

Meat Inspection and Microbiological Diagnosis on Local Anthrax of Pigs

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## INTRODUCTION:

The pathogenic forms of the pig anthrax and its pathological characteristics are different from the cases of the cattle and sheep (the septicemia of the anthrax). The authors had analysed quite a number of the anthrax cases and drew a perfect procedure of diagnosis on local anthrax of pigs, which including the first diagnosis for modern production line in the slaughter house; and the definite diagnosis in the laboratory. In the paper, some new regulars and viewpoints on the anthrax of pigs were also put forward by the authors.

Anthrax is an old-age, acute zoonosis, which has endangered human beings and domestic animals for thousands of years. It was once very popular in the most areas of the world. The ancient Hebrew, Indian, Greek and Russian had described the disease convincingly, for instance, in "the Exodus" of the Bible (1200-950.B.C.), it was recorded as "the water putule", which could almost cause the destruction to both man and animals. In traditional Chinese medical work "Huang Di's Canon of Internal Medicine" (475-221.B.C), it was called "the Gangraena acutissima" or "the yellow disease"; in traditional Chinese work of veterinary surgeon "The Collection of Curing Horses by YUAN-HENG" (the Ming Dynasty, 1608.A.D), it was regarded as "the Spleen-gangrene" or "Pian Ci Huang".

The human race and all kinds of animals, each one has a different degree of susceptibility to the anthrax, among which, the herbivorous animals catch the disease easily, and most cases are septicemia. Because of pigs' certain resistance to the disease, the most cases of pigs appear to be local anthrax. Although Kock cultured anthrax bacillus successfully in 1876, and it has been controlled in the world, however, as anthrax is a zoonotic infection, at present, with the vigorous development of the meat industry, the examination to the disease is still noticeable. People want to acquire a quick, accurate method for meat inspection of modern production in the slaughter house, especially for the microbiological diagnostic procedure to the anthracic cases of the ante mortem non-obvious symptoms.

## I. The Anthracic Epidemiology of the Pig.

A. The anthracic kinds of the pigs: In history, there were many reports on pandemic septicemic anthrax of cattle and sheep, and the epidemical characteristics were very clear. As most cases of pigs are single and local anthrax, as well as seldom numbers of them, it is difficult to estimate a representative (typical) incidence. The authors have counted up three groups of anthracic cases of the pigs. (The form is on the next page.)

The Soviet scholar, Катарован has inspected 67 cases of pig anthrax, among which, only one was septicemia (1.5%), 4 cases of intestinal anthrax (6.9%), 62 cases of pharynx anthrax (92%).

From the statistics above, we can know the incidence of pharynx anthrax makes up as high as 90%

Form:Analysis to three groups of anthracic cases of the pigs.

Groups	Amount	A P		A I		A L		A S		Else	
		Number	%	Number	%	Number	%	Number	%	Number	%
S	127	110	86.6	12	9.4			4	3.1	1	0.8
W	65	55	84.6	10	15.4						
D	62	58	93.5	2	3.2	1	1.6			1	1.6
Grand Total	254	223	87.8	24	9.4	1	0.4	4	1.6	2	0.8

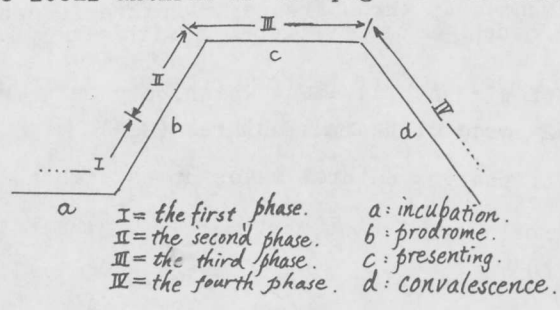
Note: AP=Anthrax of the pharynx; AI=An. of the Intestine;  
AL=An. of the Lung; AS=An. of the Septicemia.

