STUDIES ON BOVINE INFLUENZA.*

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In the year 1889 and again in 1893 there were outbreaks of an epizootic bovine disease in our country, the number of affected animals being very large. According to investigations then made by Profs. KATSUSHIMA and JANSON this disease seemed to resemble influenza in nature. Since then, however, no reports have been received on this disease until 1916 and 1919, when K. OTSUKI reported outbreaks of it among milch cows in the city and suburbs of Tokyo. The disease has been more or less prevalent there since that time; but its etiology remained unstudied. For a few years I have been working on the subject, especially its etiology, and have succeeded in isolating an organism which seems to have a causative relation with this disease. I will therefore give here a brief account of my investigations.

1. *Natural Infection.*

Although this disease may appear at all seasons of the year, in the late winter and early spring cattle seem most predisposed to it. Generally it spreads very slowly and is not so prevalent as were the cases on which Profs. KATSUSHIMA and JANSON reported. In this respect it very much resembles the GERMAN "Stallseuche." It is more apt to remain confined to an infected cow-shed from which it may spread. However, the way in which the disease naturally spreads is at present unknown but the infection seems to be spread by intermediary bearers, such as food, water, stable utensils, etc., or by persons and insects. Usually only one-fifth or one-tenth of a herd is attacked.

This disease will attack cattle of all breeds, pure or cross, and of all ages, but calves are most frequently affected and often fall victims of the disease.

2. *Symptoms.*

The initial symptoms of this disease are quite intense,

* Presented at the First Annual Meeting of the Japanese Society of Veterinary Science, Tokyo, April 9, 1922.

Usually the first symptoms noticed by the owner or caretaker are loss of appetite, dullness, and a marked languor. The affected animal stands with head down, muzzle dry, ears drooping, and appearing extremely fatigued. In the milch cow lactation diminishes or ceases altogether. The temperature rises to 40° or 41°C., and increased thirst is noted. The temperature usually drops, when the crisis is reached, on the second or third day. The pulse in the earlier stages is relatively strong and full compared with the fever, but it soon increases to 70 or 90, and becomes so weak that it can not be felt over the coccygeal artery. There is a catarrhal conjunctivitis with a copious flow of tears. Sometimes a phlegmonous conjunctivitis with marked swelling of the eyelids occurs. Later, though rarely, cataract or amaurosis appears.

The nasal mucous membrane is congested, and nasal discharge, at first serous but later muco-purulent, is a constant symptom. As a rule, the respiration is normal, but in some cases it is accelerated, and accompanied by a slight cough. The movement of the stomach becomes less active, but the rumination is not suppressed. Saliva hangs from the oral commissures in strings and increases in proportion to the progress of the disease. In the initial stage the feces are black and hard, covered with mucus or pseudo-membrane, but later a profuse diarrhea sets in, the liquid feces being mixed with fibrinous clots, rarely with blood, and sometimes having a fetid odor.

In some cases complications may be observed, such as paralysis of the hind quarters, weakness of the loin, swelling of the extremities, sheath, epigastrium, and the lower part of the chest. In the most severe cases nervous complications may develop.

3. Losses due to the Disease.

This disease can assume great economic importance on account of its greater tendency to after-effects which may impair the efficiency of the attacked animals. If this disease occurs in cows in advanced pregnancy, say, after the fifth month, abortion will almost invariably follow, and moreover if it occurs in the last month of pregnancy, the calf dropped dies immediately or

survives only for a time. This disease interferes with lactation; the secretion of milk diminishes to one-half the normal quantity, or sometimes it entirely stops. The bull becomes often incapable of performing the coital act on account of his weakness of the loin and painful swelling of the limbs, etc.

The mortality is not very high but the number of cows destroyed on account of the severity of this disease is not small. The following table shows the occurrence of this disease in 23 infected dairy farms.

	Dairies.	Cattle.	Affected.	Severely attacked.	Died.	Destroyed.	Aborted.
Total Number.	23	1051	527	72	13	35	83
Per-centage.	—	—	50.41%	13.66%	2.46%	6.64%	15.74%

4. *Morbid Anatomy.*

The post-mortem appearances vary somewhat with the severity and duration of the disease. The principal changes are as follows:—Gelatinous infiltration is observable in all parts of the body. The lungs are usually hepatized with reddish fibrinous substance or villous connective tissue on their surfaces. The liver and kidneys are enlarged and dark-colored. The spleen shows no change. The mucosa of the digestive tract is highly congested and hemorrhagic.

The autopsy of an aborted fetus resembles that of the adult animal, but more infiltration noted.

