Therapeutic Effects of Vitamin K for Hemorrhagic Disease in Pigs

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ABSTRACT. Hemostatic disorder in castration wounds or subcutaneous mass hemorrhage occurred in growing pigs weighing 20 to 35 kg on a farm from January to June, 1983. Fifty of 360 pigs displayed clinical signs and 25 pigs died. Twenty-one of 114 pigs (from 5 of 14 groups) revealed abnormal PT on screening tests. These pigs also showed prolonged APTT and decrease in the levels of factors II, VII, IX and X. They were divided into 4 groups for vitamin K administration tests. Vitamin K-1 and K-2 were injected intramuscularly into 8 pigs (K-1 group) and 6 pigs (K-2 group) in a single dose of 3 mg per kg of body weight, respectively. Vitamin K-3 was added to feed at a rate of 25 ppm and given to 3 pigs (K-3 group) for 4 consecutive days. Four pigs (control group) were not treated. Vitamin K-1 and K-2 revealed similar effects on recovery from blood coagulation defects. Thus, in K-1 and K-2 group, PT was improved at 3 hr post-injection, and APTT at 14 or 21 hr. Factors VII, IX and X were elevated to normal levels at 3 or 14 hr post-injection, and factor II at 14 or 21 hr. No pigs in K-1 group demonstrated abnormal PT and APTT on the 5th, 10th and 30th day post-injection. In K-3 group, PT and APTT improved to normal time at 21 hr after the beginning of administration, and no pigs demonstrated abnormal PT and APTT on the 5th or 30th day. In control pigs, there was no recovery from blood coagulation defects for 21 hrs. From these results, the hemorrhagic disease in this study was confirmed to result from vitamin K deficiency. The cause of vitamin K deficiency was considered to be related antibacterial drugs and ingredients of feed.—**KEY WORDS:** hemorrhagic disease, pig, vitamin K deficiency.

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The authors previously reported an outbreak of hemorrhagic disease in pigs associated with vitamin K deficiency [15]. Subsequently, some informations were obtained about outbreaks of hemorrhagic disease in Japan with clinical signs similar to our report. However, it was not known whether they were associated with vitamin K deficiency or not, because blood coagulation tests were not done in those cases.

The levels of vitamin K dependent factors (factors II, VII, IX, and X) in plasma are decreased in vitamin K deficiency. Blood coagulation factors are also decreased in severe liver diseases. Depression of coagulation factors caused by the former can be corrected in a short period by vitamin K administration, but not when caused by the

latter [2, 16]. The response to this vitamin is often used as an aid in clinical differential diagnosis between liver disease and vitamin K deficiency [16]. It has been reported that swine hemorrhagic disease responded well clinically to vitamin K administration [5, 6, 11, 12, 17], but there have been no reports on the changes in coagulation factors after vitamin K administration in swine.

Phylloquinone (vitamin K-1), menaquinone (vitamin K-2) and menadione (vitamin K-3) were produced for application in human and veterinary medicine. It has been reported that a large dose of vitamin K-3 caused hyperbilirubinemia and icterus [3], accordingly vitamin K-1 is generally used as prophylactic administration. Recently, therapeutic or prophylactic administration of vita-

Table 1. Feed composition of growing pigs

Ingredient	Feed		
	I (5-15 kg) ^{a)}	II (16-35 kg)	III (36-75 kg)
Corn	39.2%	61.0%	71.0%
Cookie refuse	10.0	10.0	2.5
Fish meal	8.2	5.0	4.5
Skim milk	25.0	0	0
Soy milk	10.0	0	0
Glucose	5.0	0	0
Defatted soybean meal	0	20.0	17.5
Beef tallow powder	0	1.0	0
Barley flake	0	0	2.5
Mineral-Vitamin mix ^{b)}	1.5	3.3	1.75
Antibacterial drugs ^{c)}	1.05	0.8	0.3

a) Body weight.

b) Salt, calcium, iron, copper, cobalt, manganese, amino acids (lysine and methionine), digestive enzymes (protease, cellulase, xylase and pectinase), vitamin (A, D, E, B₁, B₂, B₆, B₁₂, panthenic acid, niacin and choline).

c) Detail amount in Table 2.