----the most anthrax bacillus have been blocked in local infected positions of the pig; besides the quantity and power of anthracic toxin, the various kinds of clinical cases we met are relative to the initial positions of the pig where the anthracic bacillus have invaded. On grounds, we can reason: any position of the pig, where anthracic bacillus can invade, the position where the anthracic focus might take place. There are some other kinds of anthracic cases of pig collected by the authors: the anthracic cases of gastrointestine, the cases of the cecum, cases of the spleen, the cases of Ln. Subilia (Ln: lymph node) and etc. Beyond doubt, the incidence of the anthrax of the pharynx and alimentary canal was coincided with the infected food and drinking water.

**B. The Epidemical Tendency:** There were reliable reports on epidemic history and scatter of anthracic pigs in most countries. As it is an epidemic infection, since 19th century with the development of the agriculture, bivestic and the meat processing industry, there had a great pandemic in some countries. In 1940s, as sporevaccinm anthrax had been applied widely, the cases of the anthrax, special for septicemic cases, were almost controlled, the incidence of the anthrax was decreased step by step after 1960s. However, there was exception, for instance, Soviet Union PyceHa once reported that the anthracic incidence were increased by 13-16% in At present, although the research of controlling anthrax has been made a great progress in developed countries, there are still a lot of works to do in the world, especially in the developing countries.

**II. The kinds of pathology on local anthrax of the pigs and it's principle symptoms (the characteristics) in meat inspection.** The pathology of local anthrax has obvious principle symptoms. If the inspector has a good command to them, he can increase the rate of examination greatly. cases an experienced meat inspector (veterinary) diagnosed can be basically exact as the ones diagnosed in the laboratory. So the principle symptoms should be paid much attention in meat inspection.

**A. The anthrax of the pharynx:** The characteristics of pharyngeal anthrax are representative of the local anthrax of the pig. For convenience to explain, we divided the period of pathology



four phases.  
Figure 1: The four pathological phases to local anthrax of the pig. (subacute and chronic cases).

In previous records, the most anthracic reports were typical cases, which can only explain

cases of presenting period. Because of the different toxicity, the quantity of anthracic bacillus, the different resistance of the pigs and the timeliness of the infected pigs, all the cases we have met can not absolutely be presenting period or acute ones; many of them appear to be subacute or chronic; and can recover automatically, so, there might be a long incubation and a period the pathology. Since any kind of disease has a pathological period, which is entire, continuous and gradual development, and pathological characteristics at different phases are different, the inspectors must take the phase of pathology into full account, which the pig is.

The third phase: The presenting stage with typical characteristics of the disease; the *Ln mandibularis* swelled by 1-3 times, both sclerosis and fragile (non-springy) cut with a knife; well-distributed brick red colour flat sections with rough texture; no lustre but dark red or brown necrotic focus. Around the lymph node there is more or less light yellow gelatinous infiltration. The tonsil hyperemized with pseudomembrane on the surface, beneath the pseudomembrane is dark red or grey brown necrotic focus.

The first phase: The case of the pig has just been infected, the pathological period can be regarded from later stage of the incubation to the first half of the prodromal period, this moment, the colour and size of the pathological lymph node might be regular observed from the outside, only depressive dark red bleeding spots can be seen on the sections of the lymph node, which should be regarded as the initial focus just infected by bacillus anthrax, and this tiny focus is liable to be neglected in meat inspection. The virulence which was produced by the bacillus aroused the edema around the *Ln* tissue. Only on the smears of hemorrhagic focus and edema humor, the anthrax bacillus can be observed by bacteriological examination in the laboratory.

The second stage: The case has been infected for a short time, the second half of the prodromal period. The pathological lymph node swelled and turned dark red; the dark red necrotic focus can be seen on the wet sections. Around lymph nodes with obvious gelatinous infiltration, but the hardness and elasticity changed little.

