Establishment of an Attenuated Strain of Bovine Respiratory Syncytial Virus for Live Virus Vaccine

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Abstract. To develop a live virus vaccine for the prevention of bovine respiratory syncytial (BRS) virus infection in calves, an attempt was made to produce an attenuated virus. The RS-52 strain of BRS virus, isolated from the nasal secretions of a naturally infected calf, was subjected to serial passages in adult hamster lung established (HAL) cells at 30°C and the attenuated rs-52 strain as a live virus vaccine was established. The rs-52 strain multiplied better at 30°C than at 34 or 37°C in HAL cells. The differences in the highest virus titers of this strain between the culture temperature of 30°C and that of 34 or 37°C were more than 2.25 log TCID50. Colostrum-deprived newborn calves and 2–4 months old calves inoculated with the rs-52 strain manifested no abnormal clinical sings at all. However, all inoculated calves produced serum neutralization antibody. When the colostrum-deprived newborn calves immunized with the rs-52 strain were challenged with the virulent NMK7 strain of BRS virus, they exhibited no pyrexia or other abnormal clinical signs at all. An attempt was made to recover the virus from nasal secretions of these calves, but in vain. On the other hand, a nonimmunized control colostrum-deprived newborn calf developed slight fever, mild cough, and slight serous nasal discharge after challenge exposure. The virus was recovered from nasal secretions of this calf. From these results, it was considered that the rs-52 strain could be used as an attenuated live virus vaccine for prevention of BRS virus infection. —Key words: attenuated strain, BRS virus, vaccine.

In 1970, bovine respiratory syncytial (BRS) virus was isolated from outbreaks of respiratory infections of cattle in Belgium [16], Switzerland [11], and Japan [2, 3]. Since then, many papers have been published to report bovine respiratory disease caused by BRS virus infection in many countries, including Japan [20]. BRS virus infections are a major cause of respiratory disease in cattle. The infections occur every year and may be the cause of significant losses. The efficacy of commercially available BRS vaccine in preventing infection or disease was investigated in other countries [1, 6, 10, 13, 15, 17–19, 22].

The authors made an attempt to produce an attenuated strain of BRS virus for the development of a live virus vaccine by serial passages in HAL cells derived from an adult hamster lung at low temperature and succeeded in producing the attenuated rs-52 strain. The present paper deals with the process of development and the properties of the attenuated rs-52 strain of BRS virus.

Materials and Methods

Virus: The RS-52 and NMK7 strains of BRS virus were used for experiment. The RS-52 strain was isolated from nasal secretions of a naturally infected calf by one of the authors in 1977. Since then, it had been subjected to 15 passages in primary bovine kidney (BK) cells. The NMK7 strain [2–4] was supplied by the National Institute of Animal Health, Ministry of Agriculture, Forestry and Fisheries in Japan. It had been subjected to 5–7 passages in BK cells at the
author's laboratory before it was used for examination. Both strains were grown in BK cells at 34°C.

Cell cultures: BK cells were prepared from the kidney of healthy calf. Established cell lines of HAL and Vero cells were also used. HAL cells derived from an adult hamster lung have been established by the authors. Growth medium (GM) used for the propagation of these cells was Eagle's minimum essential medium (MEM) containing 0.295W/V% tryptose phosphate broth (TPB) and 10V/V% inactivated fetal calf serum (FCS). Maintenance medium (MM) for these cells was MEM containing 0.295W/V% TPB, 0.05W/V% yeast extract, 0.5W/V% sodium glutamate, 0.1W/V% glucose and 0.11W/V% bovine albumin.

Culture of the rs-52 strain in HAL cells: The rs-52 strain was passaged on a monolayer culture of HAL cells prepared in Roux's bottle. After virus adsorption at 37°C for 60 min, the culture was fed with 100 ml of MM and incubated at 30°C for 10~14 days in a stationary state. The culture fluid was collected from the bottle and centrifuged at 3000 rpm for 15 min. The resulting supernatant was stored at −70°C.