Table 2. Antibacterial drugs in feed

Drug	Feed		
	I	II	III
Spiramycin	15 ppm	15 ppm	0 ppm
Chlortetracycline	60	60	0
Sulfamethazine	60	60	60
Colistin sulfate	80	0	0
Furazolidone	200	0	0
Sulfamonomethoxine	0	200	0
Tylosin	0	0	231
Destomycine	50	50	50

min K-2 has been evaluated in hemorrhagic disease in newborn humans, because its effect is obtained quicker than vitamin K-1 [19].

This report describes an outbreak of swine hemorrhagic disease and the therapeutic effects of vitamin K administration.

MATERIALS AND METHODS

Outline of the farm: Six boars, 75 sows, 670 growing pigs and 130 suckling pigs were being reared on the farm, at the time when the hemorrhagic disease occurred. Six hundred growing pigs were crossbreeds, and the re-

mainders were Landrace and Large white.

Three kinds of mixed feed were given to growing pigs in terms of body weight (Table 1). Each feed ingredient was purchased from commercial sources at 1- or 2-week intervals, except for cookie refuse which was purchased every 1 or 2 months, and they were mixed at the farm every 4 days. Six, 5 and 3 kinds of antibacterial drugs were added to feed Nos. I, II and III, respectively (Table 2). The main composition of these feeds had been the same for 2 years, but cookie refuse and beef tallow powder were used for 2 months before the occurrence of hemorrhagic disease.

Screening tests to check for blood coagulation defective pigs: One hundred-fourteen pigs from 14 groups weighing about 5 to 35 kg (20 to 90 days of age) were examined by one stage prothrombin time (PT). Coagulation factors were measured in pigs with abnormal PT.

Vitamin K administration test: Twenty-one pigs selected by screening tests were divided into 4 groups. K-1 group (8 pigs) was injected a single dose of 3 mg of phytonadion (Eisai Co., Ltd., Tokyo) per kg of

Table 3. Screening test to check for blood coagulation defective pigs

Group No.	Breed ^{a)}	No. of pigs	No. of pigs with prolonged PT ^{b)}	Days of age	Body weight	Feed	
						Kind	Duration of feeding
1	WD	8	0	22	5- 6 kg	I	7 day
2	WD	7	0	22	5- 6	I	7
3	LWD	8	0	24	5- 7	I	12
4	LWD	9	0	30	8-10	I	17
5	WD	10	0	44	10-12	I	22
6	LWD	5	0	50	16-19	II	4
7	LW	6	0	51	14-17	II	4
8	LWD	6	1	53	16-18	II	4
9	L	10	0	74	23-25	II	30
10	WD	11	5	75	23-26	II	30
11	LW	7	3	77	25-28	II	32
12	LD	8	6	77	25-28	II	32
13	LWD	7	6	92	32-34	II	47
14	LWD	12	0	94	30-35	III	7
Control ^{c)}	WD	7	0	75	24-26	C ^{d)}	

a) W: Large white, D: Duroc, L: Landrace.

b) Over 16 seconds in prothrombin time.

c) Reared on another farm.

d) Commercial assorted feed.

body weight intramuscularly. K-2 group (6 pigs) was injected a single dose of 3 mg of menatetranone (Eisai Co., Ltd., Tokyo) per kg of body weight intramuscularly. K-3 group (3 pigs) was added 25 ppm of menadi-one sodium hydrogen sulfite (Taito Pfizer., Co., Ltd., Tokyo) to the feed and given for 4 consecutive days. Control group (4 pigs) were not treated with vitamin K preparation, and blood coagulation tests were conducted after 21 hr.

