

## EPIZOOTIC PNEUMO-PERICARDITIS IN THE TURKEY.

### A PRELIMINARY REPORT REGARDING A NEW PATHOGENIC BACTERIUM.

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ON the 1st of October 1892 I received from Mr G. E. King of Newport Pagnell two dead turkeys for *post-mortem* examination and report, and on the same day I received a letter from Mr King giving the following history:—

The turkeys were 2 of about 20 that had died in one flock. The owner, before consulting Mr King, had forwarded one of the birds to the office of an Agricultural paper in London, and in reply had received a report to the effect that "it showed every symptom of poisoning, but no trace of disease." Mr King had made a *post-mortem* examination of three fatal cases, and in all he had found strikingly similar lesions, viz., pericarditis and pneumonia; in one of the birds the mucous membrane of the intestine presented an inflamed appearance suspicious of poisoning.

Before the above letter had arrived the two turkeys had been *post-mortemed*, and the following are my notes of the cases:—

TURKEY No. 1.—The carcase, which is very lean, appears to be that of a "this year's" bird. Abdominal organs healthy in appearance, but cover-glass preparation from spleen pulp shows numerous short bacteria. Marked pericarditis with numerous flakes of yellow lymph adherent to the surface of the heart. A cover-glass preparation, stained with methyl-blue, from the pericardial lymph shows very numerous bacteria, similar in appearance to those present in the spleen, and apparently in pure culture. A cover-glass preparation from the heart-blood shows similar but fewer micro-organisms. The left lung is normal, but the right is enlarged, dark in colour, and hepatised throughout, as if from croupous pneumonia. A cover-glass preparation from the cut surface of the hepatised lung shows colossal numbers of a short bacterium, apparently the same as that present in the spleen pulp and heart-blood. The crop is half-filled with oats, and the carcase is in a fairly fresh condition.

TURKEY No. 2.—Age apparently the same as the last; carcase lean; appears to have been longer dead than the preceding one; some *post-mortem* discoloration of the abdominal parietes. Abdominal organs appear healthy. A small amount of pericardial effusion, but no fibrinous lymph. The right lung, with the exception of a small portion near the apex, is consolidated as in the case of No. 1, and a cover-glass preparation from its cut surface shows very numerous short bacteria identical in appearance with those discovered in the first bird.

Needless to say, the conditions revealed by the *post-mortem* of these two turkeys strongly suggested that the disease was caused by the short bacterium present in both cases in the hepatised lung, and I immediately wrote to this effect to Mr King, in order that he might advise such precautionary measures as appeared necessary on the assumption that the disease was infectious. At the same time cultures

were made on the surface of slanting agar tubes, which were placed in the incubator at 37° C. On the following day the surface of the agar showed numerous colonies, almost confluent in the first tube but discrete in the second, and all having the appearance hereafter described. Microscopic examination showed that these colonies were composed of bacteria morphologically similar to those present in the dead turkeys.

I may here make a digression to explain the method of inoculating the agar tubes with the object of isolating such organisms as were present in the hepatised lung. For such a purpose at least 3 tubes are required, and it is convenient to have them of considerable calibre ( $\frac{7}{8}$  of an inch or more) and the agar well sloped in order to afford a large surface for inoculation. The platinum needle used to inoculate ought to be thin and have a small loop turned on its free end, to prevent injury to the smooth surface of the agar. The previously sterilised needle, charged with a trace of the suspected material (taken with the customary precautions against accidental infection), is gently rubbed all over the slanting agar surface of tube No. 1, and, without recharging, it is in succession used to inoculate tubes No. 2, and No. 3, in the same way. This has almost precisely the effect of the ordinary plate culture, while it is less open to risk of direct contamination of the cultures, and is much more easily carried out. The first tube is generally useless for the purpose of isolating, owing to the closeness or actual confluence of the different colonies, but unless too much material has been taken on the platinum needle the second tube, or failing that the third, will almost certainly furnish discrete colonies from which pure cultures can readily be obtained. As Professor Crookshank and others have shown, this method may even be employed successfully to obtain pure cultures of tubercle bacilli from sputum.

