KOREAN J. FOOD & NUTR.
Vol. 10, No. 2, 166~173(1997)

# S-R variation and Antimicrobial Susceptibility of *Clostridium perfringens* Isolated from Necrotic Enteritis in Chickens, Enterotoxemia in Piglets and Enterotoxemia in Cattle

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# 닭의 괴사성 장염, 새끼돼지 및 소의 장독혈증에서 분리한 *Clostridium perfringens*의 S-R 변이와 항균요법제의 감수성

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## 요 약

1993년부터 1995년까지 약 3개년 동안 우리나라에서 사육하고 있는 가축의 유병율을 조사한 결과, 닭 16,200수 중에서 54수(0.3%)가 괴사성 장염에 이환되었으며 새끼돼지 620두 중에서 66두(10.6%)가 장독혈증에 이환되었고 소 65두 중에서 9두(13.8%)가 장독혈증에 이환되었다. *C. perfringens*의 분리율을 조사한 결과, 괴사성 장염에 이환되어있는 닭 54수 중 7수(13.0%)에서 본균이 분리되었으며 장독혈증에 이환되어 있는 새끼돼지 66두 중 14두(21.2%)에서 본균이 분리되었고 장독혈증에 이환되어 있는 소 9두 중 3두(33.3%)에서 본균이 분리되었다. Acriflavine을 이용하여 *C. perfringens*의 S-R변이를 조사한 결과, S-R변이는 mercuric chloride, nicotine, caffeine, cysteine, glucose 등의 순으로 나타났는데, 이중 mercuric chloride가 가장 감수성이 높았다. *C. perfringens*의 항균요법제에 대한 감수성은 cephalothin, penicillin, erythromycin, amikacin 등이 높은 감수성을 나타내었는데, S-R변이 후에는 감수성이 일반적으로 저하되는 경향이었다. 중화시험법을 이용하여 *C. perfringens*의 독소형을 분류한 결과, 총 24균주 중 22균주(91.7%)는 A형이었으며 2균주(8.3%)는 C형이었다.

Key words : *Clostridium perfringens*, prevalence, S-R variation, antimicrobial susceptibility

## INTRODUCTION

Ecology is the pattern of relations between organisms and their environment. In this instance, foodborne microbes are the organisms and food is the environment. Knowledge of this environment is the key to understanding food posioning and developing measures to control it.

*C. perfringens* type A food poisoning is widely reported[5,10,11,12,15,18,20,22,23]. The enterotoxin (ET) from *C. perfringens* type A is responsible for many cases of a mild type of food poisoning. It is produced in the intestinal tract during sporulation, after ingestion of $10^8$~$10^9$ cells[19]. An enterotoxin produced by *C. perfringens* is responsible for a common form of bacterial food poisoning[25]. Food poisoning by *C. perfringens* occurs after ingestion of food contaminated with large numbers of *C. perfringens* vegetative cells, and it is characterized by diarrhea and abdominal pain[25].

There is little information about the susceptibility of *C. perfringens* to growth-enhancing antibiotics and the eventual development of resistance has not been adequately monitored. Dutta and Devriese[8] studied on the susceptibility of strains isolated in 1979 from pigs, cattle and poultry with the growth-enhancing and therapeutical antibiotics available at that time[7].

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Human stool specimens frequently contain *C. perfringens* and some foodstuffs contain a large amount of this organism[14,26]. Since such organisms found in human and animal feces are mostly non-enterotoxigenic, it is necessary to distinguish the enterotoxigenic organisms from the non-enterotoxigenic ones to identify the true agents of food poisoning.

This study describes the prevalence, isolation rate, neutralization tests, susceptibility tests and S-R variations of *C. perfringens* are isolated from chickens with necrotic enteritis, piglets with enterotoxemia, and cattle with enterotoxemia using cysteine hydrochloride, glucose, caffeine sodium benzoate, mercuric chloride and nicotine.