5. *Bacteriological Studies.*

1. Blood. The following experiments have been made in order to determine the pathogenic property of the blood of affected animals. The blood was taken from five affected animals and then defibrinated. Three normal cows received intravenous injections with 35—150 c.c. of the defibrinated blood. In each no symptom was observed during the course of two months after the injection. Many guinea-pigs were inoculated intraperitoneally with

the same blood, and culture-media such as common media, liver broth, and ascites agar, were also used for inoculation with the blood. All of these experiments gave negative results. It may therefore be assumed that the blood of affected animals contains no pathogenic organisms:

2. Internal organs. Materials such as lungs, liver, spleen, kidneys, lymphatic glands, and subcutaneous exudate were collected from two affected cows which were destroyed on account of the severity of the disease. A normal calf was treated by subcutaneous injection with a mixed emulsion made from these organs and exudate, but manifested no symptom of the disease, except that of a large abscess at the point of inoculation.

Agar plates were inoculated from the organs such as lungs, liver, spleen, kidneys, lymphatic glands and subcutaneous exudate of four destroyed and two deceased cows. A number of bipolar bacilli were isolated, in four cases from the lungs, in one case from the liver, in two cases from the lymphatic gland. A number of mice were also inoculated with emulsions from each of these organs. In the blood from almost all of them the same bacilli as described above were found.

3. Saliva and nasal discharge. Both agar plates and mice were inoculated with saliva and nasal discharge taken from 40 sick animals in different stages of the disease. Two kinds of bacilli were isolated; one was bipolar as pointed out above, and the other like *Bacillus erysipelatis suis*. The bipolar bacillus was found in 29 cultures and in 26 animal inoculations.

As it was probable that this bipolar bacillus may be the causative agent of this disease, the following animal experiments have been made.

6. *Pathogenicity of the Bipolar Bacillus.*

Animals used in this experiment were mice, rabbits, guinea-pigs, pigeons, sheep, and cattle. They were inoculated with suspensions of eighteen hour-agar cultures of this organism in a 0.85% salt solution.

Mice: This animal was highly susceptible to this organism. It died within four days by subcutaneous inoculation with from

1/5,000,000,000 to 1/50,000 loopful.

Rabbits: These died within 12 to 24 hours after the subcutaneous or intravenous inoculation with 1/10 loopful. At autopsy, lungs were congested, thymus and liver markedly swollen.

Guinea-pigs and pigeons: These were not susceptible. In most cases a local abscess developed at the point of inoculation, sometimes arthritis appeared, but death rarely ensued.

Sheep: A sheep inoculated subcutaneously with 2 loopfuls died, manifesting the same symptoms as were observed in a naturally diseased cow. Generally when inoculated with a small quantity of the culture, the temperature rose slightly and the appetite was reduced. However, when inoculated with a large quantity, they died in from 12 to 36 hours. In sheep it was impossible to observe experimentally the typical symptoms of this disease.

Cattle: At first two calves were inoculated subcutaneously with 2 loopfuls and five days later they showed the following symptoms: dullness, loss of appetite, rise of temperature (40°C), hypersensitiveness of the skin, lameness of the hind legs, and excretion of soft feces mixed with mucus or blood. All recovered in the course of 14 days.

A calf received 2 loopfuls intratracheally. It showed the same symptoms as described above with cough. Fifteen days later, the calf was intravenously inoculated with the same dose. It died, showing the typical symptoms of this disease. The post-mortem appearances closely resembled those observed in the natural case but no general infiltration was observed. In feeding experiments loss of appetite and a slight rise in temperature were observed in a cow, which, however, completely recovered in a few days.

^o 7. *Serological Studies.*

1. Technique of complement fixation test.

Serum. This was taken from numerous sick animals and inactivated by heating at 56°C . for 40 minutes.

Antigen. Eighteen-hour agar cultures were used. 75 loopfuls of the growth were suspended in 20 c.c. of 0.85% salt

solution containing 0.5% phenol and kept at 38°C. for 7 days. This was then centrifuged and the supernatant liquid used. The antigen was diluted 1:20 and 0.5 c.c. of it used.

Complement. The serum of the guinea-pig was diluted 1:10 and 0.2 c.c. of it used.

Hemolytic system. 1 c.c. of 5% suspension of washed sheep blood corpuscles was used with 1 c.c. of diluted hemolytic serum containing 2 units of amboceptor. Normal cattle serums were used as controls.

2. Technique of agglutination test.

Serum. This was used one or two days after collection.

Antigen. This was prepared by suspending the growth of eighteen-hour agar cultures in 0.45% salt solution containing 0.5% phenol and by heating at 60°C. for one hour.

All tubes were incubated at 38°C. for eighteen hours and the results were instantly recorded.