Virus titration: Serial tenfold dilutions were made by Eagle’s MEM and 0.1 ml of each dilution was inoculated into 4 tubes of Vero cells. After virus adsorption at 37°C for 60 min, the cultures were fed with 0.5 ml of MM and incubated at 34°C using rolling drum. The cultures were observed for cytopathic effect (CPE) during 14 days. The 50% tissue culture infective dose (TCID50) was calculated by the method of Reed and Muench [12].

Physicochemical properties of the virus: The type of nucleic acid of virus was indirectly determined by using 5-iodo-2'-deoxyuridine (IUDR) [14]. The virus was examined for sensitivity to ethylether, chloroform, low pH (3.0), and heating (at 50°C for 30 min) [7]. The size of virus was estimated by filtration test [21] using Millipor membrane filter with pore sizes of 220, 100, and 50 nm.

Hemagglutinating (HA) activity: Serial twofold dilutions of the virus materials were made by veronal-buffered saline (VBS) and 0.4 ml of each dilution was mixed with 0.2 ml suspension of 0.5V/V% erythrocytes from human (type O), cattle, goat, pig, sheep, guinea pig, rat, hamster, mouse, goose and chicken. These mixtures were kept at 4°C for 18 hr and 22°C for 2 hr, respectively, and examined for HA activity.

Antisera: Guinea pigs were injected intraperitoneally 4 times with each 2 ml of the fluids containing 10⁶ TCID₅₀/ml of virus at 2 weeks intervals. They were bled 4 weeks after the last injection.

Virus growth curves: Monolayer culture of HAL cells in Roux's bottle were inoculated with virus (moi=0.01). After virus adsorption at 37°C for 60 min, the cultures were washed twice with Eagle's MEM, fed with 100 ml of MM, and incubated at 30, 34, or 37°C in a stationary state. Thereafter, at various time, the culture fluid were harvested from two bottles, pooled, and centrifuged at 3000 rpm for 15 min. The supernatant fluids were stored at −70°C until titrated.

Serum neutralization (SN) tests: Serial twofold dilutions of heat inactivated serum samples were made in GM. Each dilution was mixed with an equal volume of GM containing 200 TCID₅₀/0.1 ml of the NMK7 virus strain. The mixtures were kept at 22°C for 24 hr. One-tenth ml of each mixture was inoculated into 4 tubes of Vero cells. The cultures were incubated at 34°C for 10 days using rolling drum. SN antibody titer was expressed by the reciprocal of the highest serum dilution that had inhibited the appearance of CPE in at least two of the four tubes.

Experimental animal: Holstein calves, 2~4 months old, were used. In addition,
colostrum-deprived newborn Holstein calves, 3–5 days old, were used. All the animals were negative for antibody against BRS virus.

*Virus recovery from nasal secretions:* Nasal secretions were collected from some calves for 10 days after virus inoculation. A small sanitary tampon was inserted into nasal cavity of calf for 1 hr, and the tampon absorbed nasal secretions was extracted sufficiently with 3 ml of MM containing 1000 IU/ml of penicillin and 1000 μg/ml of streptomycin. The resulting extracts were centrifuged at 3000 rpm for 30 min and the supernatant fluids were used as specimens for virus recovery. One-tenth ml of the specimen was inoculated into tube cultures of Vero cells. The cultures were incubated at 34°C for 14 days using rolling drum. When CPE was negative, the culture fluids were harvested, reinoculated in the same manner into Vero cells and incubated further 14 days. When CPE was negative, the culture fluids were subjected to once more passage by the method described above.

*Clinical observation:* The experimental calves were observed under general clinical symptoms. Rectal temperature and the leukocyte count were taken in them for 2 weeks after virus inoculation.