To return to the disease among the turkeys. A few days after I had reported the result of my examination of the two birds first sent by Mr King, I received from that gentleman a second letter containing the following additional particulars regarding the disease. The first turkey to die was a big turkey, and at the outset of the outbreak all the victims were good birds. The first symptoms noticed were a peculiar twitching of the eyelids, stiffness of the neck and legs, drooping of the wings and tail, and ruffling of the feathers. Soon there was a discharge from the nostrils, and the affected birds made a peculiar rattling or gurgling sound in the throat, while the mouth became filled with frothy mucus. The fæces were milky-white or yellowish, and very thin in consistence. The woman who attended to the poultry said that she could readily pick out the affected birds by the drops which collected at the end of the beak. Up till this date none of the other poultry—chickens and ducks—had contracted the disease.

On the 5th of October Mr King forwarded two more turkeys and a cockerel. The whole three birds were alive at the time when they were put into the hamper, but the cockerel died on the way to the station, and one of the turkeys died before the hamper reached the Veterinary College. The following are the notes of my examination of the two dead birds:—

TURKEY No. 3.—Carcase well nourished. Abdominal organs

healthy. Severe pericarditis, the pericardium being everywhere adherent to the heart wall through the medium of a layer of cream-coloured fibrinous lymph; a cover-glass preparation from this lymph shows enormous numbers of a short bacterium similar to that found in the first two cases. A cover-glass preparation of blood taken from one of the large veins shows some similar micro-organisms. The lungs are œdematous, and show a few patches of collapse. Trachea, throat, and other organs normal. Agar tubes inoculated from the pericardium yielded colonies of a short bacterium, apparently in pure culture.

COCKEREL.—Carcase fairly nourished. Abdominal organs normal. No pericarditis. Left lung is almost entirely hepatised and the right partly so. The bronchi contain firm fibrinous yellow casts. Cover-glass preparations showed that the hepatised lung and the heart-blood contained numerous short bacteria similar to those found in the turkeys.

The living turkey was kept under observation. It did not present very urgent symptoms—was dejected in appearance, did not feed very well, and frequently shook its head and emitted a noise suggestive of an effort to get rid of something in its nose or throat. It gradually recovered, and under a liberal diet it grew rapidly and became very fat. It was subsequently killed with a pure culture of the bacterium hereafter described (*see* Experiment II.).

A subsequent letter from Mr King informed me that the above-mentioned cockerel had been purchased a short time before the disease appeared among the turkeys, and that the attendant now recollected that this bird did not seem very well when it arrived, its unthrifty appearance at the time being set down to the journey. Since the date of the previous letter about a dozen turkeys had succumbed to the disease, and it was reported that several fowls were now affected.

I now entertained no doubt that the cause of the disease was the short bacterium which was found in the lesions in each of the five cases examined, but, of course, the final and conclusive evidence of the correctness of this supposition had still to be proved by experiment.

The bacterium was cultivated through successive generations, and prove to be extremely virulent when inoculated into the rabbit, but in order to prove its causal relationship to the primary disease it was necessary to experiment with turkeys.

#### EXPERIMENTS WITH TURKEYS.

EXPERIMENT I., 19th December 1892.—Inoculated a young turkey with mixed gelatine and agar cultures of the bacterium isolated from a spontaneous case of pneumo-pericarditis in the turkey. The slanting agar culture (2nd generation from lung of turkey) had been incubated for 72 hours at 37° C. The gelatine culture had the date 6/10/92, but it had been incubated at 37° for the preceding 3 days; in the now liquid condition it was poured into the agar tube, and the growth on the surface of the agar was detached and mixed with the gelatine by means of a sterilised platinum needle. About 20 drops of the turbid gelatine was injected by means of a sterilised (by heat) hypodermic syringe underneath the skin at the side of the sternum. A portion of the gelatine was at the same time poured into the turkey's mouth.

The turkey was found dead on the morning of the 22nd December (66 hours after inoculation).

