

Porcine Eperythrozoonosis-like Disease in Korea: Epidemiological Observations

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한국에서의 돼지 Eperythroozon 증양 질병 : 역학적 연구

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초록 : 한국에서 돼지에 감염되고 있는 Eperythroozon 증양 질병의 역학적 생물학적 기초연구를 위해 본 질병에 자연감염 발병한 돼지 157두와 정상인 돼지 240두에 대하여 조사하였던 바 다음과 같은 결과를 얻었다.

혈액표본에서 관찰한 본 병원체의 감염율 형태 및 크기는 계절에 따라 변화를 보였는데, 정상인 돼지는 년평균 54% 감염율을 발병한 돼지는 모두 감염된 상태였다.

5종의 형태를 보이는 본병원체는 크기가 0.5~5.0 μ 이었는데 감염 혈액을 0°C에 1주 이상(혹은 -20°C에 24시간) 냉장하는 경우 그 크기가 1.4~8.5 μ 까지 커졌으며 세포질내에 염색질 과립을 보이는 예도 발견되었다.

본 병원체는 적혈구막에 부착되거나 혈장내 유리된체 관찰되었으며 병든 돼지의 위장관과 방광내용물 중에서도 다수의 병원체가 발견되었다. 인공감염 실험에서 접종 감염된 돼지는 2개월간 정도의 체온상승과 빈혈상을 나타내면서 혈액내에 전형적인 병원체를 보유하고있으며 집중시킨 쥐에서는 12개월이상 동일한 병원체의 잠복 감염상태를 유지하였다. 또한 병든 돼지 주위에서 포획한 파리과 모기의 장내용물 중에서 전형적인 본 병원체를 관찰할 수 있었다.

Introduction

Eperythrozoonosis of pigs is an infectious disease caused by *Eperythroozon suis* or *parvum* which belong to the family *Anaplasmataceae*.

The disease is characterized clinically by a severe hemolytic anemia combined with icterus^{7,8,11,16,18,19,23}.

From the first observation of an eperythrozoonosis-like disease of swine in Korea in 1971⁹, this disease entity has revealed increasing morbidity with high

mortality every year since 1956¹⁵. The affected pigs revealed the typical symptoms of eperythrozoonosis, but had similar or somewhat larger microorganism than genus *Eperythroozon* in the blood.

However, no biological and epidemiological investigation on the pathogen or the disease regarding clinical symptoms have been established in Korea. The purpose in the present report is to describe the basic epidemiology of disease and some biological characteristics of the causative organism.

Materials and Methods

Animals and blood: One hundred and fiftyseven pigs, 1–21 month old and affected by spontaneous eperythrozoonosis-like disease, were obtained from 10 different farms and the heparinized blood samples of two hundred and forty normal pigs were collected from 4 slaughter houses in the period 1982~1983 (Table 1).

Clinical Examination: As eperythrozoonosis was found associated with typical clinical symptoms like fever, anemia, icterus, hemolysis and diarrhea by early investigators, these symptoms of the affected animals were verified with routine clinical examination methods^{5,10,21,25}.

Infection Rates and Morphological Inspection:

In order to clarify seasonal variation of infection rates and morphology of the organisms, thin blood films of each seasonal group which was composed of 50~70 animals were prepared for microscopic examination by staining with Giemsa stain. Five morphological types of the organisms were confirmed with percent as exhibited in table 2 and the sizes of organisms were measured through long diameter or length with micrometer. The blood samples of 12 affected pigs in cold group were examined with the same method after they had been stored at 0°C for more than 1 week or at -20°C for 24 hours.

Inoculation Test: Three intact Landrace piglets which were 50 days old (one of them was splenectomized 3 days before inoculation) were inoculated naturally infected blood. 5ml of the blood stored at 0°C for 5 days were injected through the ear vein of each pig and then the blood films of inoculated pigs were inspected every 5 days for 2 months.

Ten rats (mean body weight: 200gm) were injected intraperitoneally 0.2~0.5ml of the same blood and the blood of the rats were inspected every 5 days for more than 12 months.

Cultivation: The blood samples of naturally infected cases were tested for cultivation in blood agar media or broth directly from field collection or after being preserved at low temperatures.

Detection of Transmission Route: Among the species of blood sucking insects only biting flies and

mosquitoes were captured in the stables around of affected pigs and these insect specimens were stained with Giemsa stain after being prepared as pressed smears. In order to detect the cotamination route of the organism from the affected animals, the contents of gastro-intestinal tract and urinary bladder of the affected pig cadavers freezed under -20°C for more than one day were stained with the same method and then the presence of the typical organisms were checked under microscope.