In K-1 and K-2 groups, PT, activated partial thromboplastin time (APTT), and the level of coagulation factors II, VII, IX and X) before and at 3, 14 and 21 hr after vitamin K injection were measured. In addition, in the K-1 group, PT and APTT were measured on the 5th, 10th and 30th day after vitamin K administration in order to ascertain possible blood coagulation defects. PT and APTT were measured in K-3 group at 21 hr, 5th and 30th day after vitamin K administration.

Blood coagulation tests: PT, APTT and coagulation factors were obtained by the

same methods described in our previous report [15]. Seven pigs (75 days of age) being reared on another farm with commercial feed, were used as control blood coagulation tests.

Mycological examinations: Feed and their four ingredients (cookie refuse, corn, fish meal and soy milk) were examined to detect mycoses using potato dextrose agar to which 7.5% salt was added. Cookie refuse growing with mycoses was examined to detect aflatoxin by gas chromatography.

RESULTS

Pigs weighing about 30 kg (about 60 days of age) had sporadically died of hemorrhage from castration wounds, between January and March, 1983. This hemorrhage did not occur after the age of castration was changed from 60 to over 90 days of age. However, subcutaneous swelling purple in color and the size of a fist developed at the site of vaccination, and oozing from the wounds by ear tags, subcutaneous mass hemorrhage in hindlimbs,

Table 4. Blood coagulation tests in pigs

Group	No. of animals		PT (sec)	APTT (sec)	Fibrinogen (Factor I) (mg/dl)	Calcium (Factor IV) (mg/dl)
Abnormal in PT	18	Mean	21.1	43.4	195.6	10.2
		SD ^{a)}	5.3	18.2	39.5	1.3
		Range	16.0~35.1	20.9~83.7	156~297	8.9~11.6
Control	7	Mean	13.0	17.6	218.7	9.5
		SD	0.7	2.3	38.4	0.4

a) Standard deviation.

Fig. 1. Changes in prothrombin time (PT) and activated partial thromboplastin time (APTT) after vitamin K administration.

●: K-1 group, □: K-2 group, △: K-3 group, ▲: control group, ±: normal range.
Standard deviation is shown as vertical bars.

forelimbs, axillar and mandibular regions, occurred in pigs weighing 20 to 35 kg after April. Other clinical signs were lameness, progressive emaciation, recumbency and eventual death. Fifty of 360 pigs demonstrated the clinical signs and 25 pigs died during 6 months. Affected pigs (weighing 20 to 35 kg) were limited to animals given the feed No. II; most of them were crossbreed,

Fig. 2. Changes in blood coagulation factors after vitamin K administration.

●: K-1 group, □: K-2 group, ▲: control group.
Standard deviation is shown as vertical bars.

but a few were Landrace.

Screening tests were conducted on July 7. Since pigs with over 16 sec in PT were revealed prolongation in APTT and decrease

with abnormal prothrombin time

Factor					
II (%)	V (%)	VII (%)	VIII (%)	IX (%)	X (%)
13.9	78.8	30.2	111.5	64.2	48.8
12.9	12.7	22.3	30.0	21.5	31.1
4~55	61~110	3~55	70~192	19~107	13~113
92.9	94.9	97.2	109.1	99.3	118.1
16.6	8.8	15.0	21.2	17.0	38.0

of blood coagulation factors, over 16.0 sec was regarded as abnormal PT. Twenty-one (18%) of 114 pigs (from 5 of 14 groups) revealed abnormal PT (Table 3). All these pigs were given the feed No. II, and no abnormal pigs were seen in groups given feed Nos. I and III. In 18 pigs with abnormal PT, mean APTT was also prolonged in 43.4 sec and mean levels of factors II, VII, IX and X were demonstrated in 13.9%, 30.2%, 64.2% and 48.8%, respectively. These values were significantly low compared with control value. Fibrinogen (factor I), calcium (factor IV) and factor VIII were within the normal range, and factor V was slightly decreased (Table 4).

There was complete recovery from blood coagulation defects in treated groups, but not in the control group within 21 hr (Figs. 1 and 2).

