

研究成果の刊行に関する一覧表

雑誌

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## Production of rabies neutralizing antibody in hen's eggs using a part of the G protein expressed in *Escherichia coli*

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### Abstract

In an attempt to produce anti-rabies immunoglobulin affordable for people living in developing countries, we have immunized layer chickens with a part of the G protein of rabies virus expressed in *Escherichia coli*. Immunoglobulin (IgY) was purified from the yolks of eggs laid by immunized hens. It was revealed in vitro that the antibody specifically bound to virions as well as cells infected with rabies virus. Moreover, the antibody apparently neutralized rabies virus infectivity. Inoculation of the antibody into mice infected with rabies virus reduced the mortality caused by the virus, suggesting that IgY directed to the part of the G protein expressed in *E. coli* could serve as a possible alternative to currently available anti-rabies human or equine immunoglobulins.

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**Keywords:** Rabies immunoglobulin; Recombinant protein; Egg yolk antibody (IgY); Post exposure prophylaxis

### 1. Introduction

Rabies is still a serious public health problem in the developing countries, especially in Asia. It is believed that at least 60,000 human deaths occur each year, and more than 10 million people are reported to receive post-exposure prophylaxis (PEP) every year throughout the world [1]. The PEP consists of three essential parts, immediate cleansing of the wound, immunization with rabies vaccine, and administration of anti-rabies immunoglobulins (RIG) around the wound.

According to the World Health Organization (WHO), administration of RIG is recommended if the patients experienced the category-III exposure [2]. Although human rabies

immunoglobulins (HRIG) are exclusively used in the developed countries, in the developing countries, immunoglobulins of equine origin (ERIG) are still produced and used because HRIG is not only unaffordable but also unavailable due to global shortage. Recently short supply of the ERIG raised another concerns, since the last remaining international manufacturer discontinued the production of the ERIG in 2001 [3]. In several developing countries ERIG is produced; however, producing large amount of RIG purified from equine sera is not easily achieved because it takes lots of time and effort. Furthermore for immunization of horses with inactivated rabies virus it is required to handle a large amount of live virus, posing a potential risk of infection on people who are involved in the manufacturing. We have thus tested a possibility that immunoglobulin (IgY) obtained from eggs of a layer chicken immunized with a part of the rabies G protein could neutralize the infectivity of rabies virus and that

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passive administration of anti-rabies IgY could confer resistance against virus infection in experimentally infected mice.

## 2. Materials and methods

### 2.1. Virus and cells

A stock virus suspension of the challenge-virus-standard (CVS-11) strain of rabies virus was propagated on mouse neuroblastoma (MNA) cells as described previously [4]. The virus and cells were kindly provided by Dr. C.E. Rupprecht, Rabies Section, Center for Disease Control and Prevention (CDC), Atlanta, GA, USA.

### 2.2. DNA cloning of a part of the G protein

Extraction of genomic RNA of CVS-11 and reverse transcription was performed as reported by Inoue et al. [4]. The cDNA for the G gene was synthesized by polymerase chain reaction (PCR) using the Expand High Fidelity PCR System (Roch, Germany). A part of the G gene encoding a truncated protein (G-F2) consisting of amino acids from positions 201 to 300 was amplified with the primers, G20/75(CVS)F2-*Bam*HI (containing a *Bam*HI site [GGG AAG AGA GGA TCC AAA GGG]) and G20/75(CVS)B2-*Sal*II (containing a *Sal*II site [AGA CGT CGA CAA CTT ACT GAC]). The amplified fragment was purified and digested with 12U of *Bam*HI and *Sal*II, respectively, and then inserted into the *Bam*HI and *Sal*II site of the pQE-30 vector plasmid of the QIAexpress system (QIAGEN). The resulted plasmid was designated pQEG-F2 and introduced into *E. coli* DH5 $\alpha$ .

Expression and purification of the recombinant G-F2 protein (rG-F2) was performed as reported previously by Inoue et al. [4].