*Post-mortem*.—Skin removed from seat of inoculation shows the subcutaneous tissue over an area of 2 square inches blanched and necrotic; around this the subcutaneous tissue is moist-looking and its vessels are injected. On section the necrosis is found to extend into the subjacent muscular tissue for the depth of  $\frac{1}{4}$  of an inch. Cardiac muscle abnormally pale; surface of heart shows a spot of commencing pericarditis. Posterior fourth of left lung in a condition of dense croupous consolidation. Abdominal organs normal. Cover-glass preparations showed immense numbers of the short bacterium in the hepatised lung, and about 1 bacterium for every 3 or 4 red corpuscles in blood from the heart. The bacteria were also fairly numerous in a cover-glass preparation made from the surface of the heart.

EXPERIMENT II., 3rd February 1893.—Inoculated a full-grown turkey-cock with an agar culture of the before-described bacterium, dated 21/1/93; this culture was the 3rd generation from the blood of the preceding turkey (Experiment I.). The growth on the surface of the agar was suspended in sterilised bouillon, about 20 drops of which were injected with a sterilised syringe underneath the skin of the neck.

4th February.—Turkey obviously ill; refuses food, wattles livid in colour, feathers ruffled, purging.

5th February.—Turkey much worse; respiration partly oral; frequent opening and closing of the beak. Found dead at 10 P.M. (53 hours after inoculation).

*Post-mortem* made at 11 P.M. Carcase very well nourished, fat abundant. No swelling at seat of inoculation. Abdominal organs appear normal. No obvious pericarditis. Blood in heart firmly clotted. Right lung almost entirely hepatised, the lesion exactly repeating that present in the spontaneous cases. Hepatised lung appears much swollen, dark in colour, and firm on section, with a slightly mottled cut surface. As regards its swollen appearance and its consistence, the hepatised lung recalls the solidification of bovine pleuro-pneumonia. Cover-glass preparations showed that the short bacteria were enormously abundant in the hepatised lung, and cultures made from the lung on agar in the manner already described yielded numerous colonies of the same bacteria, with one or two colonies from other organisms apparently accidentally present.

The turkey used in this experiment was the one previously referred to as having been sent by Mr King on the 5th October, and which was then suffering from a natural attack of the disease. This, apparently, left it in no way protected against infection by inoculation 4 months later.

EXPERIMENT III., 8th March 1893.—Inoculated a half-grown turkey with bouillon culture (6th generation) which had been incubated for  $3\frac{1}{2}$  days at  $37^{\circ}$  C. About 15 drops of the liquid injected with sterile syringe under skin of wattles.

9th March.—Slight subcutaneous thickening can be felt at seat of inoculation.

10th March, 9 A.M.—Turkey perceptibly ill; refuses food, feathers ruffled, bowels loose. 4 P.M., symptoms aggravated; frequent opening and closing of the beak.

11th March, 9 A.M.—Turkey very weak ; wings drooping ; makes a “sniffling” or sneezing noise through nose. Died at noon (72 hours after inoculation).

*Post-mortem.*—An area of dry yellow necrosis at seat of inoculation ; cover-glass preparation from this shows numerous short bacteria. A few spots of fibrinous lymph on the epicardium and under surface of the left lung. Cover-glass preparation from blood of heart shows no organisms. Left lung hepatised completely ; right shows a similar lesion involving its posterior half. The hepatised lung has the characters observed in previous cases, and cover-glasses made from it show great numbers of the short bacteria. Agar tubes inoculated from the lung yielded the same bacteria, apparently in pure culture. Small intestine and cæca show hyperæmia of the mucous membrane.

EXPERIMENT IV., 23rd March 1893.—Poured a quantity of bouillon culture of the bacterium isolated from turkey’s lung over the throat of a half-grown turkey.<sup>1</sup> The culture, which was dated 1/3/93, had been incubated for some time at 37° C., and for the rest of the time had stood at the temperature of the laboratory.

24th March.—Turkey found dead at 6.30 A.M. (19½ hours after infection).

*Post-mortem.*—No organisms visible in cover-glass preparations from blood of neck vein. Posterior half of left lung hepatised, though not quite so firmly as in previous cases. Cover-glass preparation from hepatised part shows colossal numbers of the short bacterium, and agar tubes inoculated from the lung yielded numerous colonies of the same bacterium with only one or two colonies of other species. Intense inflammatory congestion of small intestine and cæcal tubes.