PT: In K-1 group, the mean PT was 20.5 sec before phytonadion injection, but recovered in the normal range at 3 hr after injection. Thereafter, no pigs revealed an abnormal level on the 5th, 10th and 30th day post-injection. In K-2 group, similar results were observed for 21 hr. In K-3 group, PT recovered the normal range at 21 hr after beginning administration, and no animals revealed any abnormal level up to 30th day after administration.

APTT: In K-1 group, the mean APTT was 41.3 sec before phytonadion injection, but it gradually recovered after injection and reached the normal range after 21 hr in most of

pigs. Thereafter, all pigs demonstrated a nearly normal range on the 5th, 10th, and 30th day. In K-2 group, mean APTT was 52.6 sec before menatetranone injection, and similar results to K-1 group were observed until 21 hr after injection. In K-3 group, mean APTT was 39.2 sec, and APTT was in the normal range at 21 hr, 5th and 30th day after the beginning of administration in all pigs.

Factor II (Prothrombin): In K-1 group, the mean factor II level was 17% before injection and gradually recovered after injection and reached 100% after 21 hr. In K-2 group, the mean factor II level was 14% before injection, and recovered almost the normal level at 14 hr after injection, but the variation was greater than in the K-1 group.

Factor VII: The mean levels of factor VII in K-1 and K-2 groups were 30% and 40% before injection, 77% and 99% at 3 hr after injection, and 98% and 120% after 14 hr, respectively. Thus, factor VII recovered the normal level at 3 or 14 hr after injection in most pigs of both groups.

Factor IX: The mean levels of factor IX in K-1 and K-2 groups were 66% and 68% before injection, respectively, and recovered the normal level at 3 hr following injection in both groups. Then, the level of factor IX peaked at 14 hr in both groups.

Factor X: The mean levels of factor X in K-1 and K-2 groups were 39% and 78%, respectively. The factor X levels in both groups were elevated by injection and peaked

at 14 hr, while maintaining their pre-administration difference.

Wallemia, *Eurotium* *Penicillium* and *Cladosporium* were isolated from feed Nos. I and II, and some ingredients. The number of isolated mycoses per gram of feed or ingredients ranged from 10^1 to 10^4 . No *Aspergillus* could be isolated from them. Aflatoxin B₁, B₂, G₁, G₂, and M₁ were not detected in cookie refuse with growing mycoses.

DISCUSSION

The clinical signs and the results of blood coagulation tests in this study were quite similar to those in a previous report [15], and the cause of hemorrhage was considered to be vitamin K deficiency. Vitamin K dependent coagulation factors (factors II, VII, IX and X) are synthesized as a precursor in the liver. These precursors have no biological activity, but vitamin K converts the precursors to biologically active proteins. In the final stage of these factor's biosynthesis, vitamin K involves the carboxylation of selected glutamin acid residues on the precursors, so that γ -carboxylglutamic acid is formed [7, 18]. In the absence of vitamin K or the presence of antagonists for vitamin K, vitamin K-dependent carboxylase activity is inhibited, normal coagulation factors are decreased, and abnormal factors are increased in plasma, resulting in a hemostatic disorder [7, 15, 18]. When vitamin K is administered to such animals, γ -carboxylglutamic acid re-synthesized and there is rapid recovery from the blood coagulation defects [10].

In this study, PT and APTT in almost all pigs recovered normal levels within 3 or 21 hr post-administration of a single dose of vitamin K-1, K-2 and K-3. The levels of factors VII, IX and X were regained at 3 or 14 hr post-injection of K-1 and K-2, and factor II recovered within 14 or 21 hr. These results virtually agreed with the therapeutic results obtained in vitamin K deficiency in human

[10]. From these findings, the hemorrhagic disease in this study was confirmed as vitamin K deficiency.

It has been reported that vitamin K-2 shows effects quicker than vitamin K-1 in comparison with the therapeutic effects in human [19]. On the other hand, vitamin K-1 was quicker than vitamin K-2 in chicken [4]. There were no significant differences between vitamin K-1 and K-2 preparation in pigs.