### 2.3. Immunization of a chicken

A MOMIJI laying hen was immunized by intramuscular (im) inoculation of 0.36 mg rG-F2 emulsified with 1 ml of Freund's complete adjuvant. The hen was boosted intramuscularly at 2-week interval with the half dose of the protein emulsified in Freund's incomplete adjuvant. Eggs were collected every day and egg yolks were separated and stored at  $-40^{\circ}\text{C}$ . A part of egg yolks (1 ml) was mixed with the same volume of sterile PBS containing 0.05% NaN<sub>3</sub>. Purification of IgY was carried out according to the protocol reported by Hatta et al. [5].

In brief, the stored egg yolks (200 g) was mixed and homogenized with 600 ml of 0.5% NaCl. The homogenate was mixed with 400 ml of 0.4%  $\lambda$ -carrageenan solution. The mixture was left for 1 h at room temperature followed by centrifugation at  $7000 \times g$  for 30 min. The supernatant was filtered, and precipitated by 15% (w/v) sodium sulfate three times and dialyzed with 10 mM disodium hydrogenphosphate. Purified IgY fractions were stored at  $-80^{\circ}\text{C}$  until use. Anti-

rabies virus IgY was obtained from a hen immunized with cell culture-derived rabies vaccine (The Chemo-Sero Therapeutic Research Institute, Kumamoto, Japan). After the initial im injection of 1 ml vaccine solution, the bird received booster shots of 1 ml vaccine 2 and 4 weeks later. Non-immunized chicken egg yolks were also collected and purified as described above.

### 2.4. Neutralization test

The rabies virus-neutralizing titer of anti-rG-F2 IgY was determined with the rapid fluorescent focus inhibition test (RFFIT) [6].

### 2.5. Immunofluorescent antibody assay

MNA cells were infected with  $2 \times 10^2$  FFU of rabies virus for 48 h. Then the cells were fixed in 4% formaldehyde and permeabilized for 30 min at room temperature in PBS containing 1% Triton X-100 (SIGMA). The primary antibody (anti-rG-F2 IgY) was added and incubated for 30 min at room temperature. After washing with PBS(-), the cells were incubated at room temperature for 30 min with FITC-conjugated anti-chicken IgG (whole molecule) (SIGMA). Cells were then washed, and the fluorescent focus was observed under an UV microscope.

### 2.6. Immunohistochemistry

BALB/c mice were intracranially infected with  $1 \times 10^3$  FFU of the CVS-11. The organs obtained after 7 days post infection were fixed in 10% neutral-buffered formalin in PBS then processed as reported by Inoue et al. [4] except that anti-rG-F2 IgY and horseradish peroxidase-conjugated anti-chicken IgG (ICN Biomedicals, Inc., Aurora, OH) were used as the primary and secondary antibodies, respectively.

### 2.7. Western blotting

The purified rG-F2 was subjected to SDS-PAGE using 16% gel. After electrophoresis, western blot analysis was performed as reported previously by Inoue et al. [4] except that 1:10,000 diluted anti-rG-F2 IgY and 1:1,000 diluted alkaline phosphatase-conjugated anti-chicken IgG (SIGMA) were used for detection of proteins.

### 2.8. ELISA

ELISA was performed using cell culture-derived rabies vaccine (The Chemo-Sero Therapeutic Research Institute, Japan) as antigen. Anti-rG-F2 IgY, anti-rabies virus IgY and non-immunized IgY diluted in PBS(-) containing 0.5% bovine serum albumin, 0.05% Tween20 (0.5% PBST) were added and incubated for 1 h at room temperature. After

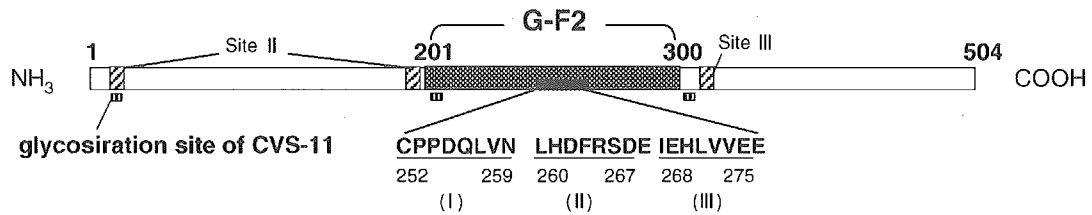


Fig. 1. Schematic representation the G protein of rabies virus. The positions of G-F2 and three octapeptides were indicated.