The results of these tests are shown in the following table:

		Complement Fixation Test.							Agglutination titer.
		0.1	0.05	0.02	0.01	0.005	0.002	0.001	
No. of serum from Affected cattle.	1	—	—	—	—	+++	+++	+++	1:160
	2	—	—	++	++	+++	+++	+++	1:320
	3	—	++	++	+++	+++	+++	+++	1:160
	4	—	—	—	+	+++	+++	+++	1:640
	5	—	—	—	—	+++	+++	+++	1:160
	6	—	—	—	—	+++	+++	+++	1:320
	7	+	+	+++	+++	+++	+++	+++	1:160
	8	+	—	—	+	+++	+++	+++	1:360
	9	—	+	+	+++	+++	+++	+++	1:160
	10	—	—	—	++	+++	+++	+++	1:80
	11	—	+	+	++	+++	+++	+++	1:160
Normal cattle serum.	1	+++	+++	+++	+++				1:20
	2	+++	+++	+++	+++				1:80
	3	+++	+++	+++	+++				1:20
	4	+++	+++	+++	+++				1:40
	5	+++	+++	+++	+++				1:40
	6	—	+++	+++	+++				1:80

The serum of the affected cattle produced a complete or partial fixation of the complement in the quantities of from 0.1 to 0.005 c.c. and the agglutination test gave positive results in a dilution of from 1:160 to 1:640. Besides those shown in the above table, a large number of the tests with the normal cattle serum has been made. In the complement fixation test any quantity of the normal cattle serum did not fix the complement except in a few cases giving a complement fixation in the quantity of 0.1 c.c. Agglutination tests with the normal cattle serum also gave positive results in the dilution of 1:80. From the results of the above-mentioned tests, it was ascertained that some anti-bodies for this organism are contained in the serum from affected animals, being quite different from the serum from normal animals.

8. *Immune serum.*

As described above, the bipolar bacillus has an intimate connection with this disease; thus I hyperimmunized an ox by injecting the bacilli and obtained from it an immune serum. The immune serum was injected into 41 sick cows in 4 herds. Of these cows 12 head, manifesting grave symptoms, were injected subcutaneously with from 300 c.c. to 700 c.c. of the Serum in two or three doses. In half the cases the injection was very effective, but in others no effect was observed. Twenty-nine cows, in the earlier stages of the disease, received one or two subcutaneous injections of the serum in doses of from 100 c.c. to 200 c.c. Most of them recovered in a short time, but no effects were observed in 4 cases.

From the above experiments, it will be manifest that the immune serum has obviously a curative power for this disease, especially if used in the early stages of the disease.

9. *Discussion.*

Bipolar bacilli are well-known organisms as a group of bacilli of hemorrhagic septicemia in rabbits, wildseuche, swine plague, infectious pleuropneumonia in calves, etc. The morphological, cultural, and biological properties of these organisms

are quite similar, but their pathogenicities vary according to the animal treated. Babes, Lignières, Hutyra, and Pfeifer reported the bipolar bacillus as the cause of equine influenza; Löffler and Guérin, as the cause of fowl diphtheria; Nocard, as the cause of malignant catarrh in the cattle. This organism was found not only in sick animals, but also in healthy animals. Moore reported that it was found in the upper air passages in 48% of pigs, in 80% of cows, in 50% of sheep, in 16% of horses, in 90% of cats, and in 30% of dogs. From the above reports it will be concluded that the bipolar bacilli are widely distributed in a saprophytic state and some of them are highly virulent, while others are avirulent. The virulence of this bacillus is generally intensified by passing through animals bodies.

Galtiere and Violet say that putrified food will mechanically cause a septicemia in domestic animals, and moreover, the former insisted that equine influenza is caused by a bipolar bacillus found in putrified vegetable foods, thus giving it the name "pneumo-enterite infectieuse." Some breeders believe that the bovine influenza has an intimate relation to food, attributing the cause of this disease to the food intoxication. From the above descriptions, it is quite certain that the bipolar bacillus must have an intimate etiological relation to the bovine influenza.

Further investigations are necessary to decide whether this organism is the primary causative agent of the disease or not.

10. *Summary.*

1. A bipolar bacillus was isolated from saliva and nasal discharge, and the internal organs of the cattle affected with influenza.

2. This organism was highly virulent for mice, guinea-pigs, rabbits, sheep, and cattle.

3. In the blood of the cattle affected with influenza were demonstrated anti-bodies specific for this organism.

4. The serum from the animals hyperimmunized against this organism has a curative power for this disease.

I wish to express my best thanks to Prof. N. NITTA for his valuable suggestions and interest in this work.

