

## Zentralblatt für Bakteriologie – 100 years ago An outbreak of fowl plague in Tyrol in 1901

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One hundred years ago Lode and Gruber (1901) from the Institute of Hygiene at the “Imperial and Royal University at Innsbruck” reported in this journal on a severe outbreak of a disease among chickens with a lethality of up to 95 %. According to the clinical symptoms described it was fowl plague. The authors got the first information by a notice in a local newspaper where the disease was named “Hühnerpest”. It was noticed that the epidemic was imported by an Italian poulterer named Salvatori. The disease was characterized by dyspnoea and diarrhea, the feathers were ruffled, the crest and the wattle became dark blue “but they don’t stop feeding nearly up to the death”.

The epidemic was not restricted to the upper valley of the river Inn; from March to July 1901 more than 2000 cases were observed in 16 Austrian districts, and officially the estimated number of unreported cases was considered to be even higher. – The origin was in all cases Italy, mostly the province of Padua. The distribution followed the way of the poulterers; in Kufstein f. i. diseased and dead chickens were sorted out before the export to Germany and the outbreak started from this place.

The authors described a personal observation. “In the last week of June the poulterer came through the village of Telfs in the upper valley of the river Inn and rested for three days at the inn ‘Zur Post’. During that time he placed his cart with the poultry in a shed of the pub. Shortly after his departure the chickens of the owner of the inn fell ill and numerous died so that out of about 80 only two survived. – From Telfs the merchant followed the way to the Fernpass and went to the village of Obstein. There the innkeeper ‘Zum Löwen’ bought 9 geese, two of them died shortly afterwards. In Nassereit the same poulterer sold chickens to some

farmers and almost all of them died within a short time. The innkeeper’s wife ‘Zur Post’ bought 24 chickens which fell victim to the plague, additionally 15 of her hens imported from Styria died. On his way back the merchant passed again Obsteig at the beginning of August. Two to three days later 25 of the chickens in the inn ‘Zum Löwen’ fell ill and died. Ducks and pigeons in the infected farmsteads did not become ill”.

Lode and Gruber had no problems to reproduce experimentally the illness for many generations.

Clinically they observed “about 24 h after infection an obvious faintness. The animals let the wings droop, the feathers are ruffled and the body is formed into a ball. Soon afterwards an intense somnolence evolves, the head is covered by the feathers, the eyes are closed. After strong irritations only, like shouting or touching, the animals startle briefly, but fall in the same state of sleepiness after a few minutes . . . The agony lasts up to 24 h. Very often we observed secretions at the nares and conjunctivas. Mucous saliva extravasates from the mouth. During the state of preagony tonic-clonic spasms of neck and wing muscles were observable. At the beginning the temperature was slightly increased (42–43 °C) but fell down during agony to 27.5 °C . . . Very remarkable were the crest and the wattle: at the beginning pale they became later on dark blue, a characteristic sign of the disease and striking also for the layman”.

Lode and Gruber tried to isolate the causative agent. Smears from organs were stained without success by Gram’s method and with Möller’s spore stain. No microorganisms could be detected. Attempts to isolate bacteria were also unsuccessful. Sterility was found with the use of beef, calf and chicken broth (with and without addition of gelatine or agar), as well as with serum, grape juice and wort media.

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However, in a few cases they were able to isolate identical microorganisms, which were found to be pathogenic in the first culture for chickens, pigeons, mice and rabbits, when greater amounts of culture fluid were used.

Microscopically the bacterium was Gram-negative, non-motile, often bipolar stained, and the authors thought of an atypical form of fowl cholera bacteria. "The diagnosis became doubtful by the different behaviour of our microorganisms in dextrose broth, where it produced gas", furthermore it did not produce H<sub>2</sub>S. "The diagnosis proved to be absolutely erroneous, when peritrichous flagella were demonstrated in spite of the non-motility". Filtrates of several-week-old broth cultures were found to be non-toxic, even in amounts of several millilitres. "In consideration of the cultural and microscopic results and the fact that this microorganism was cultivated predominantly from animals contaminated by faeces we believe it to be a member of the Coli-bacteria". The same organism could also be isolated with easiness from the contents of the small intestine and the caecum. "In our opinion one thing is settled, the described microbe is only an accidental and unessential finding. The real agent remains as invisible and mysterious as before".

The authors believed first that an extremely toxic microorganism was involved though they were unable to find it. To prove this they filtrated fluids from the organs through a Berkefeld filter.

Nordtmeyer (1891) introduced 10 years before these filters made of "Kieselguhr" named for the owner of the mine, Berkefeld, where this diatomaceous marl was found in the Lüneburg Heath.

Organs like liver, brain or muscles of dead chickens were ground with a small amount of sterile water in a mortar and diluted with 100–200 ml of water. Chickens could be infected (intoxicated?) with small amounts of the filtrates and died 2 to 3 days later. On the assumption that the toxin was evenly distributed in the whole body and that f. i. the liver contained 5 % of the toxin ("an assumption which is surely too high") the authors calculated the toxic dose. By this way the hens given the highest dilutions should have been died by  $48.96 \times 10^{-18}$  g toxin. "Further calculations would reach numbers which are so small that it is not necessary to do so [i. e. to calculate]. In that case... the toxins would be billions more toxic than the most toxic toxins known until now". Furthermore, the time between application of the filtrates and the death was about the same whether using low or high dilutions.