The fourth phase: The obsolete, chronic or convalescent pathology, in this moment, the pathological characteristics are extremely complicated: the blood from the lymph node was absorbed, the colour of necrosis focus turned from dark red to black or grey red; on account of the gelatinous infiltration around lymph node which has swelled, the lymph node adhered to the peripheral tissue, and difficult to be separated. It is noticed the views that "There might not be anthrax bacillus in pyogenous focus." is worth to be discussed again, which has been reported by many scholars. The authors have cultured anthrax bacillus from the *Ln mandibularis* of the pyogenous focus for three times, one of the case, the lymph node was wrapped with a bindweb cyst which is about 1 mm thickness, having been cut open, the most of the lymph node had been pyogenesis with pus fluid, and obsolete necrosis focus could be seen on the surplus sections. The authors consider that it was owing to the chronic local anthrax secondly infected by pyogenic bacteria, for pigs' oral cavity is easily infected by pyogenic bacteria.

The emphasis must be noticed: The pathology of lymph node above, sometimes, occurred on the *Ln mandibularis* of one side of the pig body, the lymph node on other side is almost regular looking

from outside; sometimes, it only occurred on local position of the Ln mandibularis, as the phenomena are often met, the inspectors should pay much attention to it.

B. The anthrax of the intestine: The bacillus anthrax often attacks the first half of the duodenum and jejunum, the intestinal seromembranous was obviously congested and bled; the intestinal mucous membrane swelled and bled; the aggregated lymph node which turned from necrosis ulcer to form brown coal crust swelled greatly with purplish red to become intestinal anthrax malignant carbuncle; the related Ln mesentery happened the pathology the pharyngeal anthrax. (The characteristics just like the form of pharyngeal anthrax.); the lymphatic vessel connected the lymph node appears the state of red-line because of the bleeding; the little focus can be seen on the longitudinal sections.

C. The anthrax of the lung: The pathology occurred in the lobe of the pig's one side, one or several round dark red lumps (the size between the thumb and the egg) with a obvious threshold to the around tissue can be seen; the sections are brick red with compact texture, both sclerotic and fragile; a few grey black necrosis focus scatter on the sections; the Ln bronchus of the affected side appears the characteristics just like the form of pharyngeal anthrax with obvious gelatinous infiltration.

D. The anthrax of the stomach-intestine: The bacillus anthrax mainly attacks the Ln gastrica and the first half of the Ln mesentery, which swelled just like the size of a walnut or egg. It bears the characteristic form of the pharyngeal anthrax. The size of the ulcer is as large as a pea on the stomach fundus. There is a few or over ten malignant carbuncles on the duodenum and jejunum mucous membrane.

E. The anthrax of the spleen: The colour of the spleen is regular, no swelling, only a few hemorrhagic infarcts prominent off the surface of the spleen; besides the edge of the spleen is untidy, the sections appear to be black or brick red mixed up with grey black necrosis focus. Even if, the cases were septicemic anthrax, the typical swelling spleen might not occur, this feature is difference to the herbivorous animals.

In addition, there are some other cases of Ln subiliacus, the cases of the blind intestine and etc, which also appear the characteristics of the pharyngeal anthrax. Once the inspectors master the characteristics of the pharyngeal anthrax, they will be able to diagnose any other kinds of cases of the anthrax.

### III. The laboratory diagnosis.

A. The form of the local bacillus anthrax and its culture characteristics: It is known that the typical state of bacillus anthrax is big Gram positive bacillus; the ends are smooth or depressive, which can exist in the way of single or short chains and up the capsule in the bodies of men and animals; the long chains just look like the bamboo which can form the spore easily on the culture medium. However, the state of the bacillus anthrax of pigs vary greatly, such as bacillary, crooked, zigzag, S-shaped, folded forms and etc. Especially, the smears made of the obsolete focus, there would be a various shapes of the bacillus the bacillus joints are not clear; the stained colour not homogeneous; some bacillus seem to

longer, one end of other bacillus blurred; the cases of different bacillus with different thickness of the capsules, generally speaking, the capsules of acute, typical cases are thicker, the thickest one can be thick as 2-3 times as the bacillus body, meanwhile, the bacillus body itself seems almost like a narrow thread observed by the microscopy; some non-typical cases, the capsules are very thin. In the putrid, chronic, obsolete pathological mass, the various shapes of "Bateria Shadow" (the wreckage of the bacillus) are obvious symptom of the pig anthrax.

One exception the authors had coped with, the case of Ln submaxillary, the most bacillus on the smears were short or long chains; on the contrary, the bacillus on the culture smears were shorter chains with scattered bacillus bodies.