EXPERIMENT V., 17th April 1893.—Inoculated turkey with bouillon holding in suspension culture of bacterium isolated from turkey’s lung, and poured part of the same bouillon over the turkey’s throat. The culture was detached from the slanting surface of 5 or 6 agar tubes, the reason for using so many being the unexpected discovery that several tubes, tested by attempts to start fresh cultures from them, were dead. The cultures thus mixed were of various ages and generations.

19th April.—The turkey died in the afternoon (48 hours after infection).

*Post-mortem.*—Small necrotic lesion at seat of inoculation. No bacteria visible in cover-glass preparation from blood. Firm hepatisation involving three-fourths of each lung ; cover-glass preparations from the lung lesions show enormous numbers of short bacteria, apparently in pure culture. Inflammatory congestion of intestinal mucous membrane, most marked in cæcal tubes. Agar tubes inoculated from the lung lesion yielded pure cultures of the bacterium.

The foregoing experiments constitute the last link in the chain of evidence necessary to prove that the outbreak of turkey disease occurring in Mr. King’s practice was caused by a bacterium, viz., that which microscopic examination had revealed in the lesions of the turkeys forwarded for examination on the 1st of October 1892. It now remains to describe the characters of this organism (1) as regards its effect on other species than turkeys, and (2) as regards its morpho-

<sup>1</sup> My record of this experiment unfortunately omits to state the generation of the culture.

logical and staining characters and its mode of growth in the common media of culture.

#### EXPERIMENTS WITH RABBITS.

EXPERIMENT I., *11th May* 1893, 7 P.M.—Inoculated a rabbit subcutaneously with about 15 drops of a bouillon culture of the turkey bacterium which had been incubated for 16 days at 37° C.

*12th May*.—Rabbit found dead at 5 P.M.

*Post-mortem* made at 7 P.M. Vascular injection at seat of inoculation. Cover-glass preparation of blood shows colossal numbers of short bacteria. Thoracic and abdominal organs normal in appearance. Cultures in agar made from the heart-blood grew pure.

EXPERIMENT II., *15th July* 1893, 4 P.M.—Inoculated a rabbit subcutaneously with culture on agar (1st generation from guinea-pig) of turkey bacterium. The agar tube had been incubated at 37° C. for 3 days, and the growth was suspended in sterile water.

*16th July*.—Rabbit found dead at 8 A.M.

*Post-mortem* made at 2 P.M. Much vascular engorgement of subcutaneous tissue over right side of abdominal wall (seat of inoculation); tissue abnormally moist in appearance, but no distinct œdema; a few blanched streaks of necrosis. No visceral lesions. Cover-glass preparations from local lesion and heart-blood show short bacteria, enormously abundant in latter: Agar tubes inoculated from heart-blood yielded the bacterium in pure culture.

EXPERIMENT III., *31st July* 1893, 7 P.M.—Inoculated a young rabbit subcutaneously with culture on agar of the turkey bacterium. The agar culture represented the second generation from a rabbit, and had been started from a discrete colony in a gelatine plate culture (1st generation); it had been inoculated for 24 hours at 37° C. The growth on the surface of the agar was suspended in sterile bouillon, about 10 drops of which were used for inoculation.

*1st August*.—Rabbit found dead and stiff at 10 A.M.

*Post-mortem*.—Some vascular engorgement at seat of inoculation. No visceral lesions. Cover-glass preparations of blood show great numbers of short bacteria. Bacteria recovered from heart-blood in pure culture on agar.

EXPERIMENT IV., *27th September* 1893, 8 P.M.—Inoculated a half-grown rabbit with a liquified gelatine culture (2nd generation from rabbit, date 3/7/93) of turkey bacterium.

*28th September*.—Rabbit died at 9.30 A.M.

*Post-mortem*.—Vascular distension and slight watery appearance of subcutaneous tissue at seat of inoculation. No visceral lesions. Cover-glass preparations from blood of ear-vein and heart show the short bacteria in indescribable numbers.

EXPERIMENT V., *4th October* 1893, 4.30 P.M.—Inoculated a three-quarters-grown rabbit subcutaneously with a few drops of a bouillon culture (2nd generation from rabbit of preceding experiment) which had been incubated for 24 hours at 37° C.

*5th October*.—Rabbit dead and stiff at 9 A.M.