"The transfer [of the disease] should be more understandable on the assumption that a reproducing virus" is present, passing the pores of the Berkefeld filter. Here

the authors used for the first time the designation "virus". As known, at that time this term did not cover the connotations of the modern word "virus", it was used in the sense of an agent causing disease.

Pigeons fell ill, some died after injection of these filtrates, but guinea pigs and rabbits did not show signs of the disease.

Lode and Gruber remarked that at the same time Centanni published a study of a disease, epidemic in the region of Ferrara, which he named "La peste aviaria" (*La clinica veterinaria*, Milano, XXIV, No. 24, 1901). The clinical symptoms were the same and he was also able to transfer the disease with Berkefeld- or Chamberland filtrates of diluted blood.

Of course, the studies of Loeffler and Frosch regarding the foot-and-mouth disease (FMD) were known to Lode and Gruber. Loeffler and Frosch were also able to transfer the disease with Berkefeld filtrates of the "lymph" from the blisters. They also discussed two theories: the presence of a toxin or of an agent passing the filter and being invisible (Loeffler and Frosch, 1898). With the same mathematical calculations as Lode and Gruber used, they concluded that a toxin could not be the cause of FMD: "Such a toxic efficacy would be simply incredible", and "Thus we cannot reject the assumption that the effect of the filtered lymph is not caused by a soluble substance, but rather by a germ with the ability to multiply" ("Es läßt sich deshalb die Annahme nicht von der Hand weisen, daß es sich bei den Wirkungen der Filtrate nicht um die Wirkungen eines gelösten Stoffes handelt, sondern um die Wirkung vermehrungsfähiger Erreger", p. 100 and Schmiedebach, 1999).

Lode and Gruber cited also Beijerinck's experiments from 1888 concerning the filterability of the "contagium" of tobacco mosaic disease (Beijerinck, 1899), but they ignored Ivanovski's findings of 1892, republished in the same volume of the *Zentralblatt für Bakteriologie* as Beijerinck's paper, dealing also with the filterability of the infectious agent (either a small bacterium or a toxin; Ivanovski, 1892, 1899; Schlegel and Köhler, 1999; Schlegel, 1999).

Our authors doubted the existence of some sort of a filterable virus. "We guessed that filters made of porous masses like Kieselguhr or clay are not efficacious by the smallness of the pores alone. The pores are mostly much larger than our biggest microbes". As to the authors, the filters should be active by adsorption ("Flächenattraktion"). They wondered why only the above mentioned organisms (from fowl plague and from tobacco leaves) and no saprophytes, much more distributed in nature than pathogens, had passed the filters. "It might be that we cannot see these small individuals but they should be observable by other signs of growth (turbidity, sediment, colouring, gas produc-

tion or other signs of metabolism). But nothing is known...". Lode and Gruber concluded, if one believes in the filter passage of the "enigmatical germs", then it must be caused by a change in the "state of aggregation" of the rigid bacterial body. "We think of the semifluid protoplasm of a plasmodial small body, the many adaptations of which with respect to the form of the body can be followed microscopically" ("Wir denken hierbei an das halbflüssige Protoplasma eines plasmodialen Körperchens, bei welchem wir die mannigfaltigsten Anpassungen hinsichtlich der Körpergestalt mikroskopisch verfolgen können").

Lode and Gruber speculated about another explanation for the filterability of the causative agent. "For quite a long time there exists a dispute between humoral and cellular pathology. In the sense of the first it could be assumed that the virus of these enigmatic infections is not cellular ["körperlich"] but rather a soluble multipliable substance with an enzyme-like character, causing destruction processes in the animal body without destroying itself" ("Vom Standpunkt des Ersteren könnte man sich vorstellen, daß das Virus bei diesen rätselhaften Infektionen gar nichts körperhaftes ist, sondern eine gelöste, mit Vermehrungsfähigkeit begabte Substanz, etwa von enzymartigem Charakter, die durch die Zersetzungs Vorgänge, welche sie im Tierkörper hervorruft, wirkt, ohne sich selbst dabei aufzuzehren").

The authors did not believe in such an enzyme-like substance but they still believed in very small, invisible organisms, filterable by changes of the rigid body. And they emphasized the view "of the above mentioned authors" (i. e. Loeffler and Frosch) "that similar enigmatic agents might play an aetiologic role for the unexplored infectious diseases like syphilis, measles, scarlet fever, carcinoma, and the reason that they are still unknown is their invisibility".

Lode and Gruber were not satisfied with the name fowl plague ("Hühnerpest") given by Centanni since "this is a synonym of fowl cholera". In regard to the discolouration of the crest and the wattle they proposed an own designation: *Kyanolophia gallinarum*.

Fowl plague was first observed in 1878 by Perronci in Italy (Gerlach, 1929). In 1898 the disease was spread from (Upper) Italy to Tyrol and South Germany, and in 1901, the year of Lode and Gruber's publication, a severe outbreak was observed in Northern Germany, following a poultry show in Braunschweig ("Braunschweiger Geflügelseuche"). Some publications from the early 20<sup>th</sup> century reported on the filterability of the causative agent (cf. Gerlach, 1929).

The real nature of the fowl plague was identified many years after Lode and Gruber's publication. Mrowka (1913), a governmental veterinarian in Tsing-

tau, described the virus of the Hühnerpest to be a globulin and he concluded "that the proteinaceous nature of the virus is the reason for the unsuccessful search for microscopically visible germs as well as for the failure of experiments to cultivate the virus in a visible manner". The detection of avian influenza viruses as causes of the European fowl plague is connected with the names of Dinter (1949), Schäfer (1955) and Rott and Schäfer (1960).

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