lusignan,' was sent for observation to the veterinary school at Toulouse on suspicion of dourine, because of symptoms which he presented, and which consisted principally of an extensive œdema of the sheath and scrotum. Lately he presented on the sides, on the neck and on the croup rounded protuberances having the character of hematomas which appeared and disappeared at irregular intervals. Some lameness of the hind limbs supervened, accompanied by paralysis of the crural muscles and loss of power in the hind-quarters. This stallion finally recovered. Different inoculations with fresh blood were in very large doses administered to dogs and rabbits, but gave no result. Microscopic examination of the blood was constantly negative. This stallion having served 37 mares, Professor Leclainche

willingly invited us to examine them at certain places where they were collected. Four mares were declared dourine suspects. The symptoms which they presented were vague enough, but the stallion which had served them presented such evident signs of dourine that less could not be done than to put them under the supervision of the sanitary service. Blood taken from the tip of the ear and from the vagina of two of these mares showed, after long and minute examination some very sparse trypanosomes. A dog and a rabbit received respectively the first 50 c.c., the second 20 c.c., of blood from the jugular of another of these suspected mares. The rabbit died some days afterwards from septicaemia. On the dog, which was carefully watched every day, there appeared on the seventh day, at the point of inoculation, a swelling about the size of a hazel nut in the sero-sanguineous fluid of which we found trypanosomes in sufficient numbers, which we submitted to the confirmatory examination of Messrs. Leclainche and Laveran. To this swelling, supervening at the point of inoculation, the symptoms of dourine in the dog were confined.

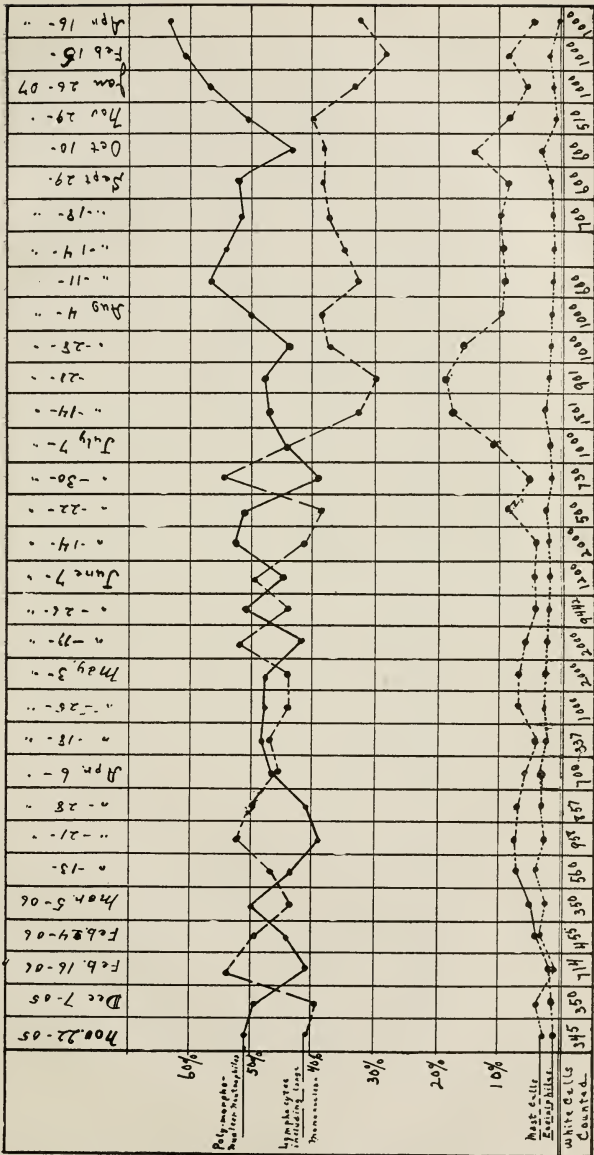
Let us add that three of the mares declared suspected by the sanitary commission died after having presented the typical symptoms of dourine. A private stallion was also castrated for dourine in the same district as that in which the affected mares lived. We desire above all to deduct from this enzootic, interesting on more than one account, the difficulty of bacteriological diagnosis and the positive discovery of the *Trypanosoma Rougeti* in the dourine of France.

In Hungary, after having failed for a long time in his search for the trypanosome, Prof. Marek has finally found it in the blood of a stallion affected with dourine and has willingly announced to the International Congress of Veterinary Medicine at Buda Pesth, that he would withdraw the reservations which he had expressed on the subject of a specific trypanosome in Hungarian dourine.