RESULTS

*Establishment of the attenuated rs-52 strain:* As an original strain was used the RS-52 strain. An attempt was made to adapt the RS-52 strain to HAL cells. It was cultured at 34°C for the first to the 5th passage and at 30°C for the 6th to the 15th passage. At the 11th, 12th, and 13th passage levels, cloning was performed by the limiting dilution method. As a result, the strain showed better growth at 30°C. The RS-52 strain at the 15th passage level in HAL cells was referred to as the rs-52 strain (Fig. 1), and used for further experiment.

**Physicochemical properties and hemmagglutinating (HA) activity:** The rs-52, RS-52, and NMK7 strains were used. The replication of these viruses was not inhibited with IUDR, indicating that the nucleic acid type of these viruses was RNA. They were completely inactivated by the treatments of 20V/V% ethylether, 5V/V% chloroform, and pH 3.0 solution, respectively, at 22°C for 60 min, and heating at 50°C for 30 min. They passed through membrane filter of 220 nm in pore size, but not through 100 nm filter. These viruses did not agglutinate the erythrocytes from human type O, cattle, goat, pig, sheep, guinea pig, rat, hamster,
mouse, goose and chicken.

Cross neutralization test: Serological relationships among the rs-52, RS-52, and NMK7 strains were examined. In cross neutralization test, the three strains were quite closely related with each other. They were serologically indistinguishable.

Virus growth in HAL cells at different temperatures: Growth curves of the rs-52, RS-52, and NMK7 strains at different temperatures are shown in Fig. 2. The rs-52 strain multiplied better at 30°C than at 34 or 37°C. The highest virus titer at 30°C was 2.25 log TCID₅₀ higher than that at 34°C and 4.5 log TCID₅₀ higher than that at 37°C. In the cases of the RS-52 and NMK7 strains, the differences in the highest virus titers between the culture temperature of 30°C and that of 34 or 37°C were less than 1 log TCID₅₀. At 30°C, the rs-52 strain multiplied better than the RS-52 and NMK7 strains. The differences in virus growth at 30°C between the rs-52 strain and the RS-52 or NMK7 strain were more than 2.0 log TCID₅₀ in the highest virus titers. The growth characteristics of the rs-52 strain were considered to be available as a maker in vitro.

Experiment on passage of the rs-52 strain in HAL cells at 34°C: The rs-52 strain was subjected to 3 or 5 consecutive passages at 34°C to examine for stability of the marker in vitro. Its growth characteristics in HAL cells at 30, 34 and 37°C remained after the passages at 34°C, indicating that the virus multiplied better at 30°C than at 34 or 37°C (Table 1).

Inoculation of colostrum-deprived calves 3–5 days old: Four colostrum-deprived calves were divided into 2 groups and

![Graph showing growth curves of the rs-52, RS-52, and NMK7 strains in HAL cells at different temperatures.](image)

**Fig. 2.** Growth curves of the rs-52, RS-52, and NMK7 strains in HAL cells at different temperatures. Infected cell cultures were incubated at 30°C (●—●), 34°C (○—○) or 37°C (△—△).

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of passages</th>
<th>Virus yield* in HAL cells at 34°C in HAL cells</th>
<th>30°C</th>
<th>34°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs-52</td>
<td>0</td>
<td></td>
<td>6.0b)</td>
<td>3.75</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>6.75</td>
<td>4.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>6.5</td>
<td>4.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

a) Virus titer 7 days after infection.
b) TCID₅₀/ml (log).

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inoculated with $10^6$ TCID$_{50}$ of the rs-52 strain by the intranasal or intramuscular route, respectively. They exhibited no pyrexia, leukopenia, or abnormal signs at all. In the colostrum-deprived calves, SN antibody titers were 1:8 to 1:64 five weeks after inoculation. SN antibody titer was higher in the intramuscular group than in the intranasal group. Besides, no contact infection was recognized in two calves which had been raised in the same barn with the inoculated group to serve as control. These SN antibody titers were always less than 1:1 in the control calves (Table 2).