*Post-mortem*.—Slight local lesion as in preceding cases. No visceral lesions. Bacteria numerous in blood.

EXPERIMENT VI., *20th November* 1893, 5 P.M.—Inoculated full-

grown rabbit subcutaneously with agar culture (2nd generation from rabbit) of turkey bacterium suspended in sterile water.

21st November.—Rabbit dead and stiff at 9 A.M.

*Post-mortem.*—Much vascular disturbance (hyperæmia and hæmorrhage) at seat of injection. No lesion elsewhere. Heart-blood contains bacteria in large numbers. Bacterium recovered from heart-blood in pure culture on agar.

EXPERIMENT VII, 25th November 1893, 4 P.M.—Inoculated a three-quarters-grown rabbit subcutaneously with agar culture (3rd generation from rabbit) of turkey bacterium suspended in sterile water.

26th November.—Rabbit found dead at 9 A.M.

*Post-mortem.*—Vascular distension, slight œdema, and incipient necrosis at seat of inoculation. Internal organs normal in appearance. Heart-blood very rich in bacteria. Pure cultures on agar obtained from the heart-blood.

EXPERIMENT VIII., 23rd December 1893, 4 P.M.—Inoculated a full-grown rabbit subcutaneously with a few drops of a liquified gelatine culture (1st generation from rabbit) of turkey bacterium.

24th December.—Rabbit found dead at 8 A.M.

*Post-mortem.*—Congestion and slight œdema at seat of inoculation. No visceral lesions. Bacteria very numerous in cover-glass preparation from blood of auricular vein. Agar tubes inoculated from heart-blood yielded pure cultures.

EXPERIMENT IX., 25th December, 8.30 P.M.—Inoculated a full-grown rabbit subcutaneously with agar culture (1st generation from rabbit of preceding experiment) of turkey bacterium.

26th December.—Rabbit seen alive but moribund at 2 P.M., and found dead at 2.30 P.M.

*Post-mortem.*—Subcutaneous tissue at seat of inoculation slightly œdematous, and vessels injected. No visceral lesions. Blood swarming with short bacteria.

The foregoing experiments abundantly prove the extreme virulence of this organism for the rabbit. In that species the disease which it excites when inoculated into the subcutaneous tissue is a typical septicæmia, if by that term we understand a disease in which the causal microbe rapidly invades the whole volume of blood and causes death by the poisonous products which it generates there. In the rabbit the organism showed no tendency to become localised in the lung or pericardium, and the following experiment (the only one of the kind yet made) indicates that infection does not take place readily, if at all, by way of the alimentary canal.

EXPERIMENT X., 24th December 1893.—About 5 cc. of a liquified gelatine culture of the turkey bacterium (the remainder of the tube used to inoculate the rabbit of Experiment VIII.) were poured over a cabbage leaf, the whole of which was afterwards eaten (between 1 and 3.30 P.M.) by a full-grown rabbit. The result of the experiment was absolutely negative, the rabbit being alive and apparently quite well at this date (30th Dec.).

#### EXPERIMENTS WITH GUINEA-PIGS.

EXPERIMENT I., 8th March 1893.—Inoculated an adult guinea-pig subcutaneously with about 15 drops of a bouillon culture (6th generation)

of the turkey bacterium (same culture proved fatal to turkey (*see* Experiment III.).

13th March.—Guinea-pig found dead at 9 A.M.

*Post-mortem*.—Area of necrosis of subcutaneous tissue about the size of a shilling at seat of inoculation, and around this wide-spread œdema with some hæmorrhages. Cover-glass preparation from this local lesion shows numerous bacteria, fibrinous pericarditis and bilateral pleurisy. Bacteria very numerous in lymph from surface of heart and pleura. Lungs and other organs normal.

EXPERIMENT II., 17th April 1893. Inoculated two adult guinea-pigs subcutaneously with mixed agar cultures of turkey bacterium (*see* Turkey Experiment, No. V.).

19th March.—Both guinea-pigs found dead at 9 A.M.

*Post-mortem*.—Wide-spread vascular disturbance and œdema around seat of inoculation. Cover-glass preparations from this show numerous bacteria.