We will not close this note without tendering our hearty thanks to Prof. Leclainche for the great obligation he has rendered us in facilitating our researches and in permitting us to demonstrate the single nature of dourine.



588 Sleepy Chronic Dourine



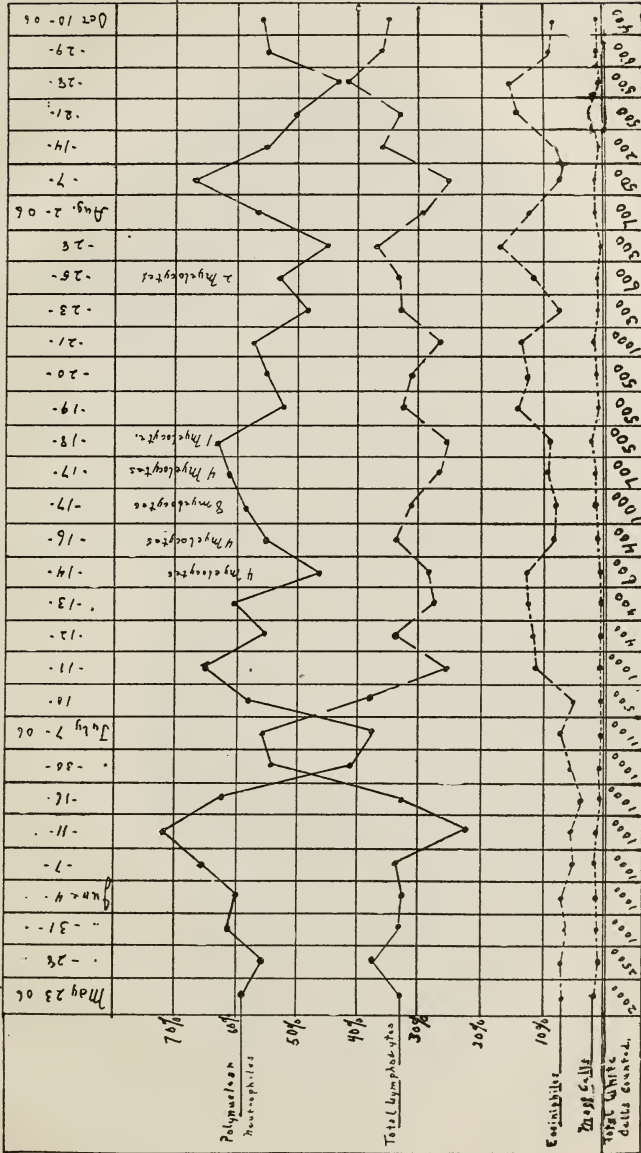








650 Healthy Stallion exposed to Dourine.







Maladie du Coût—Pure Breed Clyde Show Mare. Note the position of off hind leg. See Photograph of acetabulum of the affected limb, (Plate II). Photo. by C. H. Higgins.





Maladie du Coit.—Extensive ulceration of the acetabulum, a result of the disease. See Photo. of Mare from which this joint was removed, (Plate I). Photo. by C. H. Higgins





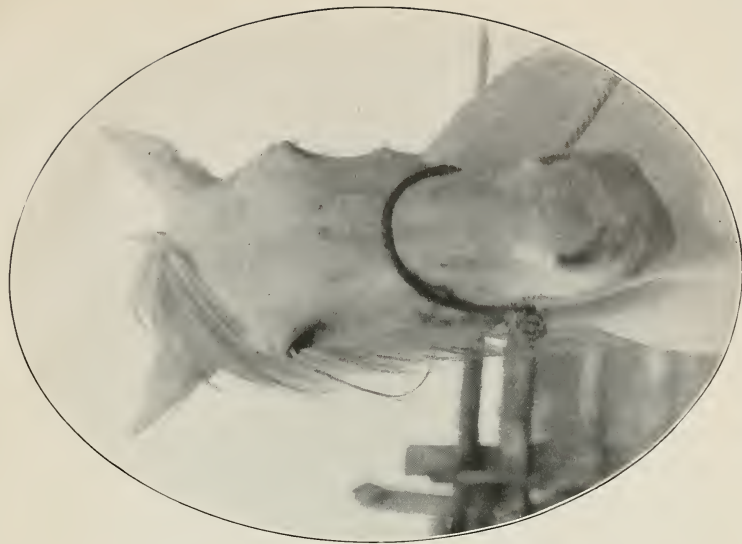
Maladie du Coit.—Brucefield Prince. Photo, by C. H. Higgins.







*Fig. a.*—Maladie du Coit.—Brucefield Prince. Note the paralysis of the ear and upper lip with accompanying distortion.



