

THE INFECTIVITY OF BLOOD OF ANIMALS SUFFERING FROM CHRONIC GLANDERS.

By Dr. ARNOLD THEILER, C.M.G.

IN the course of experiments on horse-sickness after the hyperimmunization of both horses and mules with virulent horse-sickness blood, some observations were made—quite as an unexpected side issue—as indicated by the title of this article, which from a scientific and practical point of view were both interesting and important.

It is generally admitted, that in the case of a horse suffering from an acute attack of glanders the blood will prove virulent. Likewise in chronic glanders, certain events may be surmised when the blood would contain bacilli. This must have been the case when metastatic foci were formed in various organs subsequent to a primary infection.

This admitted, it is apparent that to prove the virulency of such blood one must happen to tap the animal at the right moment, namely, when the bacilli are present. But even now the inoculation of only a small amount of blood may fail to give positive results, viz., when the bacilli are very few in number. The various writers on the subject allude to these various possibilities and mention the usual negative results from blood inoculation of chronically glandered horses.

* *Nocard* and *Leclainche*, for instance, state as follows :—

“ Dans la morve aigue la virulence est disséminée dans tout l'organisme et toutes les régions peuvent recéler le virus. Chez le cheval le sang recueilli dans la grand circulation n'est virulent que par exception ; dans tous les cas le bacille morveux est très rare et sa présence ne peut être révélée que par la culture et par l'inoculation de grandes quantités du liquide.” (Lœffler, Cadéac et Malet, Lissitzine, Prusse.)

† *Friedberger* and *Frohner* state as follows :—

“ Die Verbreitung des Rotzprocesses von einem Localherd aus geht in derselben Weise vor sich, wie bei der Tuberkulose. Im Anfang erfolgt die Weiterverbreitung regelmaessig auf dem Wege der zunaechst liegenden Lymphgefasse und Lymphdruesen ; bei chronischem Verlaufe kann der Process lange auf die Lymphdruesen als letzte Etappe beschaenkt bleiben. Die Bacillen koennen jedoch auch mit Benuetzung der Blutbahn nach entfernten Organen, so besonders in die Lunge und in die Haut verschleppt werden.”

‡ *A. Bonomic* as a result of experimental research comes to the following conclusion :—

“ Nel sangue dei cavalli affetti da morva cronica il baccillo è molto difficilmente dimostrabile col mezzo dell'esame batteriologico diretto, eseguito sopra quantità anche abbondanti di sangue estratto dalla vena giugulare. In sei cavalli l'esame ripetuto da 2 a 19 volte per ogni cavallo, in svariate circostanze, riuscì sempre negativo.

* Les maladies microbiennes des Animaux. (Morve.)

† Lehrbuch der speciellen Pathologie u Therapie der Hausthiere.

‡ Patogenesi e Trasmissibilità della Morva Chiusa.

“ Non si può però negare che in piccolissima quantità e forse anche, temporaneamente il bacillo si trovi nel circolo sanguigno, poichè altrimenti non si saprebbero spiegare certi fatti: come il passaggio del bacillo in alcuni secreti fisiologici (urina, latte, saliva), a ghiandole non mostranti noduli morvosi; nè si riuscirebbe a rendersi ragione del riprodursi di certe localizzazioni e dell'insorgenza di ricorrenti accessi febbrili. Malgrado ciò riesce difficilissimo cogliere il momento in cui tale bacillo si trova nel circolo sanguigno del cavallo. A spiegare la difficoltà che si incontra nel dimostrare batteriologicamente la presenza del bacillo specifico nel sangue concorre verisimilmente l'azione deleteria che alcuni speciali anticorpi, le agglutinine, esercitano sul detto bacillo.”

The observations which form the subject of this article give further illustration of facts already known, viz. (1) that the blood of a chronically glandered horse is not always infective; (2) that in such a horse the disease may become acute, or at least that in chronically glandered horses the blood can become infective when the horse is submitted to some obnoxious influence, for instance, the removal of a large quantity of blood.