**Inoculation of calves 2–4 months old:** Two calves, 3 months old, were inoculated with $2 	imes 10^6$ TCID$_{50}$ of the virulent NMK7 strain by intranasal route manifested a mild clinical response including pyrexia, slight serous nasal discharge, mild cough, and leukopenia. In this calf, SN antibody titer was 1:64 five weeks after inoculation (Table 2).

**In Experiment on passage of the rs-52 strain in cattle:** The serial passage tests using antibody negative calves, 2–3 months old, were carried out for the purpose of studying the change of pathogenicity of the rs-52 strain. A calf was inoculated with $3 	imes 10^6$

Table 2. Pathogenicity of the rs-52 and NMK7 strains for colostrum-deprived calves

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Strain Route</th>
<th>Dose (TCID$_{50}$)</th>
<th>Clinical signs of calves</th>
<th>SN antibody titer</th>
<th>Weeks after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fever</td>
<td>Leukopenia</td>
<td>Respiratory symptoms</td>
</tr>
<tr>
<td>1</td>
<td>rs-52</td>
<td>in $10^6$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>rs-52</td>
<td>in $10^6$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>NMK7</td>
<td>im $10^6$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>NMK7</td>
<td>im $10^6$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Cohabitating control</td>
<td>$10^3$</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Cohabitating control</td>
<td>$10^3$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Cohabitating control</td>
<td>$10^3$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

a) in: Intranasal, im: Intramuscular.
b) Cough and nasal discharge.
c) Cohabitating calves (Nos. 6 and 7) were raised in contact with four calves, Nos. 1, 2, 3 and 4.
Table 3. Pathogenicity of the rs-52 strain for 3-month-old calves

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Strain</th>
<th>Route</th>
<th>Dose (TCID$_{50}$</th>
<th>Virus inoculation</th>
<th>Clinical signs of calves</th>
<th>Virus recovery</th>
<th>SN antibody titer</th>
<th>Weeks after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>rs-52</td>
<td>in, im</td>
<td>2×10$^6$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
<td>&lt;1 1 8 8 8 8</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;1 &lt;1 4 16 32 32</td>
</tr>
<tr>
<td>13</td>
<td>Cohabitating control$^{(c)}$</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
<td>&lt;1 &lt;1 &lt;1 &lt;1 &lt;1 &lt;1</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td>&lt;1</td>
<td>&lt;1 &lt;1 &lt;1 &lt;1 &lt;1 &lt;1</td>
</tr>
</tbody>
</table>

a) in, im: Intranasal and intramuscular simultaneously inoculation.
b) Cough and nasal discharge.
c) Cohabitating calves (Nos. 13 and 14) were raised in contact with two calves, Nos. 11 and 12.

Table 4. Clinical signs and SN antibody responses of 2~4-month-old calves inoculated with the rs-52 strain

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Strain</th>
<th>Route$^{(a)}$</th>
<th>Dose (TCID$_{50}$</th>
<th>Virus inoculation</th>
<th>Clinical signs of calves</th>
<th>SN antibody titer</th>
<th>Weeks after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td></td>
<td>in</td>
<td>10$^4$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>in</td>
<td>10$^5$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>17</td>
<td>rs-52</td>
<td>im</td>
<td>10$^4$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>im</td>
<td>10$^5$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>19</td>
<td>sc</td>
<td>10$^4$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>20</td>
<td>sc</td>
<td>10$^5$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

a) in: Intranasal, im: Intramuscular, sc: Subcutaneous.
b) Cough and nasal discharge.