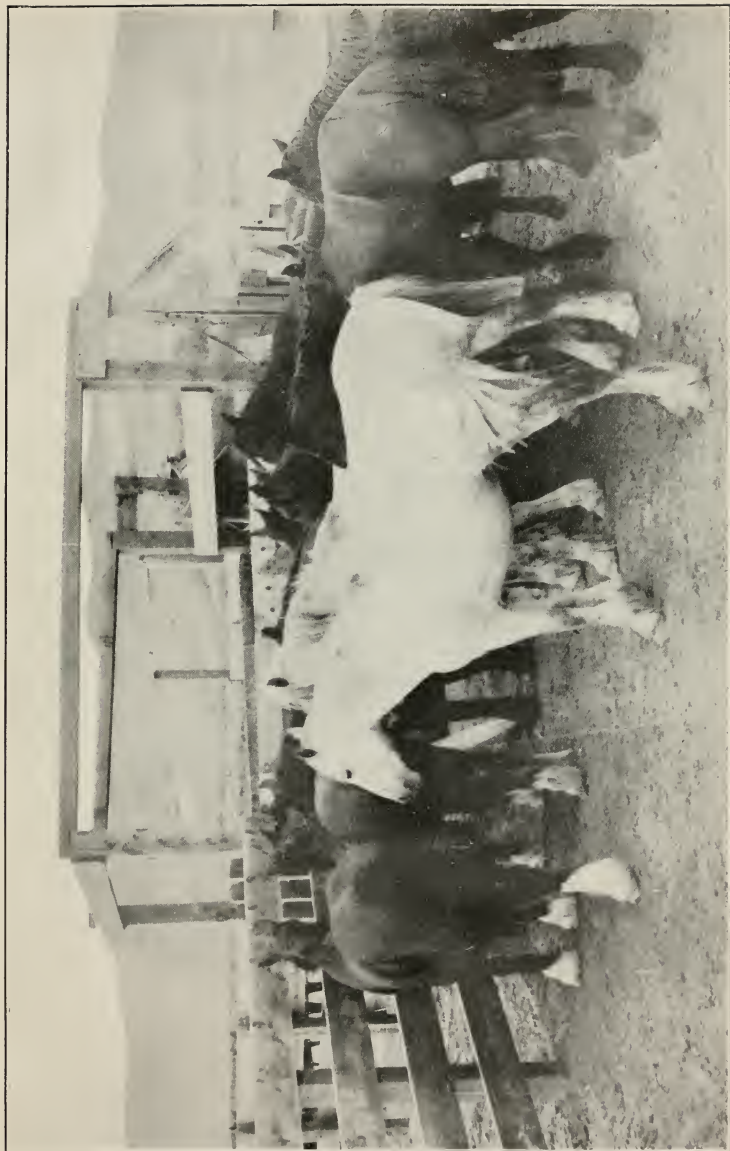
*Fig. b.*—Maladie du Coit.—A Grade Mare in the Medicine Hat District. Note the paralysis of ear and hip with accompanying distortion.





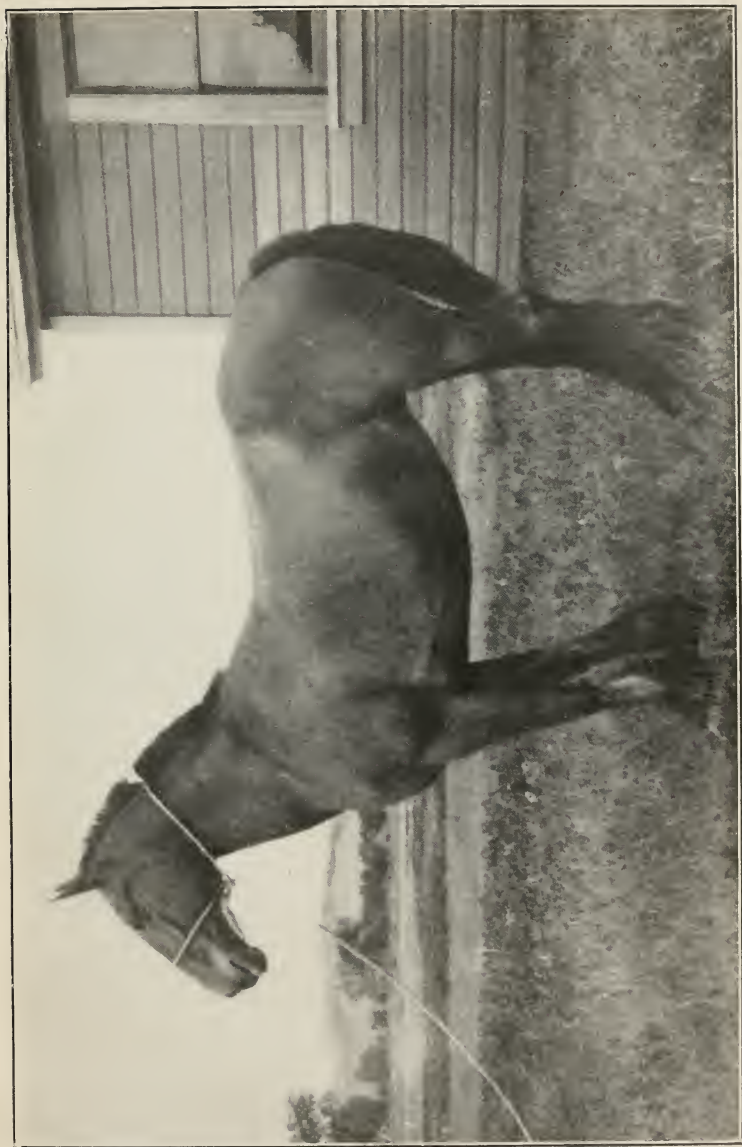
Maladie du Coit.—A group of affected Mares at the Lethbridge Quarantine grounds. Photo, taken May, 1905. Photo, by C. H. Higgins.