For the purpose of hyperimmunizing an immune horse or mule, the blood of a horse or mule suffering from horse-sickness is transfused from the latter into the former animal. The amount of blood infused is judged by the time of flow, an average of about 500 c.c. passing in a minute through the connecting trocars and pipe. In two or three intervals, in the course of forty-eight hours or less, in this way, an amount of from 8 to 12 litres is infused.

(1) *Observations on Horses.*

Horse 3273, together with a number of others of a lot amongst which glanders had broken out in Johannesburg, was picked out by means of mallein reaction and kept in quarantine for some time. It was then decided to use these reactors for experiments and to destroy them in due course.

After their removal from Johannesburg into the stables of the Government Veterinary Laboratory at Daspoort, and after a short delay only, the Mallein test was again applied. In this particular instance the test proved positive on horse 3273, the reaction reaching a maximum of 104 8° F. After seventeen hours a typical swelling had formed at the seat of injection. A careful clinical examination revealed no outward symptoms of glanders; the horse appeared to be in the best of condition, and his temperature taken subsequently was normal. It was then inoculated with *Piroplasma equi* and did not show any febrile disturbance, although on one occasion *Piroplasma equi* was noted.

On 29th February, 1908, this horse was injected with horse-sickness virus. After an incubation time of three days the reaction started, and on the fourth day it was considered fit to be used for infusion.

On the 4th March the following horses were infused:—

Horse 3063 with 5·000 c.c. of blood.

„ 3033 „ 5·000 c.c. „

„ 444 „ 5·000 c.c. „

On the following day the following infusions were made:—

Horse 444 with 5·000 c.c. of blood.

„ 3086 „ 5·000 c.c. „

„ 3189 „ 5·000 c.c. „

On 6th March horse 3189 was infused with a further amount of 4·5000 c.c. of blood.

Horse 3273 died of horse-sickness and the *post-mortem* examination was made on the 7th March, 1908, and was as follows:—

Condition good. The flesh was pale; there was a yellow gelatinous infiltration of the subcutaneous tissue of the neck.

The liquid of the pericardial sac was slightly increased. The lungs were in inspirium, hyperaemic, and oedematous; studded throughout the whole parenchyma were numerous calcareous nodules reaching up to the size of a split pea. The bronchial and mediastinal glands were swollen and contained also calcareous nodules. The trachea at the bifurcation showed some scars the size of a shilling, one joining the other in the longitudinal direction of the trachea; the lowest ones were apparently healed up; next to the top ones was a superficial ulceration of the mucous membrane with a haemorrhagic circumference. Similar lesions were found on the upper portion of the schneiderian membranes of the septum.

The left ventricle of the heart showed some sub-endocardial haemorrhages. *Capsula glissoni* showed some fibrous filaments; the parenchyma of the liver was normal. The fundus of the stomach was hyperaemic.

The intestines and rest of organs were pale.

The cause of death was horse-sickness, the anaemic condition being due to the severe bleeding. There were glanders lesions in nose, trachea, and lungs.

The conclusion from this observation was that we would have to expect an outbreak of glanders in the five infused horses. Accordingly they were isolated and closely watched.

The result was that horses 3063 and 3033 never showed any temperature reaction from the hyperimmunization and no symptoms of any disease were observed. On the 8th April, 1908, they were subjected to the Mallein test and gave negative results, they were then released from quarantine and used as serum horses.

Horse 3086.—Infusion of this horse took place on the 5th March, 1908, with blood of horse 3273 amounting to 5·000 c.c.

On the 9th March the temperature rose to 104·6° F. and remained high, with the exception of two morning remissions. On the 15th March the submaxillary glands appeared nodulated. They were a few buds on both sides of the body. On the 17th March a watery discharge from the nostrils was noticed. The lymphatic vessels on off side of neck and body were swollen and nodules were present.

