Virulence and Immunogenicity of Plasmid-cured Salmonella serovar Enteritidis AL1192 against Cattle

Masayuki NAKAMURA, Shizuo SATO, Shoko SUZUKI, Yutaka TAMURA, Osamu ITOH, Tetsuo KOEDA, and Sumio IKEDA

National Veterinary Assay Laboratory, Kokubunji, Tokyo 185, and 1 Zen-Noh Institute of Animal Health, Sakura 285

(Received 30 October 1987/Accepted 17 February 1988)

ABSTRACT. The virulence of Salmonella choleraesuis subspecies choleraesuis serovar enteritidis (S. enteritidis) AL1192, a plasmid-cured derivative from virulent S. enteritidis AL1190, was tested. Except for fever no serious adverse clinical signs were observed in the 4 cattle inoculated subcutaneously or intravenously with S. enteritidis AL1192, although two of them showed positive fecal cultures. Next, it was also investigated that this strain was capable of protecting 7-month-old cattle against subsequent intramuscular challenge with Salmonella choleraesuis subspecies choleraesuis serovar typhimurium (S. typhimurium) L-535 of which O antigenic structure has common component with S. enteritidis AL1192. S. typhimurium L-535 used as a challenge strain was not fully virulent to the cattle in this experiment, even though it was isolated from mesenteric lymph node of a dead calf with salmonellosis and fully virulent to ddY mice. This prevented us from evaluating S. enteritidis AL1192 as a vaccine. This observation suggests that symptoms in 7-month-old cattle are not invariably observed although young calves are very susceptible to Salmonella. Moreover, S. enteritidis AL1192 could not prevent even infection with the challenge strain which was not so virulent for the aged cattle, judging from the development of specific H antibody against challenge strain.—KEY WORDS: cattle, immunogenicity, plasmid-cured S. enteritidis, S. typhimurium, virulence.

In Japan, there have been many reports concerning salmonellosis in cattle [12, 17, 22, 23] and among them, salmonellosis due to the infection with Salmonella choleraesuis subspecies choleraesuis serovar typhimurium (S. typhimurium), serovar enteritidis (S. enteritidis), serovar dublin (S. dublin), and serovar naestved (S. naestved) were dominant in cattle [16]. Although it has been required to develop vaccines for the prevention of this disease, there has been no vaccine in Japan.

In foreign countries, many kinds of vaccines had been developed. Although few of various killed bacterial vaccines protected calves [1, 15], live vaccines, for example, rough mutant of S. dublin, protected challenge exposure of virulent strains [13, 18].

Recently, modified live strains of smooth type S. typhimurium and S. dublin that were genetically altered [to be deficient in the biosynthesis of aromatic compound (aro−)] have been reported as an effective live vaccine and moreover a cross protection between S. typhimurium and S. dublin was also reported [4, 5, 19, 20].

On the other hand, there have been several reports describing that virulence was associated with the presence of a plasmid in S. typhimurium, S. enteritidis, and S. dublin [6, 9, 24]. Because curing the plasmid of these serovars resulted in a decrease in virulence for mice, it is possible to assume that plasmid-cured derivatives from these strains may be effective as live vaccines.

Accordingly, we tested the ability of S. enteritidis AL1192, a plasmid-cured derivative of virulent S. enteritidis AL1190 iso-
lated from spleen of a dairy cow, to protect mice against subsequent challenge with AL1190 and several wild type strains of S. dublin, S. naestved, or S. typhimurium, of which O antigen structure has a common component. It was demonstrated that AL1192 given subcutaneously provided significant protection in mice against oral, subcutaneous or intraperitoneal challenge by virulent wild-type strains of not only S. enteritidis but also S. dublin, S. naestved and S. typhimurium [11].

This report describes the virulence of plasmid-cured S. enteritidis AL1192 against cattle and the trial challenge test with S. typhimurium of which O antigenic structure (O-type 1, 4, 5, 12) has a common component with S. enteritidis AL1192 (O-type 1, 9, 12).