Treatment: Naturally affected thirty pigs of 5 animal farms and 28 carrier pigs of 2 farms were divided into two groups. The pigs in one group received 0.02% 3-nitro-4-hydroxyphenyl-arsonic acid (=3-Nitro, Salsbury Lab. U. S. A.) of the feed for 6 days, while the pigs in the other group were given Oxytetracycline (Pfizer, U. S. A.) 3mg/kg body weight intramuscularly for 10 days.

Results

Clinical Observations: High fever (39.5°C to 42°C), decreased PCV (14 to 26) and diminished amount of hemoglobin were commonly observed in acutely affected swine and the long duration of the disease appeared to enhance the degrees of these symptoms. Also in the most cases, anoraxia, thin watery blood, redish yellow serum, orange-yellow discoloration of the urine and feces and diarrhea were typical symptoms. Icterus was prominent in some subacute and chronic cases.

Infections Rates: A total of one hundred and fiftyseven affected pigs revealed 100% infection rates with acute or chronic symptoms as illustrated on table 1 by detection of organisms in stained blood films. Hog Cholera vaccination enhanced apparently the acute occurrence of the disease in two animal farms, meanwhile the affected pigs in the other farm were observed to be undergoing coincidence with hog cholera in respect of symptoms and pathological findings.

The blood samples of two hundred and forty normal pigs were tested and approximately 54% of the animals were found positive, 74% in summer and 21% in winter, respectively.

Morphology and Distribution: In stained blood

Table 1. Eperythrozoon-like organism in swine-prevalence at blood examination

Pig Group	Location	No. of Animal	No. of Positive	(%)	Age (months)	Remarks
Affected pigs						
	Iri A	9	9	(100)	2-4	Subacute clinical symptom (among 300 pigs)
	B	16	16	(100)	1-2	Chronic diarrhea with anemia (among 200 pigs)
	C	5	5	(100)	2	Acute clinical symptom in a litter after hog cholera vaccination
	Jeonju A	2	2	(100)	2-3	Acute clinical symptom with posterior paralysis
	B	27	27	(100)	3-8	Chronic clinical symptom, Antibiotics treatment for 1 month
	C	34	34	(100)	2-5	Acute clinical symptom after hog cholera vaccination
	D	23	23	(100)	1-4	Coincidence with hog cholera (among 62 pigs)
	E	28	28	(100)	1-21	History of enzootic area for more than 5 years
	Chungbug	7	7	(100)	2-5	Subacute clinical symptom
	Chungnam	6	6	(100)	5-11	Chronic diarrhea and anemia
Normal pigs						
	Jeonbug (from 4 slaughter houses)	240	130	(54)	7-28	74% in summer; less than 21% in winter

Table 2. Seasonal variation of the organism in form with size

Form		Spring	Summer	Fall	Winter	Cold*
Discoid	%	1	3	4	9	1
	μm	1.6-2.5	1.1-2.5	1.1-2.5	0.5-3.5	1.6-3.0
Coccoid	%	91	68	69	61	76
	μm	0.5-2.5	0.5-2.0	0.5-2.5	0.5-2.5	1.4-2.5
Ring	%	2	6	4	1	0
	μm	1.6-2.0	0.5-2.0	0.5-2.0	1.1-2.0	0
Rod	%	2	4	6	17	19
	μm	1.1-4.0	1.1-3.5	0.5-3.5	1.1-5.0	1.4-8.5
Irregular	%	4	19	17	12	4
	μm	0.5-3.5	0.5-2.0	0.5-2.0	0.5-2.5	1.5-3.5

* Keep the blood under 0°C more than 1 week or -20°C 24 hours

films the organisms appeared to be discoid, coccoid, ring, rod and irregular form with sizes of 0.5-5.0 μ . Although the coccoid form was predominant over the year, the rate of each organism form varied depending on the season, and the number of the rod form was highly increased in winter or when the blood was kept under the low temperatures (Table 2).

The organisms were loosely attached to the surface of the erythrocyte or free in the plasma. The sizes of the organisms increased to 1.4-8.5 μ with multiplication when the blood was stored under

0°C for longer than one week or -20°C for 24 hours. One or two chromatin-like granules could be readily seen in some enlarged organisms (Fig. 1, 2).

Inoculation and Cultivation: Normal and splenectomized piglets inoculated with blood from field cases failed to develop any typical symptoms, however mild fever 39.2-40.5°C with slightly reduced PCV and hemoglobin than normal values of the animal did occur from the 5th day for 2 months. The same organisms were inspected from the blood films of the inoculated piglets over all experimental term, but no difference was presented between the

two pig groups. The similar organisms had been found from the inoculated rats for more than 12 months (Fig. 3). But no organism from the infected blood samples had been successfully cultivated in blood agar media or broth.