TCID$_{50}$ of the rs-52 strain by the intranasal, intratracheal, and intramuscular routes. It did not show any symptoms, and SN antibody titer was 1:8 after 1 month of inoculation. Nasal secretions and bloods were collected daily from it for 10 days after inoculation. These materials were pooled respectively, and 10 ml of the nasal secretion were inoculated into nasal cavity, and 10 ml of the blood were injected simultaneously into vein of a calf. Nasal secretions and bloods were also collected from it. Virus recovery from these materials was all negative. It showed no symptoms, or no antibody response, thus we could prove no evidence of infection of the rs-52 strain.

Resistance of colostrum-deprived calves immunized with the rs-52 strain to challenge with the virulent NMK7 strain: Two colostrum-deprived calves (Nos. 2 and 3) were inoculated with 10$^6$ TCID$_{50}$ of the rs-52 strain by the intranasal or intramuscular route, respectively. After 5 weeks later, they were inoculated (challenge exposure) with 2×10$^7$ TCID$_{50}$ of the virulent NMK7 strain by the intranasal and intratracheal routes. Calves Nos. 2 and 3 had SN antibody titer of 1:4 and 1:64, respectively, at the time of challenge. SN antibody titer was less than 1:1 in a challenge control calf (No. 6).
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Table 5. Resistance of colostrum-deprived calves immunized with the rs-52 strain to challenge with the NMK7 strain

<table>
<thead>
<tr>
<th>Group</th>
<th>Challenge inoculation</th>
<th>Clinical signs</th>
<th>Virus recovery</th>
<th>SN antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calf No.</td>
<td>Strain</td>
<td>Route</td>
<td>Dose (TCID_{50})</td>
</tr>
<tr>
<td>Immunized^{a)}</td>
<td>2/3</td>
<td>NMK7</td>
<td>in, it</td>
<td>2×10^{7}</td>
</tr>
<tr>
<td>Challenge control</td>
<td>6</td>
<td>NMK7</td>
<td>in, it</td>
<td>2×10^{7}</td>
</tr>
</tbody>
</table>

a) Calves Nos. 2 and 3 were inoculated with 10^{6} TCID_{50} of the rs-52 strain by the intranasal or intramuscular route, respectively. After 5 weeks later, they were challenged with the virulent MKK7 strain.
b) in, it: Intranasal and intratracheal simultaneously inoculation.
c) Cough and nasal discharge.

The clinical signs of infection, virus recovery from nasal secretions, and antibody responses of calves after challenge are summarized (Table 5). After challenge, the immunized calves manifested no abnormal clinical signs of infection at all. Virus recovery from nasal secretions was negative in these calves. In them (Nos. 2 and 3), SN antibody titers were 1:32 and 1:128 four weeks after challenge. On the other hand, a challenge control calf (No. 6) showed a mild clinical response including rectal temperature 40.0–40.5°C, slight serous nasal discharge, and mild cough 5–6 days after challenge. SN antibody titer rose to 1:256 four weeks after challenge.

DISCUSSION

The attenuated strain in use for live virus vaccine was usually obtained from serial passages in cells originated from an animal other than natural host of the virus at low temperature. This is because such procedures led to reduce or disappear its pathogenicity for the relevant host. Most of these attenuated strains have been practically used for live virus vaccines including Akabane and infectious bovine rhinotracheitis live virus vaccines [5, 8, 9] in Japan.

As for BRS virus vaccine, Several attenuated vaccine strains have been developed by repeating passages of the field type BRS virus in bovine cell cultures [6, 13, 17, 22]. But, these strains were not a low temperature adapted strain produced by using the cells originated from an animal other than a natural host of BRS virus.

The authors subjected the RS-52 strain to serial passages in HAL cells derived from an adult hamster lung at low temperature (30°C). The low temperature adapted rs-52 strain which replicated better at 30°C than at 34 or 37°C in HAL cells was obtained. Physicochemical properties of the rs-52 and parental RS-52 strains were in agreement with the NMK7 strain of BRS virus isolated by Inaba et al. [2–4]. Inaba et al. [2, 4] reported that the NMK7 strain did not agglutinate erythrocytes from cattle, sheep, goat, guinea pig, mouse, chicken and goose. Also, in the present study, the rs-52, RS-52, and NMK7 strains did not agglutinate these erythrocytes.