Maladie du Coit.—A group of affected Mares at the Lethbridge Quarantine Grounds. Photo, taken May, 1905. Photo, by C. H. Higgins.





Mare 33, "Tiffin."

It was in material taken from this mare that Drs. Watson and Gallivan first demonstrated trypanosomata Feb. 11, 1907.--- Photo by C. H. Higgins, June 12, 1907.

DOUBINE.







**DOURINE.**

Sleepy (588) and Puss (589). Naturally affected with Dourine. Note the position of the near hind leg of Puss. — Photo by C. H. Higgins, May 15, 1906.





FIG. 1.





DOURINE.

Corneal opacity appearing about one year after affection. No. 35—Stallion naturally infected.—Photo by E. A. Watson.

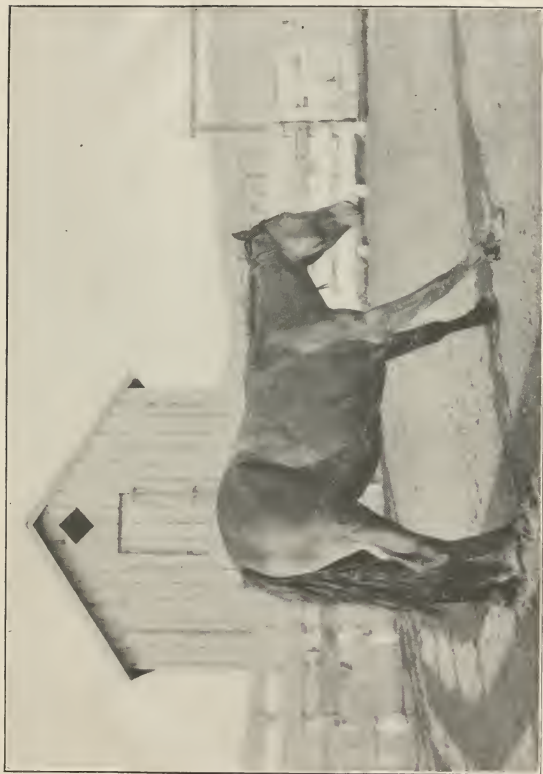




DOURINE.  
Stallion No. 35. — Naturally infected. — Photo by C. H. Higgins, June 12, 1907.







DOUBINE.

No. 74—Gelding. Photo by E. A. Watson, Aug. 11, 1907. Neuro-musculas in co-ordination.









THIS BOOK IS DUE ON THE LAST DATE  
STAMPED BELOW

AN INITIAL FINE OF 25 CENTS  
WILL BE ASSESSED FOR FAILURE TO RETURN  
THIS BOOK ON THE DATE DUE. THE PENALTY  
WILL INCREASE TO 50 CENTS ON THE FOURTH  
DAY AND TO \$1.00 ON THE SEVENTH DAY  
OVERDUE.

Biology Library

NOV 30 1942

NOV 1 1 1943

LD 21-100m-7,'40 (6936s)

Q138  
Q133

299574

BIOLOGY  
LIBRARY

*Handwritten signature*

UNIVERSITY OF CALIFORNIA LIBRARY