The aspect of the culture feature should be mentioned: The more typical the case is, the more clear the curly hair state of the colony will be; the more adhesive the colony and the longer the hyphae are, the more powerful the bacteria toxin will be.

B. The method of quick diagnosis for post mortem of the meat inspection. (the first diagnosis)

If the equivocal anthrax case was discovered in the slaughter house, the veterinary should be able to make a decision in the shortest time. Thereby, the microscopy becomes very important method, and yet, as this method belongs to the morphological examination of bacteria, the result by the method can not be regarded as the definite diagnosis. Once the inspector masters the formal symptoms of the bacillus anthrax of the pigs skilfully, he can greatly increase the reliability of the first diagnosis. Due to the characteristics of the local anthrax of pigs, the following three keys must be mastered: Firstly, the preparations should be sampled from obvious pathological positions of the pig (the necrosis focus is best), the smears should be made from many parts of the mass, at least, ten smears should be made from each sample, among which, four smears of the Gram stain, six smears of the capsule stain. Secondly, the capsule is the principle symptom of the bacillus anthrax, it must be distinguished accurately, as the capsule thickness of different bacillus anthrax varies greatly, the inspectors should use 2-3 kinds of capsule stainings at the same time in the examination. It proved that the methods of Olt and Loeffler's methylene blue stains are convenient and efficient, the contrast colours between the bacillus bodies and the capsule is sharp. Thirdly, as there is a great difference in bacillus number on each smear, at least thirty visual fields should be examined on every smear: one time, the author had observed over 50 bacillus on each visual field of one smear; another time, among ten smears, only one smear on which, a few bacillus with unclear capsule were observed, the pure culture had not been obtained until the focus was re-isolated many times. So, the microscopy to the samples should be carried out carefully.

One rich experienced veterinary was able to finish the examination above within 30-40 minutes. Once the big Gram positive bacillus with capsule were examined at lab by microscopy, the laboratory should report: "The anthrax by the first diagnosis", the slaughter house dealt with the emergence according to the relevant stipulations, meanwhile, the further examination should be carried out at the laboratory (to make a definite diagnosis).

The key to the examination is to increase the rate of coincidence between the first diagnosis and the definite diagnosis, and it's best to reach as accurate as 100 percent.

C. The method of the laboratory diagnosis (to make a definite diagnosis).

The procedure for meat inspection according to the figure 2. (The figure 2 is on the pages 13.

The following aspects should be noticed:

\* As the samples of the examination, sometimes, with more hybrid, sometimes, with less bacillus anthrax, thus, the multiple re-implants are needed to acquire the pure culture.

\* The experiment of phage lysis with a high degree of specificity, in general, the bacteriophage plaque can be observed within 8-10 h, the phage PA 631 made in China has been used for over years, except bacillus anthrax split, any other kind of bacillus split has not been found out.

\* The experiment of little white mice: The different kinds of white mice has different resistance to the bacillus anthrax, among which, the Swiss, Webster and LCR-JCL(F50) are best for the experiment. The authors had injected 18 hybrid white mice with debile-toxin-stock, fourteen of them died naturally within 56 h (77.8%); the rest died of man. In all 239 smears had been made, there were 207 smears on which the bacillus anthrax were observed (86.6%), 127 smears on which there had been over 30 bacillus bodies in each visual field (61%), the rest smears with no bacillus anthrax (13%).