washing the plate, horseradish peroxidase-conjugated rabbit anti-chicken IgG (ICN Biomedicals, Inc.) diluted in 0.5% PBST was added and incubated for 30 min. Absorbance at 450 nm was measured after incubation with the substrate (100  $\mu$ g/ml tetramethylbenzidine, 0.003%  $H_2O_2$  in HRP color buffer containing 0.05 M  $Na_2HPO_4$  and 0.025 M citrate, pH 5.0) for 15 min.

### 2.9. Synthetic peptides

Three octamer peptides corresponding to the regions consisting of amino acids 252–259 (I), 260–267 (II) and 268–275 (III) of the G protein were custom synthesized by NeoMPS, Inc. (San Diego, CA) (Fig. 1). Peptide I and II were dissolved in a small amount of 0.1% ammonium solution, and then diluted with PBS at a concentration of 1 mg/ml. Peptide II was dissolved in PBS to give a final concentration of 1 mg/ml. They were stored at  $-30^\circ C$  until use.

### 2.10. Competition of neutralization activity of anti-rG-F2 IgY by the synthetic peptides

Neutralizing activity of anti-rG-F2 IgY was determined in the presence of varying amounts of the synthetic peptides.

### 2.11. Effect of anti-rabies IgY on experimental rabies virus infection of mice

Six-week-old BALB/c mice were intramuscularly inoculated with 50  $\mu$ l of the CVS-11 strain of rabies virus ( $2 \times 10^7$  FFU/100  $\mu$ l). After 30 min post inoculation, anti-rabies virus IgY, or different amount of purified anti-rG-F2 IgY (80  $\mu$ l) was intramuscularly injected into the site different from but within a distance of 5 mm from the virus inoculation site. Infected mice were observed for 3 weeks, and serum samples were obtained from survived mice. Determination of virus neutralization titer of serum samples from survived mice was carried out as described above.

## 3. Results

### 3.1. Preparation of rG-F2 and anti-rG-F2 IgY

Although expression of viral proteins in *E. coli* is comparatively cheap and convenient, it is usually difficult to produce

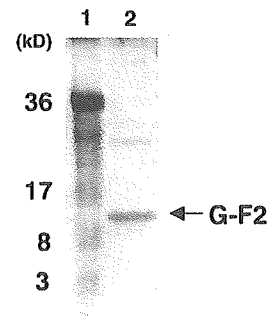


Fig. 2. SDS-PAGE analysis of rG-F2 protein expressed in *E. coli* DH5 $\alpha$ . The 16% polyacrylamid gel and tris–tricine buffer were used for electrophoresis. Lane 1, molecular weight marker; lane 2, purified rG-F2.

viral glycoproteins in bacterial cells because of aggregation of proteins. Since it was reported that certain monoclonal antibodies directed to the linear epitopes of rabies virus neutralize virus infectivity [7–11], we have attempted to express a part of the G protein (rG-F2) containing these epitopes (Fig. 1).

The rG-F2 expressed as (His) $_6$ -tagged protein was subjected to the SDS-PAGE analysis (Fig. 2). A single band with a molecular mass of 16–17 kDa was detected. By Western blotting, it was shown that anti-rG-F2 IgY obtained from the hens immunized with this polypeptide specifically bound to the rG-F2 protein (Fig. 3).

### 3.2. Detection of virus antigen with anti-rG-F2 IgY

To ascertain that IgY raised against the rG-F2 recognized the G protein of rabies virus, virus-infected cells were incubated with anti-rG-F2 IgY followed by FITC-labeled anti-chicken IgG. The fluorescent antigens were detected on

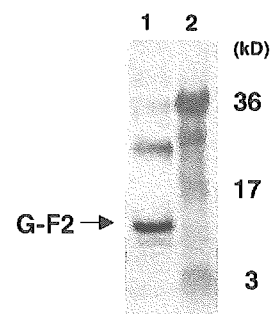


Fig. 3. Western blot detection of rG-F2 protein with purified anti-rG-F2 IgY. Lane 1, purified rG-F2; lane 2, molecular weight marker.