This study hopes to help increase the productivity of animal farms and promote the nation's health by preventing food poisoning due to *C. perfringens* of necrotic enteritis of chickens, enterotoxemic piglets and enterotoxemic cattle.

## MATERIALS AND METHODS

### 1. Bacterial strains

A total of 24 samples in this study were examined for causative agents isolated from the 7 chickens with necrotic enteritis, as well as 14 piglets and 3 cattle with enterotoxemia from 1993 to 1995 in Korea. The control group of *C. perfringens* NCTC 8791 (Hobbs serotype 1; $ET^+$), NCTC 8238 (Hobbs serotype 2; $ET^+$), NCTC 8239 (Hobbs serotype 3; $ET^+$), and NCTC 8235 (Hobbs serotype 8; $ET^+$) was supplied by Teizo Tsukamoto, Osaka Prefectural Institute of Public Health, Japan. Also, *C. perfringens* ATCC 3629 ($ET^-$) and *C. perfringens* type A, B, C, D, and E were supplied by Veterinary Research Institute, Rural Development Administration, Korea.

### 2. Test for variation by chemical compounds

Medium A (agar 20g, beef extract 5g, bactopeptone 10g, NaCl 5g, d.d.w. 1ℓ) and BHIA medium (brain-heart-infusion agar$^+$ 1% thioglycollate, DIFCO) were prepared with 0.1 percent, 0.25 percent, 0.5 percent, and 1.0 percent chemical compounds (cysteine hydrochloride, Sigma; dextrose, Junsei; caffeine sodium benzoate, Sigma; mercuric chloride, Junsei; nicotine, Fluka) respectively.

Two hours incubation in the water bath might make it possible to discern strong positive reactions, but this would not be enough time to detect moderate and weak reactions. Therefore, overnight setting at room temperature after 2 hours incubation at 37℃ was necessary to get definite results, from strong positives to weak ones. If the time for observation was longer than the above, it would not bring about any particular change in the results.

A slide agglutination test was performed using 0.2% acriflavine solution (Sigma) and normal saline (control) for confirmation of the test organisms, normal S-forms, intermediate variant SR-forms, and variant R-forms[1,2].

### 3. Susceptibility test

The antimicrobial susceptibility test performed was a standardized disc susceptibility test[9,16]. Antimicrobias were chosen based on activity against *C. perfringens* isolated from necrotic enteritis of chickens, enterotoxemia of piglets, and cattle, and they included amikacin (AN, BBL), cephalothin (CF, BBL), erythromycin (EM, BBL), gentamycin (GM, BBL), kanamycin (KM, BBL), nalidixic acid (NA, BBL), penicillin (PP, BBL) and tetracycline (TC, BBL).

### 4. Neutralization test

Two mice were injected intraperitoneally with 0.5ml of the supernatant fluid of washings or serum and observed for three days. The remainder of the serum or washings were held in the refrigerator. If the mice died during the three-day period, the toxin was identified by neutralization with type-specific antitoxin[3,21]. The antitoxins were used were from the Veterinary Research Institute of the Korean Rural Development Administration.

## RESULTS

The prevalence was observed in 54 cases of necrotic enteritis (0.3%) among 16,200 chickens, 66 cases of enterotoxemia (10.6%) among 620 piglets and 9 cases of enterotoxemia (13.8%) among 65 cattle. *C. perfringens* was isolated from 7 cases (13.0%) out of the 54 necrotic enteritis of chickens, 14 cases (21.2%) out of the 66 enterotoxemic piglets and 3 cases (33.3%) out of the 9 enterotoxemic cattle in Table 1. Table 2 shows the results of *C. perfringens* agglutination in the presence of various concentration of cysteine, glucose, caffeine, mercuric chloride and nicotine. R and SR variants by the 0.2% acriflavine solution were typically agglutinated by mercuric chloride, nicotine, caffeine, cysteine, and glucose, in that order. Mercuric chloride ions were most sensitive in differentiating the variation degrees of *C. perfringens*.