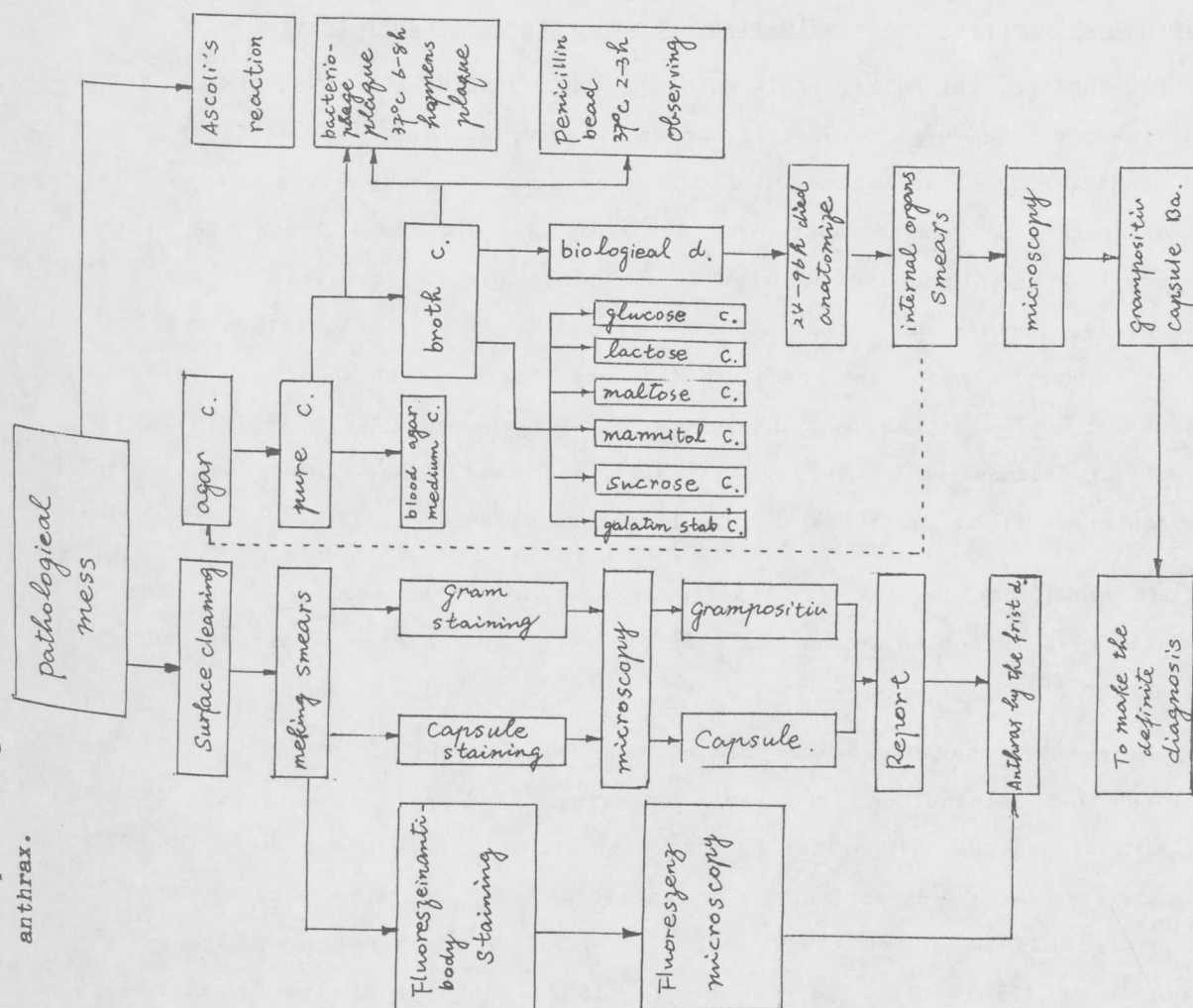
\* The experiment of the penicillin bead: The method includes the agar, the broth and paper strip with high degree of the specificity. The main keys to the experiment is to control the concentration of the penicillin, and the observing time to the beaded bacillus; beforehand, bacillus bodies don't expand; behind schedule, bacillus bodies split.

\* The experiment of the immunofluorescence: The method of fluorescence microscopy can be carried out after the indirect stain of the fluorescent antibody of sheep-anti-rare, which has a high specificity. Using this way, the result can be acquired within two hours.

\* Ascoli's Precipitation reaction is specially suited to the putrid pathological mass and the one which can not be cultured, but the definite diagnosis can't be based only on this experimental result.

\* In reality, the report of definite diagnosis should be written on the conclusion of the synthetical analysis to all kinds of the experiments above.

Figure 2.  
The processing illustration of the definite diagnosis to pig anthrax.



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