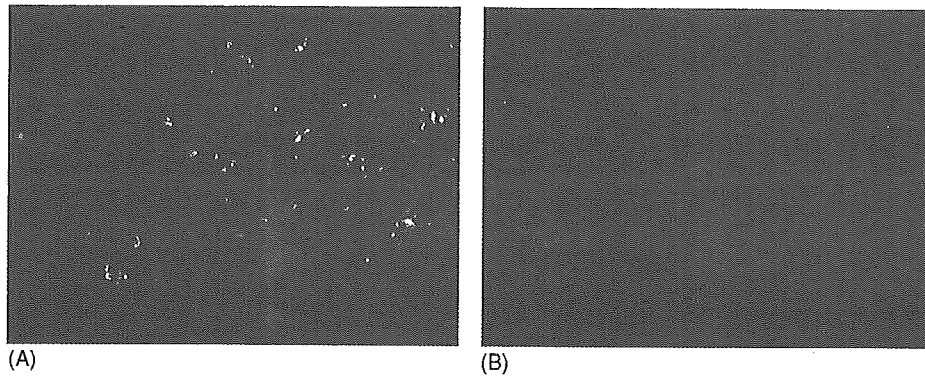


Fig. 4. Detection of viral antigen by anti-rG-F2 IgY. MNA cells infected with CVS-11 (A) and non-infected cells (B) were stained with anti-rG-F2 IgY followed by FITC-labeled anti-chicken IgG.

the surface of virus-infected cells (Fig. 4). Furthermore, the trigeminal ganglion obtained from a mouse infected with rabies virus seven days before was subjected to immunohistochemical staining using anti-rG-F2 IgY as the primary antibody. As shown in Fig. 5, viral antigens were detected in the perinuclear regions of neurons. These findings suggest that anti-rG-F2 recognized the authentic G protein of rabies virus.

3.3. Binding of the anti-rG-F2 IgY to rabies virus

To further confirm that anti-rG-F2 IgY bound to rabies virus, ELISA was performed using the rabies virus-coated plate. Anti-rG-F2 IgY was shown to bind to rabies virus as well as anti-rabies virus IgY, whereas no binding of normal IgY was observed (Fig. 6).

3.4. Neutralizing activity of anti-rG-F2 IgY

We next attempted to determine whether anti-rG-F2 IgY showed virus neutralization activity against the CVS-11 strain of rabies virus. Fig. 7 clearly shows that egg yolks obtained from the hen immunized with the rG-F2 contained IgY capable of neutralizing rabies virus infectivity. The anti-

body titer increased after the booster immunizations, reached its peak at 6 weeks after the first immunization, and then gradually declined. Yolks of 14 eggs collected from 6 weeks through 8 weeks after immunization were pooled and IgY

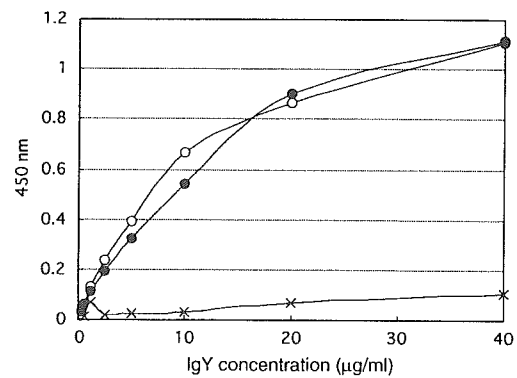


Fig. 6. Binding of egg yolk antibodies to rabies virus measured by ELISA. Anti-rG-F2 IgY (○), anti-rabies virus IgY (●), and non-immunized IgY (×) were incubated with rabies virus prepared in an ELISA plate. HRP-conjugated anti-chicken IgG was used as the second antibody. Absorbance at 450nm was measured after incubation with substrate for 15 min.