The selection of pure S-forms from doubtful S-forms is possible only through the tube agglutination technique with mercuric chloride ions, even through the slide agglutination test with acriflavine may indicate rough difference between the S and SR. Besides, acriflavine reactions on slide agglutination tests are influenced by the high concentrations of chemical compounds to be stronger than low concentrations, and stronger BHIA medium (brain-heart-infusion) than medium A (agar 20g, beef extract 5g, bactopeptone 10g, NaCl 5g, d.d.w. 1 ℓ) generally. The titers of this reaction were almost parallel with the degree of acriflavine reaction on the slide.

It was observed that *C. perfringens* was susceptible to both CF (95.8%) and PP (95.8%), as well as EM (75.0%), and AN (71.0%) as shown in Table 3. In after S-R variation by cysteine, glucose, caffeine, mercuric chloride and nicotine, the antimicrobial susceptibility of *C. perfringens* appeared to decrease somewhat (Table 3).

Table 4 shows the *C. perfringens* toxins as categorized by the neutralization test. Both the 7 chickens with necrotic enteritis and 3 enterotoxemic cattle were types-A enterotoxin (100.0%). The piglets were 12 types-A toxins (85.7%) and 2 types-C ones (14.3%) from the 14 strains infecting them.

Overall, 22 types-A toxins (91.7%) and 2 types-C toxins (8.3%) were isolated from the 24 strains studied.

## DISCUSSION

*C. perfringens* is widespread in the soil and sewage, and is commonly found in the intestinal tract of human and various animals. It is an opportunistic organism and causes great economic losses by death due to necrotic enteritis of chickens, enterotoxemia of piglets and enterotoxemia of cattle[4,13,17]. Type-A *C. perfringens* food poisoning

**Table 1. Outbreaks patterns of necrotic enteritis in chickens, and enterotoxemia in piglets and cattle**

| Cases | Number of animals | Number of cases (%)* | Number of isolated *C. perfringens strains*(%) |
|---|---|---|---|
| Necrotic enteritis of chickens | 16,200 | 54( 0.3) | 7(13.0) |
| Enterotoxemic piglets | 620 | 66(10.6) | 14(21.2) |
| Enterotoxemic cattle | 65 | 9(13.8) | 3(33.3) |
| Total | 16,885 | 129( 0.8) | 24(18.6) |

$$\text{* Prevalence} = \frac{\text{Total number of cases, new and old, existing at a point in time}}{\text{Total population at that point in time}} \times 100$$

**Table 2. Influence on S-R variation of *Clostridium perfringens* by chemical compounds**

| Cases(strains) | Medium | Chemical compounds | Cysteine | | | | | | | | Glucose | | | | | | | | Caffeine | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.1% | | 0.25% | | 0.5% | | 1.0% | | 0.1% | | 0.25% | | 0.5% | | 1.0% | | 0.1% | | 0.25% | | 0.5% | | 1.0% | |
| | | | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac |
| Control (NCTC 8239, ET+) | Medium A | S | - | | - | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | | SR | | + | | + | | | | | | | | | | | | | | | | | | | | |
| | | R | | | | | | | | | | | | | | | | | | | | | | | | |
| | BHIA | S | | | - | | - | - | - | - | - | | - | | - | | - | | - | - | | - | | - | | |
| | | SR | + | ± | | + | | | | | | + | | + | | + | | + | | + | | + | | ± | | |
| | | R | | | | | | | | | | | | | | | | | | | | | | | | |
| Necrotic enteritis of chickens (n=3 strains) | Medium A | S | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | | SR | | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| | | R | | | | | | | | | | | | | | | | | | | | | | | | |
| | BHIA | S | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 |
| | | SR | | | | | | | | | | | | | | | | | | | | | | 1 | | 1 |
| | | R | | | | | | | | | | | | | | | | | | | | | | | | |
| Enterotoxemic piglets (n=3 strains) | Medium A | S | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | | SR | | | | | | | | | | | | | | | | | | | | | | | | |
| | | R | | | | | | | | | | | | | | | | | | | | | | | | |
| | BHIA | S | 2 | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 |
| | | SR | 1 | 1 | 1 | | 1 | | | | | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | | 1 |
| | | R | | | | | | | | | | | | | | | | | | | | | | | | |
| Enterotoxemic cattle (n=3 strains) | Medium A | S | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 |
| | | SR | 1 | | 1 | | | | | | | | | | | | | | | | | | | | 1 | |
| | | R | | | | | | | | | 1 | | | | | | | | | | | | | | | |
| | BHIA | S | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 |
| | | SR | 1 | 1 | | | | | | | | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | | |
| | | R | | | | | | | | | | | | | | | | | | | | | | | | 1 |