EXPERIMENT III., 11th May 1893.—Inoculated a guinea-pig subcutaneously with about 15 drops of a bouillon culture of the turkey bacterium (same material fatal to rabbit in 22 hours).

29th June.—Guinea-pig found dead (49 days after inoculation).

*Post-mortem*.—Much inflammatory thickening and necrosis of textures of abdominal wall (seat of inoculation). Cover-glass preparation shows numerous short bacteria.

EXPERIMENT IV., 29th June 1893.—Inoculated a guinea-pig subcutaneously with a few drops of a liquified gelatine culture (4th generation) of the turkey bacterium. A rabbit inoculated at the same time as a control.

30th June.—Rabbit found dead at 9 A.M. The *post-mortem* revealed the usual conditions.

12th July.—The guinea-pig was reported to be weak, and like dying. Killed by chloroform.

*Post-mortem*.—Extensive local lesion spreading from seat of inoculation over whole abdominal wall and forward between fore legs. Whole textures of abdominal wall thickened by inflammatory exudate, and to a large extent necrotic. Not much vascular disturbance and no trace of suppuration. Brachial lymphatic glands enlarged to size of pea. Liver pale and fatty; kidney ditto. No other lesion. Cover-glass preparations from the seat of inoculation showed numerous short bacteria, which were recovered in pure culture on agar tubes.

As these experiments show, this organism is pathogenic for the guinea-pig, though it is much less virulent for that species than for the rabbit and the turkey. It is very interesting to observe that here, as in the case of many other diseases, the extent of the local lesion is a measure of the resistance which the animal organism offers to the invading microbe; in other words, the more virulent the microbe the slighter the lesion at the seat of inoculation. The interpretation of this law is obvious, viz., that the inflammatory reaction at the seat of inoculation, or at the primary seat of invasion when the disease is naturally contracted, is a salutary phenomenon, representing a battlefield in which the animal cells (phagocytes) make a stand against the bacteria, and for a time at least prevent the invasion of the bloodstream and the body in general.

It will be observed that in these four experiments on guinea-pigs the



effects produced were not so constant as in the case of rabbits and turkeys; whether this was due to variations in the virulence of the inoculating material, or to varying degrees of resistance on the part of the guinea-pigs, it is impossible to state.

#### EXPERIMENTS WITH PIGEONS.

EXPERIMENT I., *1st October* 1892.—Inoculated a pigeon with a speck of fibrinous lymph from the surface of a turkey's heart (turkey No. 1, page 334). The lymph, which, as previously mentioned, teemed with the short bacteria, was inserted into a small puncture, made with the usual precautions, into the pectoral muscles.

*Result.*—Entirely negative.

EXPERIMENT II., *11th May* 1893.—Injected about 15 drops of a bouillon culture of the turkey bacterium (same material fatal to a rabbit in 22 hours) underneath the skin of a pigeon.

*Result.*—Entirely negative.

EXPERIMENT III., *15th July* 1893.—Inoculated a pigeon subcutaneously on breast with agar culture (1st generation from guinea-pig) of the turkey bacterium. The same material was fatal to a rabbit in 18 hours.

*17th July.*—Pigeon seen alive, but dull, at mid-day, and found dead at 2 P.M. (46 hours after inoculation).

*Post-mortem.*—Small necrotic area at seat of inoculation, but very little vascular injection. Cover-glass preparation from heart-blood shows very numerous short bacteria. These recovered in pure culture on agar tubes.

EXPERIMENT IV., *20th November* 1893.—Injected about 15 drops of water holding in suspension an agar culture (2nd generation from rabbit) of the turkey bacterium underneath the skin of the breast of a pigeon (a rabbit inoculated with the same material died in 16 hours.)

*25th November.*—Pigeon, which had been dull and moping during the previous three days, found dead at 9 A.M.

*Post-mortem.*—Tissues at seat of inoculation, of the volume of a horse bean, in a condition of dry firm necrosis. No visceral lesions. Cover-glass preparation of heart-blood shows moderately numerous short bacteria. These recovered in pure culture on agar.

EXPERIMENT V., *25th November* 1893.—Injected about 15 drops of water holding in suspension an agar culture (3rd generation from rabbit) of the turkey bacterium under the skin of the breast of a pigeon (a rabbit inoculated with the same material died in 17 hours).