Transmission Route: The typical organisms which had characteristic staining property and forms were found in microscopic inspection of the pressed smear specimens of the biting flies and mosquitoes (Fig. 4).

The contents of gastro-intestinal tract and urinary bladder also demonstrated a number of the typical organisms with characteristic staining and forms which could be observed especially in the frozen blood.

Treatment: Six days administration of 3-Nitro had completely eliminated or effectively reduced blood infection of the organisms with recovery from the symptoms. In some field cases the pigs appeared to be subclinical after the treatment, however the pigs displayed moderate numbers of the organisms in the blood after a few weeks. The animal group being treated with Oxytetracycline showed diminished clinical symptoms but did not decrease the number of the organism in the blood.

Discussion

The naturally affected cases of the current studies had revealed the typical symptoms of eperythrozoonosis accompanying peculiar discoloration of the urine and feces, but there were a number of various reports on other symptoms including "extreme pallor of the skin and mucous membrane without jaundice or hemoglobinuria" and "Chronic bacterial pneumonia and enteritis"^{7,22,26}.

Also eperythrozoonosis was reported as a cause of infertility in swine⁴, but Zinn *et al*²⁶ reported that eperythrozoonosis is clinically observable at four stages of swine production: 1) reproductive failure; 2) anemia, mild icterus and weakness in newborn pigs; 3) delayed marketing syndrome; and 4) the classic icteroaemia of stressed feeder pigs.

Smith (1978)¹⁷ reported that the infection rate was 24% in Illinois with the swine samples submitted by practitioners and approximately 36.4% (1971, Seoul area)⁹ and 63% (1976, Jeonbug area)¹⁵

of the normal pig population were positive for eperythrozoonosis in Korea, however, in this study naturally affected pigs were found to be all positive and normal pigs exhibited comparatively high infection rate of 54% with seasonal variation of 74% in summer and 21% in winter.

There were two pig farms showing acute heavy infection of the organism after hog cholera vaccination as previous reports^{14,20} and the other one farm revealed coincidence of the disease with hog cholera. It is assumed that visible evidence of the disease is readily acquired when the animals were allowed some kind of stress or decreased resistance.

With Giemsa stain the organisms appeared to have peculiar staining property and five morphological forms which were greatly different from other organisms, but it was greatly difficult to distinguish the organisms from stain particles or Howell Jolly body.

In general, the size of rod form of the organism discovered in Korea was larger than the sizes of various organisms belonged to genus *Eperythrozoon* as were previously reported^{6,12,13,25}. The predominant form was coccoid through the year, however the most organisms grew up (1.4–8.5 μ) when the blood samples were checked in the winter season or after stored in cold condition. These abnormally enlarged forms would assist greatly in identification of the organisms and diagnosis of the disease.

It had been reported that direct inoculation of the blood from naturally affected pigs resulted in mild infection with subclinical symptoms (Splitter)²³ and also inoculation of the infected blood refrigeration on -4°C for 10 days or frozen blood samples could show no more symptoms than mild fever and anemia (Bierstein)⁹. However, the pigs to which *Eperythrozoon parvum* frozen at -79°C for 35 days was inoculated, developed clinical symptoms of the disease¹³. In this artificial infection tests the infected pigs showed no symptom other than mild fever and anemia from the 5th day for 2 months.

It was probable that the presence of spleen of the pig would not give marked effect to the disease course considering the results of the inoculation to splenectomized and intact animals.

Although it was known that the organism had not been adequately infected to other animal species, very similar organisms could be inspected in the blood films of rats from 5th day for more than 12 months following intraperitoneal inoculation of the infected pig blood.

These cultivation attempts were unsuccessful as no worker has successfully cultivated Eperythrozoon in culture media or in a cell culture system. In general, it was widely believed that arthropods would be the vectors^{2,24)} and hog lice (*Haematopinus suis*) was noted as vector by Henry⁷⁾. However, no louse was found from these tested animals but only biting flies and mosquitoes were captured around the farms. The typical organisms were detected from the pressed specimens of the arthropods, which proved them to be vectors of the organism.

It is believed that the excreta are implicated in transmission of the organism because a great number of the typical organisms were found from the contents of the gastro-intestinal tract and urinary bladder. Furthermore the close investigation has to be undertaken in order to clarify the infection cycles of the organism including the excretory contamination.

As arsenilic acid and the sodium salts had been used to improve the disease conditions by previous workers^{1,5,19)}, 3-Nitro(feed additive) also was very effective to the organisms. Oxytetracycline might be of help by decreasing the secondary infection but of no effect to the pathogens. Manufacturing of legally useful arsenicals or substitutive materials of

the drugs is greatly needed.