| Cases(strains) | Medium | Chemical compounds | Mercuric chloride | | | | | | | | Nicotine | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.1% | | 0.25% | | 0.5% | | 1.0% | | 0.1% | | 0.25% | | 0.5% | | 1.0% | |
| | | | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac | Sa | Ac |
| Control (NCTC 8239, ET+) | Medium A | S | | - | | - | | - | | - | | | | | | | | |
| | | SR | | | | | | | | | | | | | | | | |
| | | R | | ++ | | ++ | | +++ | | +++ | | | | | | | | |
| | BHIA | S | - | | | | | | | | - | | - | | - | | - | |
| | | SR | | | + | | + | | | | | + | | + | | + | | + |
| | | R | | ++ | | ++ | | +++ | ++ | +++ | | | | | | | | |
| Necrotic enteritis of chickens (n=3 strains) | Medium A | S | | 3 | | | | | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |
| | | SR | 1 | | 1 | 1 | | 1 | | | | | | | | | | |
| | | R | 2 | | 2 | 2 | 3 | 2 | 3 | | | | | | | | | |
| | BHIA | S | 3 | 1 | 3 | | 2 | | 2 | | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 |
| | | SR | | | | 1 | 1 | | 1 | | | 1 | | 1 | | 1 | | 1 |
| | | R | | 2 | | 2 | | 3 | | 3 | | | | | | | | |
| Enterotoxemic piglets (n=3 strains) | Medium A | S | 3 | 2 | 3 | 1 | 3 | | 3 | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | | SR | | 1 | | | | | | | | | | | | | | |
| | | R | | | | 2 | | 3 | | 3 | | | | | | | | |
| | BHIA | S | 2 | 1 | 2 | 1 | 2 | | 2 | | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 |
| | | SR | 1 | | 1 | | 1 | | | | | 1 | | 1 | | 1 | | 1 |
| | | R | 2 | | 2 | | 3 | 1 | 3 | | | | | | | | | |
| Enterotoxemic cattle (n=3 strains) | Medium A | S | | 3 | | 3 | | 1 | | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | | SR | | 2 | | 1 | | | | | | | | | | | | |
| | | R | | 1 | | 2 | 2 | 3 | 2 | 3 | | | | | | | | |
| | BHIA | S | 2 | 1 | | | | | | | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 |
| | | SR | 1 | 1 | 2 | 1 | 1 | | 1 | | | 1 | | 1 | | 1 | | |
| | | R | 1 | 1 | 2 | 2 | 3 | 2 | 3 | | | | | | | | | 1 |

Remarks : 1) Sa : 0.9% saline solution, and Ac : 0.2% acriflavine solution.

2) S(−) : normal, SR(±, +) : intermediate variant, and R(++, +++) : variant.

3) n : Strain numbers of isolated from cases.