*1st December.*—Pigeon found dead at 8 A.M.

*Post-mortem.*—Pea-sized necrotic area at seat of inoculation. No visceral lesions. Blood contains numerous bacteria. Organism recovered in pure culture from blood.

#### EXPERIMENTS WITH FOWLS.

EXPERIMENT I., *8th October* 1892.—Injected about 10 drops of water holding in suspension an agar culture (2nd generation from turkey) of the turkey bacterium underneath the skin of the breast of an adult fowl (hen). The culture had been incubated for 48 hours at 37° C.

During the next 3 days the fowl appeared rather dull, generally sat on perch, with ruffled feathers and livid comb.

*12th October.*—Fowl appears more lively; down from perch and looking for food.

During the following days it recovered appetite, but was weak and lost condition. It was killed on the 7th November.

*Post-mortem.*—Carcase very emaciated. Small necrotic area at seat of inoculation; cover-glass preparation from this shows no organisms. No lesions elsewhere. No bacteria visible in cover-glass preparation from blood.

EXPERIMENT II., *25th November 1893.*—Injected about 10 or 15 drops of sterile water holding in suspension an agar culture (3rd generation from rabbit) of the turkey bacterium underneath the skin of the breast of a fowl (hen). A rabbit inoculated with the same material died in 17 hours.

*Result.*—Entirely negative. The fowl never showed any signs of disturbed health, and is apparently quite well at this date (30th December).

EXPERIMENT III., *25th December.*—Poured about 5 cc. of water holding in suspension an agar culture (1st generation from rabbit) of the turkey bacterium over the throat of a fowl (hen). Fifteen drops of the same material injected under the skin of a rabbit proved fatal in 18 hours.

*Result.*—The fæces passed on the following day were rather thin in consistence, but otherwise the fowl was in no way disturbed, and it remains alive and apparently healthy at this date (30th December).

#### EXPERIMENTS WITH OTHER ANIMALS.

A sheep, a calf, a pony, and a pig were subcutaneously inoculated (25/11/93) with 10 or 15 drops each of an agar culture (3rd generation from rabbit) of the turkey bacterium. The same material proved fatal to a rabbit in 17 hours. In the calf a subcutaneous swelling as large as a pigeon's egg formed at the seat of inoculation, and disappeared within a few days. A smaller lesion formed at the seat of inoculation in the pony. No effect was produced in the sheep or pig.

#### THE CAUSAL BACTERIUM.

Since pure cultures of a micro-organism isolated from the lung lesions of turkeys reproduced the disease, with the characteristic lesions, in experimental turkeys, it cannot be doubted that the micro-organism in question was the actual cause of the outbreak in Mr King's practice. The following are the principal characters of that organism in respect of morphology, mode of growth, and staining properties.

In stabbed cultures in 10 per cent. gelatine, kept at a temperature of 25° C., the growth is distinctly visible along the needle track after 48 hours, provided the material used to inoculate has been rich in bacteria, as is always the case with the blood of rabbits dead from this disease. At the lower end of the needle track the whitish streak when examined with a powerful pocket lens is seen to be made up of small discrete spherical colonies. These colonies soon lose their

smooth contour, and acquire a granular surface. In old cultures (2 months) the line of growth acquires a brownish tinge, and when magnified this brown coloration is seen to belong to the separate colonies, more especially the central older part. The surface of these old colonies is always more or less botryoidal, due to the development of bud-like colourless projections from the brown central mass. The gelatine is not liquified, and the surface growth is slight. In a tube which has been incubated at 20° C. for 2 months the surface growth is only slightly raised above the gelatine, and its diameter does not exceed 2 mm.

Grown as a streak culture on slanting gelatine the bacteria develop in the form of a whitish line which does not spread far from the needle track. After a month's incubation at 25° C. its breadth is only from 1 to 2 mm. When grown on the slanting surface of agar in tubes incubated at the body temperature the culture develops as a thin translucent pellicle. The colonies when discrete are at first circular, but with advancing age they acquire a festooned or scalloped edge, and a dark more opaque centre. The growth always remains thin, and in old cultures is difficult to detach from the surface of the agar.