MATERIALS AND METHODS

Bacterial strains: S. enteritidis AL1192 [11] was used for the virulence test and S. typhimurium L-535 was used as a challenge strain, which was isolated from mesenteric lymph node of a dead calf [10] and of which LD50 was less than 10^1.9 for intraperitoneal challenge and 10^3.5 for subcutaneous challenge against mice. Six-hr shaking cultures in Heart Infusion Broth (HIB, Difco, Detroit, Mich.) of these strains were mixed with 10% skim milk and stocked at −70°C before use.

Cattle: Eleven young cattle of around 7–8 months old were used. Nine of them were male Holstein cattle and two Japanese black cattle. They were raised in individual pens and fed on hay cube and concentrated feed ad libitum. All cattle had negative fecal culture for Salmonella and negative serum antibody response for Salmonella.

Virulence test against cattle: Two cattle were inoculated with S. enteritidis AL1192 intravenously, two cattle subcutaneously, and another two cattle were kept as non-inoculated control. S. enteritidis AL1192 for the inoculation was prepared from 6-hr shaking culture in HIB. After centrifugation, the culture was suspended in phosphate buffered saline (pH 7.2). The number of viable bacteria was determined by plate colony count, and 1.8×10^9 and 1.8×10^10 bacteria were inoculated intravenously and subcutaneously, respectively.

Challenge exposure with S. typhimurium L-535: Two Holstein cattle and one Japanese black cattle were inoculated with 1.6×10^9 bacteria of AL1192 intramuscularly and one Holstein and one Japanese black cattle were kept as non-inoculated control. After 14 days of inoculation, these five cattle were challenged with 2.5×10^9 bacteria of S. typhimurium L-535 subcutaneously.

Daily monitoring: After inoculation, cattle were observed daily. Rectal temperature, appetite, attitude and character of feces were recorded.

Bacteriology: Blood samples were taken on 1st, 2nd, 3rd, 6th, 9th and 14th days after inoculation in the virulence test and on 1st, 2nd, 4th, 8th, 11th and 16th days after challenge with S. typhimurium. Five ml of blood was inoculated into HIB for enrichment, cultured at 37°C overnight and subcultured on deoxycholate hydrogen sulfide lactose (DHL, Eiken, Tokyo, Japan) agar plates. Feces were collected daily from the rectum and one gram of each feces was inoculated into 10 ml of Selenite Cystine Broth (SCB, Difco, Detroit, Mich.) and Hajna Tetrathionate Broth (HTB, Eiken, Tokyo, Japan) and DHL agar plates [8]. After 14 days of the virulence test and 16 days of challenge exposure, cattle were sacrificed and duodenum contents and lymph node, ileal contents and lymph node, colon contents and lymph node, cecum contents and lymph node, rumen contents, spleen, liver,
kidney, lung, bile were examined for *Salmonella*. One gram of contents of alimentary tract was inoculated into SCB and HTB as described above and subcultured on BG and DHL agar plates. Tissue samples and bile were directly cultured on DHL agar plates. From all of DHL and BG plates, suspected colonies were purified and identified as *Salmonella* [8]. Slide agglutination tests using O antisera prepared against groups O4 and O9 were carried out for identification of *S. typhimurium* and *S. enteritidis*, respectively.

**Serology:*** Tube agglutination tests against O and H antigen of *S. typhimurium* and *S. enteritidis* were carried out.

**Pathology:** At the time of sacrifice, duodenum and lymph node, ileum and lymph node, colon and lymph node, cecum and lymph node, rumen, spleen, liver, kidney and lung were fixed with 10% formalin. After the preparation of paraffin sections, these were stained with Haematoxylin and Eosin.