When the serological properties of the rs-52 strain were examined, in the cross neutralization test, there was no difference in antigenicity between the rs-52 strain and the parental RS-52 strain or the NMK7
strain.
It has been known that the attenuated strains of live virus vaccines exhibit characters different from those of the virulent strains. In the growth experiment in HAL cells, the rs-52 strain multiplied better at 30°C than at 34 or 37°C. The differences in the highest titers of this strain between the culture temperature of 30°C and that of 34 or 37°C were more than 2.25 log TCID₅₀. At 30°C, the rs-52 strain grew better than the parental RS-52 and virulent NMK7 strains. The growth characteristics of the rs-52 strain were considered to be available as a maker in vitro. Its marker was stable even after five passages in HAL cells at 34°C.
In inoculation of 3–5 days old colostrum-deprived newborn calves by the intranasal or intramuscular route, they rapidly produced SN antibody. However, they manifested no abnormal clinical sings, and there were no contact infections in cohabiting control calves. On the other hand, a colostrum-deprived newborn calf which was inoculated with the virulent NMK7 strain by the intranasal route manifested a mild clinical sings of respiritory disease. Accordingly, the rs-52 strain was distinctly attenuated, as compared with the NMK7 strain. When calves, 3 months old, were inoculated with the rs-52 strain by the intranasal and intramuscular routes, they manifested no abnormal clinical sings or no virus discharge in nasal secretions. In cohabiting control calves, no contact infections were observed. The rs-52 strain manifested no pathogenicity for calves, 2–4 months old, regardless of the route of inoculation. Moreover, no pathogenicity recurred when the rs-52 strain had been subjected to two consecutive passages in cattle. These results indicated that the rs-52 strain was safe for calves.
To study the ability of the rs-52 strain to prevent BRS viral infection in calves, colostrum-deprived newborn calves were inoculated with this strain and challenged later with the virulent NMK7 strain. After challenge exposure, they manifested no abnormal clinical sings of infection, or no virus discharge in nasal secretions. On the other hand, a nonimmunized control calf manifested a mild clinical sings of respiritory disease. Virus was recovered from nasal secretions of this calf. From these results, it is presumed that calves immunized with the rs-52 strain can be prevent from infection with a virulent BRS virus.
From the results mentioned above, it is concluded that the rs-52 strain can be used as live virus vaccine for prevention of BRS virus infection.

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REFERENCES

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要約

生ワクチン用牛 RSウイルス弱毒株の樹立：久保田道雄・福山新一・児玉和夫・佐々木文雄 (病態生物化学研究所)——牛 RSウイルス感染症を予防するための生ワクチンを開発する目的で、弱毒ウイルスの作出を試みた。呼吸器症状を呈した野牛のサポート系より分離したRS-52株を成熟ハムスター肺由来培養(HAL)細胞に30℃で連続接代することにより、30℃でより増殖性の高いrs-52株を樹立した。rs-52株の30℃での増殖性は34℃又は37℃での培養に比較し、明らかにすぐれており、感染力は最高2.25 log TCID₅₀以上の差が認められた。牛 RSウイルス抗体変性性の初乳未摂取人工補育牛および2-4ケ月齢の子牛にrs-52株を接種したところ抗体産生はみられたが、臨床上の異常はまったく認められず、同居感染も成立しなかった。rs-52株で免疫した初乳未摂取人工補育牛での感染防止試験では、強毒ウイルスの鼻腔内および気管内接種に対して発病は完全に阻止され、鼻汁からのウイルス回収はすべて陰性であった。一方、強毒ウイルスで攻撃した非免疫対照牛では、攻撃後、軽度の発熱、咳および鼻汁などの定型的な呼吸器症状を示し、鼻汁からのウイルス回収も陽性であった。