Bouillion tubes or flasks inoculated with the bacterium become uniformly turbid within 24 hours when incubated at 37° C. With continued incubation a somewhat ropy deposit collects at the bottom of the liquid, the latter ultimately becoming clear.

On the surface of solid blood serum the growth is more elevated, white, and opaque than on agar. No appreciable growth takes place on potato.

The germ is motile; this is shown by the rapidity with which it renders bouillion turbid, and is further evident when the bacteria are examined in a "hanging drop" preparation. It is a facultative anærobe, growing abundantly in bouillion flasks in which hydrogen has been substituted for air.

The morphology of the organism does not demand a long description. Whether in blood, tissues, or artificial culture medium, it presents itself as a short ovoid bacterium, not distinguishable either by shape or size from the germ of fowl cholera. Like that organism, it stains with aqueous solution of any of the basic aniline dyes, but the methods of Gram and Gram-Weigert leave it unstained. In preparations stained by aqueous solution of methyl-blue the great majority of the organisms are short, almost ovoid, bacteria, deeply stained at the poles and unstained at the centre; but in every preparation a number of the bacteria are decidedly longer than the prevailing ovoid or diplococcus form, and this want of uniformity is apt at first to suggest the possibility of impurity. As is well known, however, a similar irregularity is exhibited by the germ of fowl cholera.

It need hardly be said that from the outset of the investigation into the cause of the turkey epizootic the possibility of the disease being fowl cholera was present to my mind, though the facts communicated by Mr King made it improbable that such could be the case. Chief among the clinical facts scarcely compatible with the view that the disease was fowl cholera were, (1) that although the owner kept a mixed flock of turkeys and common fowls over 20 of the former had succumbed to the disease before a fatal case had occurred among

the latter ; (2) that Mr King, on inquiry after the outbreak was over, learned from the owner that the only fowl lost from the disease was the cockerel referred to on a previous page ; and (3) that pneumonia was an almost constant lesion. I am aware that pneumonia and pericarditis are met with in cases of fowl cholera, but not with such constancy as in this turkey disease.

It has already been said that in respect of morphology the turkey bacterium is not distinguishable from that of fowl cholera, and it must be admitted that in their mode of growth when artificially cultivated the two germs present a close resemblance. On the other hand, judged by their effects on different species of animals the two organisms are clearly differentiated, and deserve to rank as distinct species.

The history of the outbreak and the experiments recorded in this article show that the turkey bacterium is only feebly pathogenic for the fowl, while the organism of fowl cholera is certainly and rapidly fatal by inoculation or ingestion to that species.

When the pigeon is used as the reagent a similar difference is brought out. The germ of fowl cholera is invariably fatal, generally within 24 hours, when inoculated into the pigeon, but in 5 experiments with pigeons the turkey bacterium was without apparent effect in two cases, and in the remaining three cases the period elapsing between inoculation and death varied from 46 hours to 6 days.

As regards their effects when inoculated into guinea-pigs and rabbits the two germs are strikingly similar, though for the latter species the turkey organism is perhaps a little more virulent than the other ; and, as far as one experiment warrants a conclusion, the turkey germ has no effect on the rabbit when taken into the alimentary canal, whereas the organism of fowl cholera is very fatal to the rabbit when administered with the food.

The discovery of this germ raises afresh a question of considerable interest and one that is difficult to answer. Ought micro-organisms that cannot be differentiated by morphological characters or mode of growth in artificial media, but which have different pathogenic effects, to be regarded as distinct species or as mere varieties? Excluding the disease here described, we are acquainted with at least four clinically distinct maladies caused by germs that bacteriologists have hitherto failed to distinguish by any other test than inoculation into the animal body. These are fowl cholera, the so-called rabbit septicæmia, German swine plague, and septicæmia hæmorrhagica (Wildseuche). The majority of bacteriologists have refrained from committing themselves regarding the point here raised, though Hueppe has expressed the view that the germs of these diseases are not specifically distinct, but varieties of the same organism which in natural circumstances have had impressed upon them physiological modifications (in respect of pathogenic properties) of a more or less permanent character.

I cannot conclude this article without expressing my best thanks to Mr King, not only for ascertaining the clinical history of the outbreak, but also for taking pains to provide me with experimental turkeys from stocks known to be healthy.