**Table 3. Antimicrobial susceptibility of *Clostridium perfringens* S-R variations by chemical compounds**

| Antimicrobias | No. of susceptible strains before variation(n=24), (%) | No. of susceptible strains after variation(%) | | | | |
|---|---|---|---|---|---|---|
| | | Cysteine (n=10) | Glucose (n=8) | Caffeine (n=11) | Mercuric chloride (n=91) | Nicotine (n=12) |
| Amikacin(AN) | 17 (71.0) | 7 (70.0) | 6 ( 75.0) | 7 (63.6) | 62 (68.1) | 8 (66.7) |
| Cephalothin(CF) | 23 (95.8) | 9 (90.0) | 8 (100.0) | 10 (90.9) | 86 (94.5) | 11 (91.7) |
| Erythromycin(EM) | 18 (75.0) | 7 ( 70.0) | 5 (62.5) | 8 (72.7) | 68 (74.7) | 9 (75.0) |
| Gentamycin(GM) | 1 ( 4.2) | NT | NT | 1 ( 9.1) | 3 ( 3.3) | NT |
| Kanamycin(KM) | 2 ( 8.3) | 1 (10.0) | 1 ( 12.5) | 1 ( 9.2) | 6 ( 6.6) | 1 ( 8.3) |
| Nalidixic acid(NA) | 13 (54.2) | 5 (50.0) | 5 ( 62.5) | 5 (45.5) | 48 (52.7) | 7 (58.3) |
| Penicillin(PP) | 23 (95.8) | 9 (90.0) | 8 (100.0) | 10 (90.9) | 87 (95.6) | 11 (91.7) |
| Tetracycline(TE) | 2 ( 8.3) | 1 (10.0) | NT | 1 ( 9.1) | 7 ( 7.7) | 1 ( 8.3) |

Remarks : NT; not detected.
n : Strain numbers of isolated from cases.

**Table 4. Toxin types of *Clostridium perfringens* by the neutralization test**

| Cases (Strains) / Toxin types | No. of strains isolated from necrotic enteritis of chickens(%) | No. of strains isolated from enterotoxemic piglets(%) | No. of strains isolated from enterotoxemic cattle(%) | Total(%) |
|---|---|---|---|---|
| A | 7(100.0) | 12(85.7) | 3(100.0) | 22( 91.7) |
| B | | | | |
| C | | 2(14.3) | | 2( 8.3) |
| D | | | | |
| E | | | | |
| Total | 7(100.0) | 14(100.0) | 3(100.0) | 24(100.0) |

is also widely reported[5,10,11,18]. The objective of this study was to define influence of antimicrobial susceptibility in after S-R variation of *C. perfringens* isolated from necrotic enteritis of chickens, enterotoxemic piglets and enterotoxemic cattle by cysteine, glucose, caffeine, mercuric chloride and nicotine, and then classification of toxin type by neutralization test. *C. perfringens* was isolated from the intestine of 17.8% of the chickens in one study[13]. 300 diarrheal calves out of the 1,700 examined were tested for the isolation of *C. perfringens*, and 95 strains (31.7%) were identified among the 300 samples[4].

The prevalence was observed in 54 cases of necrotic enteritis (0.3%) among 16,200 chickens, 66 cases of enterotoxemia (10.6%) among 620 piglets and 9 cases of enterotoxemia (13.8%) among 65 cattle. *C. perfringens* was isolated from 7 cases (13.0%) out of the 54 necrotic enteritis of chickens, 14 cases (21.2%) out of the 66 enterotoxemic piglets and 3 cases (33.3%) out of the 9 enterotoxemic cattle in this study. This reason was due to differences in region, year, and animal disease studied.

Ahn[1,2] reported that acriflavine reaction on slide agglutination test was influenced by the kinds of culture medium. The titers of this reaction were almost parallel with the degree of acriflavine reaction on slide, and Pb ions were most sensitive in differentiating the variation degree of *Shigella flexneri*. According to Youmans[27], many of the properties of small-colony variants can be accounted for by an over-all lowering of the metabolic rate. Clowes and Rowley[6] suggested that this explanation might account for the isolation of many of these variants from toxic environments, and proposed that the inhibitor (antibiotics, metal ions, etc.) permeates the variants at a lower rate, thus permitting their survival for a longer time than the corresponding, normally metabolizing, organisms. They have further suggested that the general lowering of the metabolic rate might be due to a decrease in cell wall permeability.