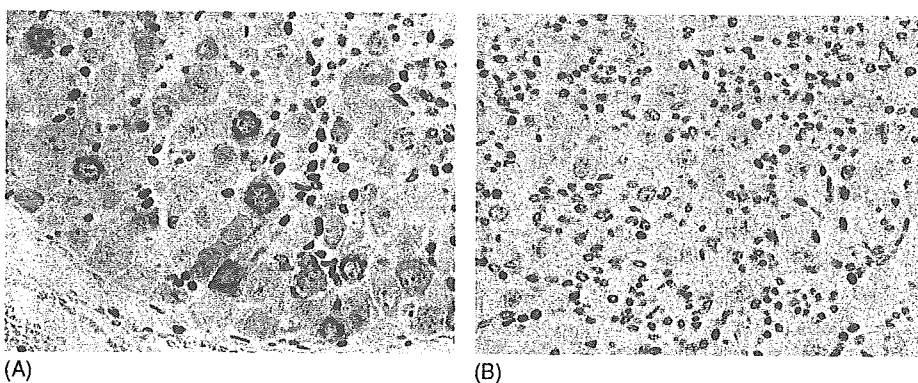


Fig. 5. Immunohistochemical detection of rabies virus in the mouse tissue by anti-rG-F2 IgY. The trigeminal ganglions of CVS-11 infected mice (A) and non-infected mice (B) were stained using anti-rG-F2 IgY.

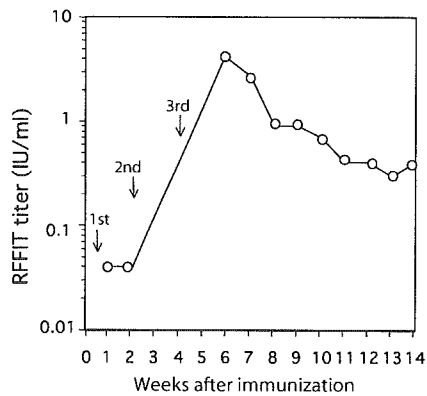


Fig. 7. Virus neutralizing activities of IgY obtained from hen at various days after immunization with rG-F2 protein. Booster immunizations were given at 2 and 4 weeks after the primary immunization.

was purified. By RFFIT assay the neutralization titer of the purified IgY was shown to be 9.9 IU/ml.

### 3.5. Competition of neutralization activity of anti-rG-F2 IgY by synthetic peptides

Since the rG-F2 contains linear epitopes defined by neutralizing monoclonal antibodies, it was of interest to determine whether anti-rG-F2 IgY recognized such linear epitopes. Viral neutralization assay was carried out in the presence of varying amounts of three different synthetic peptides. Approximately 80% decrease of neutralizing activity was observed by RFFIT when 10–20  $\mu\text{g}$  of the peptide II was added to the reaction. No reduction in neutralizing activity was observed in the presence of two other peptides (Fig. 8).

### 3.6. Effect of anti-rG-F2 IgY on development of rabies in mice infected with the virus

Now that anti-rG-F2 IgY was revealed to have neutralization activity in vitro, it appeared rational to see whether the antibody could prevent mice infected with the CVS-11 virus from developing symptoms and death. BALB/c mice

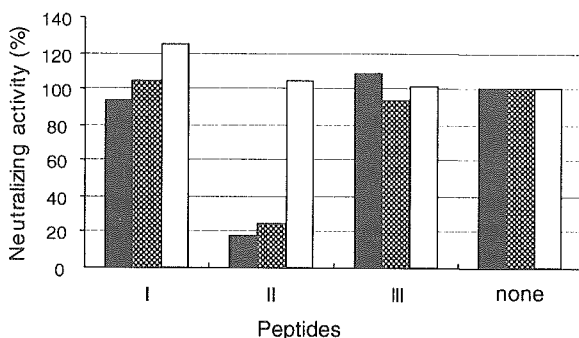


Fig. 8. Competition of neutralizing activities of anti-rG-F2 IgY by synthetic peptides. Neutralizing activities were determined in the presence of 1  $\mu\text{g}/\text{ml}$  (white bar), 10  $\mu\text{g}/\text{ml}$  (gray bar) and 20  $\mu\text{g}/\text{ml}$  (black bar) of respective peptides.