Conclusion

Basic biological and epidemiological observation on eperythrozoonosis like disease of swine in Korea was taken, using 157 naturally affected pigs and blood samples of 250 normal pigs.

Although infection rates, morphology and sizes of the organisms in stained blood films varied depending on the season. All of the affected pigs showed positive infection and the normal pigs were found 54% positive over the year. The organisms appeared to have five morphological forms with sizes of 0.5–5.0 μ but the sizes had increment to 1.4–8.5 μ with cytoplasmic granules when the blood was stored under 0°C for more than 1 week or –20°C for 24 hours.

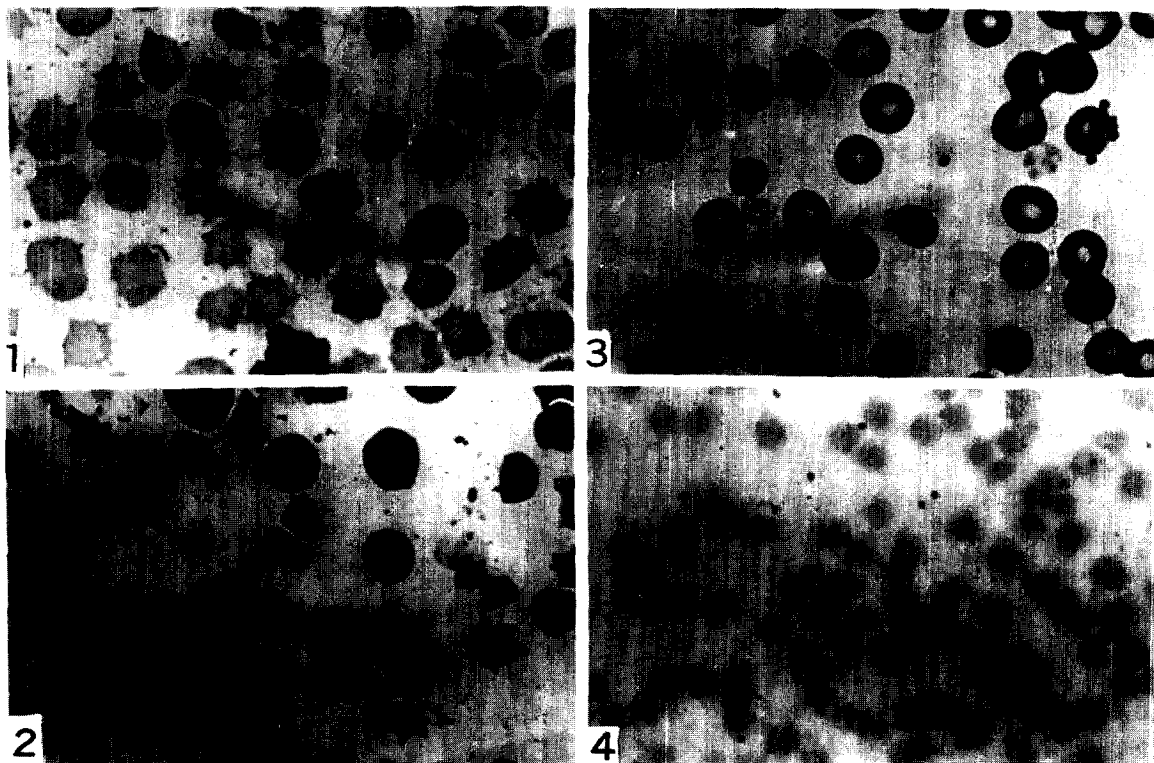
The organisms were loosely attached to the erythrocyte or free in the plasma and the contents of gastro-intestinal tract and urinary bladder of the affected animal had a number of the typical organisms.

In the artificial infection tests the infected pigs revealed the typical organisms in the blood without symptoms other than mild fever and anemia for 2 months. The similar organisms were observed from the blood films of the rats for more than 12 months following the inoculation.

Also the typical organisms were detected from the pressed smear specimens of the biting flies and mosquitoes captured around affected animal farms.

Legends for Figures

- Fig. 1.** Eperythrozoon-like organisms are observed with poikilocytes in the blood smear of naturally affected pig. Giemsa stain x1,000.
- Fig. 2.** Multiplication of the organism is shown in the blood sample which was stored at 0°C for 14 days. Giemsa stain x1,000.
- Fig. 3.** Similar organisms are observed in the blood smear of the rat inoculated with naturally infected blood, 11th month after inoculation. Giemsa stain x1,000.
- Fig. 4.** The typical organisms are found from the pressed gut content of the biting fly. Giemsa stain x1,000.



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