In general, R and SR variants by acriflavine were agglutinated by mercuric chloride, nicotine, caffeine, cysteine and glucose in that order. Mercuric chloride ions were most sensitive in differentiating the variation degree of *C. perfringens*, and the acriflavine reaction on slide agglutination test was influenced by the kinds of culture medium. The titers of this reaction were almost parallel with degree of acriflavine reaction on slide in this results. It is in agreement with findings of Ahn[1,2].

The antimicrobial susceptibility of the 30 isolates against 12 drugs demonstrated that 27 or more of 30 isolates(>90%) were susceptible to amoxicillin, AP, CF(Kim, 1994)[13]. In antimicrobial drug susceptibility tests on isolated strains, all strains were found to be highly susceptible to CF, CM and PP[4]. 12 isolates of *C. perfringens* were highly sensitive to AP, ENR, CF and PP[17]. *C. perfringens* was observed to be very susceptible to CF (95.8%) and PP (95.8%), as well as EM (75.0%), and AN (71.0%). After variation by cysteine, glucose, caffeine, mercuric chloride and nicotine, its susceptibilities appeared to decrease somewhat in this study. These findings are similar to those of Kim[13], Cho *et al.*[4] and Park *et al.*[17].

The antimicrobial susceptibility was decreased current due to antimicrobias misused and abused, generally. Although antimicrobias and growth hormons can be misused when overfed to young animals to promote growth, the also to treat and prevent animal disease. Their misuse or abuse is thus an important issue for human health.

There are five *C. perfringens* toxin types: A, B, C, D and E, but type A has been known as the main culprit in the food poisoning of humans[19]. Neutralization tests in mice to determine the toxin types of the 30 isolates revealed that 26 isolates could be classified as type A, while 2 isolates were confirmed as type C. The other 2 isolates could not be classified because of the absence of toxin produced in the cooked meat medium[13].

The toxins from isolated 40 strains were almost totally neutralized by *C. perfringens* type-A antitoxin but not by type B, C, D and E antitoxins[4]. The toxin types of isolated strains were determined to be 22 A-types (91.7%) and 2 C-types (8.3%) among the 24 strains isolated in this study. These results are similar with Kim[13] and Cho[4]. Especially noticeable is the high isolation rate for strains implicated in food poisoning outbreaks. From the stand point of food hygiene, the high rate of isolation for various A-type toxin-producing *C. perfringens* from domestic animals is a great problem.

This study is to suggest that treat and prevent animal disease, to help reduce the occurrance of food-borne disease and increase the productivity of animal farming.

## CONCLUSION

The prevalence was observed in 54 cases of necrotic enteritis (0.3%) among 16,200 chickens, 66 cases of enterotoxemia among 620 piglets (10.6%) and 9 cases of enterotoxemia(13.8%) among 65 cattle. *C. perfringens* was isolated from 7 cases (13.0%) out of the 54 necrotic enteritis of chic-

kens, 14 cases (21.2%) out of the 66 enterotoxemic piglets and 3 cases (33.3%) out of the 9 enterotoxemic cattle. Varied R and intermediate varied SR of *C. perfringens* derived using acriflavine were agglutinated by mercuric chloride, nicotine, caffeine, cysteine and glucose, in that order. Especially, mercuric chloride ions is most sensitive in differentiating the variation degree of *C. perfringens*. *C. perfringens* was observed to be highly susceptible to cephalothin, penicillin, erythromycin and amikacin, but in after, normal S-varied R of *C. perfringens* by cysteine, glucose, caffeine, mercuric chloride and nicotine, its susceptibility appeared to decrease somewhat.

The toxin types were categorized from the 22 A-type strains (91.7%), and 2 C-types (8.3%) among 24 strains.

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(1997년 4월 24일 접수)