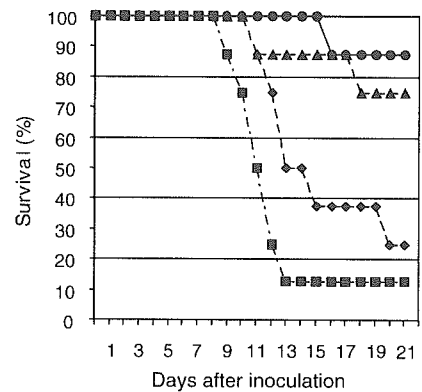


Fig. 9. Effect of administration of anti-rG-F2 IgY on the progression of rabies in virus-infected mice. Forty IU/Kg of anti-rG-F2 IgY (◆) or 80 IU/Kg of anti-rG-F2 IgY (▲) or 40 IU/Kg of anti-rabies virus IgY (●) were administered to mice infected with the CVS-11 strain of rabies virus. Mice inoculated CVS-11 without treatment were also involved (■).

were, therefore, treated with anti-rG-F2 IgY after inoculation with rabies virus. Ninety percent (9/10) of non-treated group died of rabies between day 9 and 13 after infection, and 75% of mice received 40 IU/Kg of anti-rG-F2 IgY also died of rabies. Administration of 80 IU/Kg of anti-rG-F2 IgY, however, protected 75% of mice from death attributable to rabies virus infection. This preventive effect was comparable to that of anti-rabies virus IgY achieved at a concentration of 40 IU/Kg (Fig. 9). All of mice treated with 130 IU/Kg of anti-rG-F2 IgY survived (data not shown).

## 4. Discussion

In developing countries, ERIG is frequently used for PEP instead of HRIG because HRIG is so expensive that people living in poor countries cannot afford [12]. It became also difficult to obtain ERIG because the major international supplier decided to discontinue the production of ERIG [3]. It is, therefore, urgently required to develop a substitute for HRIG and ERIG. In this study we tried to produce IgY capable of neutralizing rabies virus by immunizing a laying hen with a part of the G protein expressed in *E. coli*.

About 1.2 g of purified anti-rG-F2 IgY was obtained from about 120 g of egg yolk, which corresponded to approximately 14 eggs. It has been reported that about 1.4 g of specific antibody could be obtained from an immunized rabbit [13]. It became thus possible to obtain nearly equivalent amount of RIG, without euthanizing animals, by immunization of a hen instead of a rabbit.

Production of RIG in principle requires immunization of either humans or animals with inactivated rabies virus. Preparation of inactivated virus must be conducted in a specially designed facility where highly pathogenic viruses can be handled safely. It seems, therefore, ideal to use antigens prepared without propagating infectious virus. Recombinant DNA technology can offer several options to prepare rabies

virus G proteins such as expression of the protein in mammalian cells, insect cells, and bacterial cells. Although the expression of viral proteins in bacterial cells can be achieved most cheaply, it is well known that glycosylated proteins can rarely be expressed in bacterial cells like *E. coli*, since *E. coli* lacks some machineries for posttranslational modifications including glycosylation. It was reported, however, that linear epitopes resided at the central region (from amino acid at positions 222 to 332) of the G protein and that dogs received rabies vaccination developed antibody response directed to these linear epitopes [14]. Dietzschold et al. mapped a linear epitope recognized by the 6-15C4 monoclonal antibody (MAb) to a portion of the G protein flanked by the amino acids from positions 253 to 275 [7]. The epitope was subsequently fine tuned to amino acids at positions between 248 and 268 independently by Heijiden et al. [8], Ni et al. [9], and Luo et al. [10].