**RESULTS**

As shown in Table 1, except for fever no serious adverse clinical sign was observed in the cattle inoculated with *S. enteritidis* AL1192. Although transient increases in rectal temperature which returned to basic line within 4 days post inoculation were observed, this cattle did not develop diarrhea, and anorexia was seen only on the first day in two cattle inoculated subcutaneously. However in the bacteriological examination (Table 2), three of the 4 inoculated cattle developed positive fecal cultures for six to eight days during the period of observation and one of the 4 cattle developed positive blood culture. Three of the 4 cattle were positive for the isolation of *S. enteritidis* from several tissues sampled. Control cattle developed no positive reactions.

O and H agglutination titers are shown in Table 3. *S. enteritidis* AL1192 induced considerable rises in specific antibodies and no antibodies were developed in control cattle.

In the pathological examination, superficial cervical lymph nodes of two cattle inoculated subcutaneously swelled. However, no purulent inflammation could be observed in both lymph nodes. Other tissues sampled from cattle inoculated subcutaneously and intravenously did not show significant lesions compared with those from control cattle.

In the cattle inoculated with AL1192

---

**Table 1. Clinical signs of 7-month-old cattle inoculated with *Salmonella enteritidis* AL1192**

<table>
<thead>
<tr>
<th>Cattle No.</th>
<th>Route Dose</th>
<th>No. of days with high temperature&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of days with diarrhea</th>
<th>No. of days with anorexia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s.c. 1.8\times 10^{10}</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>s.c. 1.8\times 10^{10}</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>i.v. 1.8\times 10^{8}</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>i.v. 1.8\times 10^{8}</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> More than 40°C.

**Table 2. Bacteriologic examination of 7-month-old cattle inoculated with *Salmonella enteritidis* AL1192**

<table>
<thead>
<tr>
<th>Cattle No.</th>
<th>Rectal content</th>
<th>Blood</th>
<th>Tissue&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8/14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6</td>
<td>Ki, SCL, CeL, CeC, CoC</td>
</tr>
<tr>
<td>2</td>
<td>0/14</td>
<td>0/6</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6/14</td>
<td>1/6</td>
<td>Lu, Ki, CeL, CoL</td>
</tr>
<tr>
<td>4</td>
<td>6/14</td>
<td>0/6</td>
<td>Lu, CeL, CoL</td>
</tr>
<tr>
<td>5</td>
<td>0/14</td>
<td>0/6</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>0/14</td>
<td>0/6</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> No. of days with positive sample/No. of days examined.

<sup>b</sup> Ki: Kidney, SCL: Superficial cervical lymph, CeL: Cecum lymph, CeC: Cecum content, CoC: Colon content, Lu: Lung, CoL: Colon lymph.
intramuscularly, serious adverse clinical signs except for fever were not observed after challenge although two Holstein cattle developed positive fecal cultures for 8 and 11 days during the period of 14 days, respectively (Data not shown). After challenge with S. typhimurium L-535, no serious adverse clinical signs were observed even in control cattle. This suggests that challenge strain was not fully virulent for the cattle employed (Table 4).

In the bacteriological examination of feces, one Holstein cattle developed fecal cultures of S. enteritidis continuously for 12 days of post-challenge, but not the other cattle and Japanese black cattle (Table 5). Challenge strain S. typhimurium L-535 could be recovered from feces of one cattle challenged, but not from another two cattle. On the other hand, S. typhimurium L-535 could not be isolated from feces of control cattle.

In the bacteriological examination of tissue samples, S. enteritidis was mainly recovered from contents of alimentary tract and S. typhimurium was isolated from lymph node.

On the contrary, recovery of S. typhimurium was very rare and no S. typhimurium was detected from control Japanese black cattle.