The diagnosis was made of acute glanders, and the horse was destroyed on the same date (17th March, 1908).

Post-mortem examination.—Horse 3086. Aged gelding in good condition.

The lymphatic glands in the flanks and shoulders on off side were swollen and a number of nodules were present.

A similar condition was found in the regions of the hip and thigh. Here the lymphatic glands were also enlarged.

In the upper end of the left nostril, just above the velum palati, was a granulated ulcer, the size of a penny piece.

The lungs were in half inspirium and oedematous. There were a few fibrous filaments on the pleura of the left lung. One soft white nodule was found in the parenchyma of the right lung, the size of a pea and was surrounded by a hard capsule.

The liver was enlarged, more particularly the left lobe.

The *Capsula glissoni* showed two fibrous patches.

Under the capsule of the spleen were a few white nodules, they contained soft white matter, and were surrounded by a haemorrhagic infiltration.

Diagnosis.—Acute glanders of the skin and of the nose.

Horse 3189.—Infusion took place on the 5th March, 1908, with 5·000 c.c. blood of horse 3273 and on the 6th March, 1908, with 4·500 c.c.

On the second day after this latter infusion the temperature rose in the evening to 104·6° F.; it remained high until the horse was killed. On the 3rd day the mucous membranes of the eyes were of a dirty yellow colour, on the 5th day they were injected; the animal was noticed to cough on this date. On the 9th day, on the near side of the nasal septum, ulcerations were noted; the near hind leg showed an oedematous infiltration. The horse was destroyed on the 9th day.

Post-mortem examination.—Aged grey gelding in rather poor condition.

Ulcerations on both sides of the septum of the nose.

The pleura of the lungs was thickened in parts, and the corresponding parenchyma underneath was in a state of red hepatization. The mediastinal glands were enlarged and showed gelatinous infiltration. The bronchi contained a milky fluid.

The heart was normal.

The parenchyma of the liver was black in colour.

Under the capsule at the base of the spleen were a number of nodules averaging the size of a split pea; they were surrounded by a haemorrhagic capsula.

The mucosa of stomach and ileum was hyperaemic.

There were melanotic tumours present in the pleural and peritoneal cavities and in the subcutaneous tissue disseminated all over the body.

Localization of glanders in the nose, lungs, spleen, and near hind leg was diagnosed.

Horse 444.—This horse was the first to be hyperimmunized on the 4th of March. Since the two others inoculated subsequently on the same date did not contract glanders, it had to be concluded, that this one also would not contract the disease from the first hyperimmunization.

On the 3rd day after the second infusion (8th March, 1908), the temperature rose suddenly in the morning to 104° F. It reached 105·6° F. that evening and now remained high for the next day. On the 9th March, 1908, the mucous membranes of the eyes were of a yellowish dirty colour. On the 13th March, 1908, the lymphatic vessels on both sides of the body were swollen and nodules appeared in their tracts. Nodules also appeared in the off nostril. On 14th March, 1908, the nodules began to break down.

The diagnosis of acute glanders was made, and on the 15th March, 1908, the horse was destroyed.

Post-mortem examination.—Horse 444. Aged mare in good condition.

The lungs showed a yellow gelatinous exudate on the pleura. In the sub-pleura and throughout the parenchyma were numerous nodules from the size of a pea to a bean. These nodules were soft with creamy or cheesy contents surrounded by haemorrhagic areas; there were also red and yellow areas of a larger size in a state of hepatization. The mediastinal glands were swollen about five times their normal size.

The liver was swollen and contained a few nodules the size of a buck shot, some were soft and others were hard. There were fibrous filaments on *glissoni's* capsule.

The spleen was slightly enlarged, the base was swollen, there were sub-capsular nodules, white in colour and soft in consistence, reaching the size of a pea; some were surrounded by a haemorrhagic infiltration.

The rest of the internal organs were normal.

Erosions were found in the mucosa of the off nostril.