牛ノ流行性感冒ニ關スル研究

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余ハ東京府下ノ乳牛ニ年々發生流行セル流行性感冒ニ關シ數年間研究ヲ行ヒ次ノ結果ヲ得タリ

發病後種々ノ經過ニ在ル病牛 5 頭ヨリ血液ヲ採收シ之レヲ單獨或ハ混合シ試驗牛 3 頭ノ脈管内ニ 35—150 C.C. ヲ注射シ 2 箇月間觀察シタルニ全ク異狀ヲ呈セザリキ斃牛 2 頭及屠殺牛 4 頭ノ脾臟肝臟腎臟肺臟淋巴腺及心臟血液ニ就テ細菌學の檢索ヲ行ヒタルニ肺臟 4 例肝臟 1 例淋巴腺 2 例ニ於テ極メテ強キ毒性ヲ有スル兩極染色菌ヲ證明セリ又患牛 40 頭ノ唾液及鼻漏ヲ南京鼠ニ接種シ尙同時ニ培養ヲ行ヒタルニ前者ノ 26 例後者ノ 29 例ニ於テ前記臟器中ニ證明シタルモノト同一ノ兩極染色菌ヲ分離シ得タリ

本菌ハ南京鼠及家兎ニ對シ著シキ毒性ヲ有スるも、鳩ハ本菌ニ對シテ稍々抵抗力ヲ有ス、緬羊ハ本菌ノ注射ニヨリ 1 例ニ於テ自然病牛ニ於テ見ルガ如キ症狀ヲ呈シ遂ニ斃死セリ其他ノ試驗ニ於テハ菌量大ナル時ハ短期間ニ斃死シ少ナキ時ハ一時僅カニ食慾不振體溫上昇ヲ示スノミニシテ定型的症狀ヲ發セザリキ牛ハ比較的強キ感受性ヲ有シ 10 C.C. ノ皮下注射ニヨリ體溫上昇食慾不振血便又ハ粘液便等ヲ呈シ頗ル不良ノ症狀ヲ發セリ 1 例ニ於テ本菌ノ氣管内注射後 15 日ヲ經テ再ビ脈管内ニ注射シタルニ自然病牛ニ於テ見ルガ如キ症狀ヲ呈シテ斃死セリ而シテ其ノ解剖的變狀モ亦本病ノ爲メ自然ニ斃死セルモノニ酷似セシモ一般ニ浸潤ヲ呈セザリキ 1 例ニ於テ本菌ノ中寒天培養 2 本ヲ飼料ニ混ジテ給與セシニ數日間僅カニ體溫上昇シ且ツ食慾不振ヲ呈セリ

病牛血清ハ本菌ニ對シテ 80—640 倍ノ凝集價ヲ有シ健康牛血

清ハ 20—80 倍ノ凝集價ヲ有セリ補體結合試驗ニ於テ病牛血清ハ 0.1—0.005 c.c. ニテ完全ニ或ハ幾分補體ヲ吸收ス之レニ反シテ健康牛血清ハ僅カニ數例ニ於テ 0.1 c.c. ニテ補體ヲ吸收セシモノアリシモ概テ全ク吸收セザリキ之ニ由テ觀レバ病牛血清中ニハ明カニ本菌ニ對スル抗體ヲ有ス

本菌培養ヲ以テ高度ニ免疫セル牛ノ血清ヲ 4 榨乳所ニ於テ總計 41 頭ノ病牛ニ應用セシニ發病初期ノモノハ 100—200 c.c. ノ皮下注射ニヨリ大部分速カニ治癒シ發病後長時日ヲ經タル病牛 12 頭ニ於テハ中量及大量血清ノ注射ニヨリ 6 頭ニ於テ稍々良好ノ成績ヲ得タルモ其他ニ於テハ其ノ效力ヲ認メザリキ

上述セル實驗成績ニ據レバ本菌ハ本病ト密接ナル關係ヲ有ス尙ホ本菌ガ果シテ本病ノ一次的病原ナルカ否ヤニ就テハ今後ニ於ケル研究ノ結果ニ依テ之ヲ決定セントス (自抄)

附 圖 說 明

- 第 1 圖 發病後 20 日ヲ經タル牝牛(3 歳) 羸瘦四肢殊ニ後肢及胸
下部ノ浮腫竝ニ鼻漏ヲ示ス
- 第 2 圖 左 分離菌ノ寒天培養 (18 時間)
右 分離菌ノゼラチン培養 (10 日間)
- 第 3 圖 分離菌ノ寒天培養塗抹標本 (×1000)
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EXPLANATION OF PLATE VII

- Fig. 1. A diseased cow, 3 years old, 20 days after appearance of bovine influenza, showing emaciation, edematous swelling of the extremities and lower part of the chest.
- Fig. 2. Left: 18-hour agar slant culture of the bipolar bacillus (initial culture from the lung of a calf).
Right: 10-day gelatin stab culture of the bipolar bacillus.
- Fig. 3. Smear preparation from agar culture of the bipolar bacillus.

FIG. 1

FIG. 2

FIG. 3