Serum agglutination titers against S. typhimurium were shown in Table 6. Until 4 days after challenge, O agglutination titer appeared to indicate antibody against S. enteritidis AL1192 because H specific antibody against S. typhimurium could not be

Table 3. Serum agglutination titers in cattle inoculated with Salmonella enteritidis AL1192

<table>
<thead>
<tr>
<th>Cattle No.</th>
<th>Antibody titer (Days after inoculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 6 9 14</td>
</tr>
<tr>
<td>1</td>
<td>- - - x128 x128 x256 (-) (-) (-) (x1,600) (x3,200) (x800)</td>
</tr>
<tr>
<td>2</td>
<td>- - - x128 x128 x128 (-) (-) (-) (x1,600) (x12,800) (x6,400)</td>
</tr>
<tr>
<td>3</td>
<td>- - - x256 x256 x256 (-) (-) (-) (x800) (x800) (x1,600)</td>
</tr>
<tr>
<td>4</td>
<td>- - - x64 x128 x1,024 (-) (-) (-) (-) (x400)</td>
</tr>
<tr>
<td>5</td>
<td>- - - - - - - (-) (-) (-) (-) (-)</td>
</tr>
<tr>
<td>6</td>
<td>(-) (-) (-) (-) (-) (-) (-)</td>
</tr>
</tbody>
</table>

a) O-antibody titer.
b) H-antibody titer.

Table 4. Clinical signs of vaccinated cattle after challenge exposure with Salmonella typhimurium L-535

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Vaccination(^a)</th>
<th>Challenge(^b)</th>
<th>Clinical sign (16 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Route</td>
<td>Dose</td>
<td>Route</td>
</tr>
<tr>
<td>7</td>
<td>i.m.</td>
<td>1.6x10(^9)</td>
<td>s.c.</td>
</tr>
<tr>
<td>8(^c)</td>
<td>i.m.</td>
<td>1.6x10(^9)</td>
<td>s.c.</td>
</tr>
<tr>
<td>9</td>
<td>i.m.</td>
<td>1.6x10(^9)</td>
<td>s.c.</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>s.c.</td>
</tr>
<tr>
<td>11(^c)</td>
<td>-</td>
<td>-</td>
<td>s.c.</td>
</tr>
</tbody>
</table>

a) Salmonella enteritidis AL1192.
b) Salmonella typhimurium L-535.
c) Japanese black cattle.
Table 5. Recovery of salmonellae from vaccinated cattle after challenge exposure with Salmonella typhimurium

<table>
<thead>
<tr>
<th>Cattle No.</th>
<th>Recovery from rectal content</th>
<th>Recovery from tissue&lt;sup&gt;a)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. typhimurium</td>
<td>S. enteritidis</td>
</tr>
<tr>
<td>7</td>
<td><strong>2/16&lt;sup&gt;a&lt;/sup&gt;</strong></td>
<td>0/16</td>
</tr>
<tr>
<td>8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>9</td>
<td>0/16</td>
<td>12/16</td>
</tr>
<tr>
<td>10</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/16</td>
<td>0/16</td>
</tr>
</tbody>
</table>

<sup>a</sup>) No. of days with positive sample/No. of days examined.
<sup>b</sup>) RuC: Rumen Content, DuC: Duodenum Content, IIC: Ileum Content, CeC: Cecum content, CoC: Colon content, SCL: Superficial cervical lymph, CoL: Colon lymph, CeL: Cecum lymph, DuL: Duodenum lymph.
<sup>c</sup>) Japanese black cattle.

Table 6. Serum agglutination titers against Salmonella typhimurium in vaccinated cattle after challenge exposure

<table>
<thead>
<tr>
<th>Cattle No.</th>
<th>Antibody titer (Days after inoculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>$\times 128^a$</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
</tr>
<tr>
<td>8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>$\times 32$</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
</tr>
<tr>
<td>9</td>
<td>$\times 128$</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
</tr>
<tr>
<td>11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
</tr>
</tbody>
</table>

<sup>a</sup>) O-antibody titer.
<sup>b</sup>) H-antibody titer.
<sup>c</sup>) Japanese black cattle.

detected. On the 8th day post challenge, H specific antibody against S. typhimurium developed, and O and H specific antibodies also appeared in control cattle.