All over the trunk and the limbs were distributed a larger number of nodules, some were open and discharged a yellowish matter. On both sides of the shoulder the lymphatic vessels were swollen and prominent.

Anatomical pathological diagnosis.—Acute glanders.

Epicrisis.—It is evident from these facts that at the date of the first infusion, namely, the 4th March, the blood of horse 3273 was not virulent. The two horses infused on this date did not contract glanders; the horse which was first infused (444) was again infused on the second day and contracted the disease together with the rest. The blood of horse 3273 therefore became infected between the first and second tapping; it looks as if the tapping was the agency provoking the acute lesions of glanders which appeared in horse 3273 on *post-mortem*, where, besides chronic scars, acute ulcers had been found.

(2) *Observations on Mules.*

Mule 4865 was received at the laboratory in October, 1909. On account of a high temperature it did not pass the Mallein test; this temperature reaction did not abate, it developed to a typical curve, and during this time the microscopical examination revealed the presence of *Piroplasma equi* on the 11th November, 1909.

The clinical symptoms of biliary fever were noted. The mule again rallied, and on the 28th November it was considered to be fit for use as a virus animal, and accordingly on that date it was injected with horse-sickness virus. It must be stated here that the mule was not malleined before use, an oversight which so far occurred in this one instance only and which was succeeded by a severe lesson. Typical horse-sickness reaction ensued, and on the 5th, 6th, and 7th day after injection the blood was used for hyperimmunization of mules in the following order:—

- (1) Mule 4778 was infused on 3rd December, 1909, with 3 litres; on 4th December, 1909, with 6 litres of blood.
- (2) Mule 4774.—Infused on 3rd December, 1909, with 3 litres; on 4th December, 1909, with 6 litres.
- (3) Mule 4780.—Infused on 3rd December, 1909, with 3 litres; on 4th December, 1909, with 3 litres.
- (4) Mule 908.—Infused on 5th December, 1909, with 3 litres.
- (5) Mule 3789.—Infused on 5th December, 1909, with 3 litres.
- (6) Mule 4786.—Infused on 5th December, 1909, with 3 litres.

Mule 4865 died in the night of 5th–6th December.

Post-mortem examination.—Made in the morning of the 6th December, 1909.

Condition good.

The subcutaneous tissue was yellowish. The blood was coagulated. The pleural cavities contained some liquid, that in the pericard was also increased, and some fluid was present in the peritoneal cavity.

The lungs were in half inspirium; the pleura showed some fibrous filaments. Both lungs were oedematous, and hyperaemic and the trachea contained some foam.

Some nodules were disseminated in the right middle lobe containing a whitish centre surrounded by a haemorrhagic area. The mediastinal and bronchial glands were swollen and anthracotic.

The left endocardium was pale and showed a few sub-endocardial haemorrhages. The myocardium was friable and yellowish. There was imbibition of right endocardium.

The liver was pale and had a patchy appearance; on section it was clay coloured.

The spleen was 19 in. × 16 in. × 10 in.; some fibrous patches were present on the capsule; the pulp was yellowish and slightly softened; the trabeculae were distinct.

The stomach contained some food and the fundus was hyperaemic. The mucosa of the duodenum was slate coloured and covered with bile stained mucus; the blood vessels of the jejunum were injected, and the mucosa of the ileum showed some longitudinal streaks. The mucosa of the caecum was slate coloured and slightly swollen, and that of the colon was bile stained.

The kidneys were pale and the pelvis showed a yellowish infiltration.

Diagnosis.—Cause of death was due to horse-sickness; the nodules were considered to be of glanders nature, and the microscopical examination of them showed typical histological structure of a glanders nodule.

NOTE.—*Post-mortem* examination proved the presence of glanders in mule 4865, accordingly it had to be expected—based on previous experience—that the infused mules would also develop the disease, and they were therefore promptly isolated.

Result of infusion.