In the previous outbreak [15], it seemed likely that the vitamin K deficiency contributed to the combined effects of antibacterial drugs and consanguinity. However, the hemorrhagic disease did not appear to limit the specific consanguinity in this case. The pigs with clinical hemorrhage and blood coagulation defects were limited to the groups given feed No. II. There are some reports on swine hemorrhagic disease related to feed, but the true cause of the hemorrhage is not known in these cases, except for toxication by coumarin derivatives [5, 6, 11, 12, 13, 17]. In this outbreak, the feed contamination with coumarin derivative was excluded, because even though feed ingredients were changed, the hemorrhagic disease did not cease and rodenticid belonging to coumarin derivative had not been used in this farm. Aflatoxin is considered as one of the antagonists to vitamin K [1], but mycoses which produced aflatoxin were not isolated from feed Nos. I and II, and their ingredients, and aflatoxin was not detected from cookie refuse with growing mycoses. Sulfamonomethoxine was added to feed No. II, but not to feed Nos. I and III. Although this drug is extensively used in human and veterinary medicine, there have been no reports of vitamin K deficiency induced by sulfamonomethoxine.

Vitamin K is obtained both from the diet and from synthetic activity of intestinal bacteria in man and animals [20]. The contribution of bacteria synthesis to vitamin K bioavailability becomes important only when dietary intake of vitamin is markedly decreased.

ed [14]. However, vitamin K deficiency does not occur due to ordinary diet, since vitamin K is widely distributed in plants and animal tissues [20]. The absorption rate of vitamin K-1 is influenced by many factors in the intestine [8, 9]. It is well known that long-chain unsaturated fatty acids in the intestine cause a marked decrease in the absorption rate of vitamin K-1 *in vitro* [8, 9]. Since the addition of beef tallow powder was begun from 2 months before hemorrhagic disease occurred, it was suspected that the fatty acids containing fish meal or tallow powder contributed to the decrease in vitamin K absorption. In addition, antibacterial drugs which were added to the feed may have decreased the synthetic vitamin by intestinal bacteria, causing the vitamin K deficiency. These problems remain to be solved in further study.

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要 約

子豚の出血性疾患に対するビタミンK剤による治療試験：佐々木栄英・北川 均・石原勝也・佐野 弘¹⁾・溝口 徹¹⁾・梶尾規一¹⁾（岐阜大学農学部獣医学科家畜内科学講座，¹⁾静岡県家畜衛生研究所）——1983年1月から6月にかけて1養豚場において，去勢創の止血不良および皮下の大量出血を主徴とする疾患が発生した。発病は体重20～35 kgの子豚に限られ，360頭中50頭が発症し，25頭が死亡した。7月初旬に外見上健康な14群114頭の仔豚について，血液凝固学的検査を行った結果，5群の21例に prothrombin time (PT) および activated partial thromboplastin time (APTT) の延長およびビタミン K（以下，K）依存因子（第Ⅱ，Ⅶ，Ⅸ，Ⅹ因子）の減少を認めた。このうちの8例に K₁ 剤（K₁ 群）を，6例に K₂ 剤（K₂ 群）をそれぞれ1回筋肉内に注射（3 mg/kg）し，投薬後の血液凝固学的所見の推移を観察した。両群とも大多数例において，PTは3時間後に，APTTは14～21時間後に，また，第Ⅶ，ⅨおよびⅩ因子は3～14時間後に，第Ⅱ因子は14～21時間後にほぼ正常値に回復した。K₃ 剤の粉末を飼料中に添加給与（25 ppm，4日間）した3例（K₃ 群）では，全例で給与開始21時間後に PT および APTT は正常値に回復していた。無処置4例（対照群）では，PT，APTT および血液凝固因子量に有意な変動はみられなかった。また，K₁，K₃ 群では30日後まで PT および APTT の異常は認められなかった。以上の成績から，本症はビタミンK欠乏症であることが確認され，発病要因として抗菌物質の給与と飼料組成が疑われた。