In the pathological examination, inflammatory reaction was observed at the injection sites of three cattle inoculated intramuscularly. There were lots of cell debris in the center of the lesion and inflammatory cells gathered outside of this center. This inflammatory cells which consisted of neutrophiles, plasma cells and lymphocytes were surrounded by fibroblasts. In two cattle challenged and one control cattle, S. typhimurium could be detected, and superficial cervical lymph nodes adjacent to challenge site were swelled. Purulent inflammation, however, could
VIRULENCE OF S. ENTERITIDIS AL1192

not be observed. No significant lesions could be observed in other tissues.

DISCUSSION

The 4 cattle inoculated with S. enteritidis AL1192 subcutaneously or intravenously showed transient increase in rectal temperatures but did not develop diarrhea and depressed appetite. So far as the gram-negative living bacteria are used as a vaccine, transient increase of body temperature is inevitable, because lipopolysaccharide which is intact in this strain is a pyrogen and elicits both local and systemic inflammatory reactions that cause fever [21].

In the bacteriological examination, three out of 4 cattle inoculated with S. enteritidis AL1192 in the virulence test and three cattle in the challenge test had positive samples. In other words, S. enteritidis AL1192 was recovered from contents of alimentary tract, tissues and lymph nodes after 14 days of inoculation in the virulence test, but the strain was recovered mainly from contents of alimentary tract after 30 days of the inoculation in the challenge test. Moreover, no significant lesions could be observed in alimentary mucous membranes by the microscopic findings. These findings suggested that S. enteritidis AL1192 finally colonized and persisted in the alimentary tract and these cattle were considered to be shedder. Such a kind of strain as this should not be allowed as a live vaccine because reintroduction of virulence-associated plasmid into excreted AL1192 might happen in the field, for example, through mobilization by conjugative R plasmids. We could succeed in transferring this virulence-associated plasmid from AL1190 to Escherichia coli ML1410 and from E. coli ML1410 to AL1192 by mobilization with R plasmid RA1 (Data not shown). Although it was considered that virulence against cattle was reduced by curing the virulence-associated plasmid, the loss of colonizing ability in the gut might not be associated with curing.

S. typhimurium L-535 used as a challenge strain was isolated from mesenteric lymph node of a dead calf in 1981 in Japan [10]. To check the virulence of this strain as a challenge strain before use, LD50 was determined for ddY mice and stocked at −70°C. Judging from LD50 of this strain described above, this strain was virulent against mice. However, cattle inoculated with this strain did not reveal any severe symptoms. Although it is well-known that young calves are very susceptible to Salmonella, symptoms in adults are not invariably observed and symptomless cows excrete salmonellae [14]. It might be pointed out that S. typhimurium L-535 used in this test was not fully virulent to 7-month-old cattle to be tested although this strain was fully virulent to young calves [10].

We carried out this experiment with reference to the results by Smith et al. [19, 20] demonstrating cross protection between S. typhimurium and S. dublin using aromatic-dependent derivatives as a live vaccine. However, in order to evaluate precisely the ability of this strain as a live vaccine, first of all we should have carried out the challenge test using S. enteritidis AL1190 as a challenge strain which was virulent and the parent strain of S. enteritidis AL1192. In our experiment, evaluation of the efficacy of S. enteritidis AL1192 as a live vaccine has not been succeeded. Moreover, this strain could not prevent even infection by the challenge strain which was not fully virulent for the aged cattle, judging from the fact that specific H antibody against this strain developed. It was also suggested that the results obtained in mice [11] were not parallel to those in cattle in this test.

In case of evaluating one of the abilities of this AL1192 to protect against challenge exposure by the production of humoral antibody, cellular immune mechanisms
should be taken into consideration as well as humoral ones. Recently, Killar and Eisen-
stein [7] reported that an aromatic-
dependent live vaccine of S. typhimurium 
SL 3235 seemed to induce cellular immu-
nity, involving both nonspecific and specific 
resistance. Moreover, it had been already 
reported that cellular immunity played an 
important role in guaranteeing survival of 
challenged mice [3].