1. Mule 4778, which was infused on 3rd and 4th December with 9 litres of blood in all, did not show any signs of glanders, nor did a subsequent Mallein and complement fixing test give any positive results. The mule was tapped in the usual way for serum, and was still alive on date of writing (end of March, 1910).

2. Mule 4774 (which was infused on 3rd December, 1909, with 3 litres and 4th December, 1909, with 6 litres).

The temperature of this animal began to rise on the 4th day after infusion and remained high; the pulse increased to 66 and remained at this figure. On the 8th day lumps were noted in the skin on the rump and chest. The diagnosis of farcey was made, and the mule was killed on the 12th day after infusion.

Post-mortem.—Made on 15th December, 1909, on a mare mule, eight years old, in good condition. (Made two hours after death.)

Rigor mortis was not complete.

The lungs were in half inspirium; the pleura was smooth. Under the pleura were a number of small nodules containing a white centre with a haemorrhagic border; there were also some calcified nodules. The trachea and bronchi were normal. The bronchial glands were swollen and reddened.

The ventricles of the heart were empty, on the middle semi-lunar valve of the aorta was a small well defined tumour with calcified contents and a capsule of cartilagenous consistence.

The liver was enlarged and hyperaemic; the lobulae were distinct. There was a patchy discolouration of the parenchyma and a mosaic of light and dark coloured areas.

The spleen was 44 cm. long and 24 broad, the middle of the body showed a cicatrix of 10 cm. in length; the follicles were distinct and there were sub-capsular haemorrhages.

The stomach contained some fluid ; the fundus was covered with viscid mucous. The mucosa of the duodenum and jejunum was bile stained, and there was a patchy hyperaemia in the ileum. The mucosa of the large intestines was normal.

The top portion of the septum nasi showed some small ulcers ; there were also some on the right conchae as well as small abscesses.

Diagnosis.—Glanders of the nose and of the lungs.

3. Mule 4780.—Infused on 3rd December, 1909, with 3 litres and on 4th December, 1909, also with 3 litres of blood. The temperature of this mule began to rise on the 4th day after the first infusion and remained high. The pulse also increased. On the 8th day on the near shoulder a lump was noticed in the skin. On the 9th day a watery discharge was observed from the nostrils ; this discharge had a yellowish tinge on the 10th day, and on the following day lumps were noticed on the near side of the abdomen.

The mule was killed on the 11th day.

Post-mortem examination.—Mule 4780 in good condition ; nine years old. *post-mortem made soon after death.*

The lungs were in expirium ; the pleura was whitish and folded. Small nodules were noticed throughout the whole parenchyma with a whitish centre and a haemorrhagic area irregularly distributed. There were pneumonic infiltrations surrounded by oedematous areas. The bronchial glands were swollen, and on section yellow liquid oozed from the cut surface. There was mucus mixed with blood in the trachea and bronchi.

The heart was normal.

The liver was swollen and of a bluish colour.

On section the lobuli were not so distinct.

The spleen was 47 cm. long and 24 broad.

A few nodules were noticed in the *corpus* also a haematoma, the size of a large apple, containing a black coagulum. Near this haematoma was a cicatrix about 14 cm. long ; the spleen pulp was of light brown colour, the trabeculae were distinct.

The stomach, small and large intestines were normal, with the exception of a few hyperaemic patches in the caecum.

The capsule of the kidneys was easily detachable ; there was an injection of the glomeruli.

The septum nasi was oedematous, infiltrated and studded with small abscesses surrounded by haemorrhagic areas. The epiglottis was slightly oedematous.

Diagnosis.—Farcy. Glanders of the nose, lungs, and spleen.

4. Mule 908.—Infused on 5th December, 1909, with 3 litres of blood. The temperature began to rise on the 3rd day after inoculation and remained high. The pulse also increased. On the fifth day lumps made their appearance on the neck and body, and two days later a nasal discharge mixed with blood was noted. On the 9th day superficial ulcers were seen on the septum nasi. The mule was killed on the 9th day.

Post-mortem examination.—Mule 908 in good condition ; about eleven years old.

Under the pleura and throughout the parenchyma were numerous nodules with haemorrhagic areas. Bronchial and mediastinal glands were swollen. Trachea and bronchi contained mucus mixed with blood.

The heart was normal.

The liver was swollen and hyperaemic.

The spleen was 19 cm. long and 23 broad and contained a few nodules.

The pulp was light brown.

The fundus of the stomach was covered with mucus.

The small and large intestines were normal.

The kidney showed an injection of the glomeruli.

The septum nasi was strongly oedematous and covered with superficial ulcerations. On the tenth ring of the trachea was an ulcer with a red border.

Diagnosis.—Farcy. Glanders of the nose, trachea, lungs, and spleen.

5. Mule 3789.—Infused on 5th December, 1909, with 3 litres of blood. The temperature rose on the 2nd day and remained high; the pulse rose also. On the 5th day lumps were noted all over the body. On the 7th day nasal discharge mixed with blood was noticed. The mule was killed on the 9th day.

Post-mortem examination.—Mule 3789 in good condition; about nine years old.

The lungs were in expirium; there were a few white fibrous patches on both pleura. Under the pleura and throughout the whole parenchyma were a number of small nodules with a white centre and haemorrhagic area. The bronchial and mediastinal glands were swollen.

The heart was normal.

The liver was swollen, dark bluish in colour and hyperaemic.

The spleen was enlarged, 50 cm. long and 30 broad. The margins were rounded. There was an old cicatrix in the middle of the corpus.

There were a fair number of sub-capsular nodules and haemorrhages.

The pulp was brownish; trabeculae and *Malpighi's* bodies distinct.

The stomach, small and large intestines were normal.

The kidneys showed a strong injection of the glomeruli.

The septum nasi was oedematous and infiltrated; there was an abscess on the left side about 4 cm. long with a haemorrhagic border.

In both conchae were ulcers and abscesses. The trachea was normal.

Diagnosis.—Farcy. Glanders of the nose, lungs, and spleen.

6. Mule 4786.—Infused on 5th December, 1909, with 3 litres of blood. The temperature rose on the 2nd day after infusion and remained high. Lumps were noted on the 7th day in the skin of the neck and body. On the 9th day discharge from both nostrils was noted. One ulcer was seen in the off nostril. The animal was killed on the 10th day.

Post-mortem examination.—Mule 4786 in good condition; about eight years old.

The sub-maxillary glands were swollen and soft.

The lungs were in expirium, the pleura was whitish. In the lung parenchyma were whitish nodules with haemorrhagic areas containing pus. Bronchial and lymphatic glands were swollen.

The heart was normal.

There were some nodules in the liver.

The spleen was 47 cm. long and 23 cm. broad.

There was one prominent nodule with a haemorrhagic area in the corpus. The pulp was swollen and soft.

The stomach and intestines were normal.

The septum nasi and both conchae were spotted with small abscesses, and the conchae also showed ulcers. There was an ulcer in the twentieth ring of the trachea; the mucosa was injected.

Diagnosis.—Farcy and glanders of nose, trachea, lungs, and spleen.

Pathological anatomical diagnosis of the five cases.—Acute glanders with localization in the skin (farcy) in the lungs, and mucosa of the nose, in some cases as well in the trachea and spleen.

Epicrisis.—Of the six animals infused three were done on the 1st and 2nd days, namely, 4778, 4774, and 4780. Of these one escaped the glanders infection, namely, the one which was infused first on the 2nd day, the other two were infused after the first one became infected. We can conclude that at the date of the first infusion the blood of the virus mule was not infective and that it only became so on the 2nd day after the infusion of the first animal. Judging from the length of the incubation time, which in the first two animals lasted four days, and in the others three and two days, the severity of the infection increased with the time which elapsed after the first tapping.

Conclusions.—The blood of animals suffering from chronic glanders is not of necessity infective; it may become so after large quantities have been withdrawn.