Apart from the ability of this AL1192 as a 
live vaccine, it is required that a live vaccine 
strain has at least two specific markers or 
characters. When a live vaccine is distin-
guished by only single character from wild 
type strains and if reversion might occur in 
the field, a wild type strain coincidentally 
infecting a recently vaccinated animals may 
be suspected of being revertant of the 
vaccine strain. S. enteritidis AL1192 can be 
distinguished only by the absence of 36 Md 
plasmid from the wild parent strain and it 
has not yet been demonstrated that reintroduc-
tion of this plasmid never occurs in the 
field. It is considered that two specific 
characters which are distinguishable from 
wild type strains must be given to a live 
vaccine strain. So, we made an attempt to 
produce an amino acid-dependent mutants 
which could be distinguishable from wild 
strain using N-methyl-N'-nitro-N-nitro-
soguanidine. However, this could not be 
succeeded because all strains obtained be-
came morphologically rough.

In conclusion, in spite of our attempt, it 
could not be recommended to use this strain 
AL1192 of which virulence plasmid alone 
was cured as one of the approaches to 
develop an effective live vaccine which 
might be comparable to the effective vac-
cines, UDP-glucose-4-epimeraseless mutant 
of S. typhimurium [2] and aromatic depen-
dent S. typhimurium and S. dublin [19, 20].

REFERENCES

173: 610–613.

E., Dupont, H. L, Snyder, M. J., Levine, M. M., 
and Libonati, J. P. 1977. Evaluation of a UDP-
glucose-4-epimeraseless mutant of Salmonella 
typhi as a live oral vaccine. J. Infect. Dis. 136: 
717–723.

induced by a avirulent Salmonella in LPS-
defective C3H/HeJ mice. J. Immunol. 133: 
958–961.

Aromatic-dependent Salmonella typhimurium are 
not virulent and effective as live vaccines. Nature 
291: 238–239.

5. Johnson, E. H., Hietala, S., and Smith, B. P. 
1985. Chemoluminescence of bovine alveolar 
macrophages as an indicator of developing im-
munity in calves vaccinated with aromatic-
dependent Salmonella. Vet. Microbiol. 10: 
451–464.

and Whitefield, H. J. 1982. Association of adhe-
sive, invasive, and virulent phenotypes of Sal-
monella typhimurium with autonomous 60-
megadalton plasmids. Infect. Immun. 38: 
476–481.

to Salmonella typhimurium infection in C3H/
HeNcrBR mice: studies with an aromatic-
dependent live S. typhimurium strains as a vac-

8. Nakamura, M., Ohmoe, K., Sato, S., Suzuki, S., 
and Ikeda, S. 1985. Isolation of salmonellae from 
apparently healthy fattening male dairy calves and 
fattening pigs and stability of plasmids in the 

1985. possible relationship of a 36-megadalton 

10. Nakamura, M., Sato, S., Ohya, T., Suzuki, S., 
and Ikeda, S. 1986. Plasmid profile analysis in 
epidemiological studies of animal Salmonella 
typhimurium infection in Japan. J. Clin. Micro-

11. Nakamura, M., Sato, S., Ohya, T., Suzuki, S., 
Salmonella enteritidis AL1192 as a candidate for a 

要約

プラスミド除去 Salmonella serovar enteritidis AL1192 の牛に対する病原性と免疫原性：中村政幸・佐藤静夫11・鈴木善子・田村 慧・伊藤 治・小枝鉄雄・池田洋雄（動物医薬品検査所）――病原性プラスミドを除去した Salmonella serovar enteritidis AL1192 の7ヶ月齢牛に対する病原性を調べ、接種牛を Salmonella serovar typhimurium で攻撃して免疫効果を調べた。AL1192 接種により一過性の発熱及び排菌が認められたが、重篤な臨床症状は生じなかった。AL1192 筋肉内接種16日後にO抗原を共有するS. typhimurium L-535を皮下接種したところ、L-535に対するH抗体が認められたことから、AL1192接種により L-535 の感染は阻止されないと思われた。