We, therefore, decided to express a part of the G protein designated G-F2 (amino acid between 201 and 300) containing the linear epitope (260–267) (Fig. 1). This region of the G protein contains no or one potential glycosylation site depending on the virus strain. A potential glycosylation site at amino acid position 204 was found in CVS-11 strain. Since IgY antibody raised against the G-F2 produced in this study bound to the virus, a glycan at this position did not affect the antigenic structure. Similarly PV and ERA strains have a potential glycosylation site at 247. It was reported, however, that the monoclonal antibody recognizing a linear epitope, 6-15C4, could neutralize both CVS-11 and ERA strains of rabies virus [11]. On the other hand, a potential glycosylation site at position 237 in silver-haired bat-associated rabies virus strain, SHBRV-18 (accession no. AY705373), has recently been reported. It is not clear at this point whether the presence of the glycan at position 237 would pose any effect on the conformation of the linear epitope.

As expected the G-F2 was successfully expressed in bacterial cells and the purified protein induced neutralizing antibodies upon immunization of the layer chickens. Combining the expression of unglycosylated part of the G protein in *E. coli* and immunization of a layer chicken will be appropriate to produce a large quantity of virus-neutralizing antibody safely, easily and economically.

As it seemed interesting to see whether anti-rG-F2 recognized the octapeptide reported by Heijiden et al. [8], we have performed neutralization test in the presence of synthetic peptides. The result showed an octapeptide (LHDFRSDE) composed of deduced amino acids at positions between 260 and 267 was recognized by anti-rG-F2 IgY, supporting the observation of Heijden et al. [8].

Efficient virus neutralization by the IgY antibody in vitro led us to investigate whether post exposure administration of the antibody could prevent the diseases caused by the virus in mice. The progression of the symptoms in rabies virus-infected mice was significantly delayed by the inoculation of anti-rG-F2 IgY as PEP at the proximal site of rabies virus injection. Although the anti-rG-F2 IgY was less effective com-

pared with IgY raised against whole virion when 40 IU/kg of IgY was administered, a higher dose (80 IU/Kg) of anti-rG-F2 IgY provided protection similar to that achieved by 40 IU/kg of anti-rabies virus IgY.

The reason why protection by anti-rG-F2 IgY in vivo was less efficient than that by anti-rabies virus IgY was not clear, but it seemed likely that more efficient protection of mice was achieved by purified IgY containing antibodies directed to multiple epitopes. Nonetheless IgY antibody raised against a part of the rabies G protein provided sufficient protection against rabies virus infection when administered immediately after infection.

Anti-rG-F2 IgY antibody would shed light on people living in countries where it is difficult to obtain not only HRIG but also ERIG after exposure to life threatening rabies virus infection, although further studies for improving the efficacy of the antibody would be necessary.

## Acknowledgments

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## Short Communication

# Detection of Rabies-Specific Antigens by Egg Yolk Antibody (IgY) to the Recombinant Rabies Virus Proteins Produced in *Escherichia coli*

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**SUMMARY:** We obtained rabies-specific egg yolk antibodies (IgY) by immunizing hens with recombinant His-tagged nucleoprotein and phosphoprotein (rN, rP) of the rabies virus (CVS-11 strain) expressed in *Escherichia coli*. The anti-rN and rP IgY were shown to bind specifically to the respective proteins of the CVS-11 strain of rabies virus by Western blotting, immune fluorescent assay and immunohistochemistry, indicating that IgY to rabies recombinant proteins could serve as a reagent for diagnosis of rabies virus infection.

Rabies is a serious public health problem in developing countries, especially in Asia. Approximately 35,000 to 50,000 human deaths occur due to rabies each year (1). Administration of rabies vaccine along with anti-rabies immunoglobulin is known to prevent development of rabies; however, prompt and precise diagnosis is essential. For rabies diagnosis, direct immunofluorescence detection of the antigens of the rabies virus has been used worldwide as the most rapid and reliable method.

Most rabies-specific antibodies used for diagnosis are made from sera of immunized mammals such as mice, rabbits and goats. However, producing a large amount of specific antibodies from these animals is time-consuming and labor-intensive. Additionally, the preparation of rabies antigen is required for immunization of animals. There is a concern that handling live and large amounts of rabies virus to produce antigen may pose a potential risk of infection to laboratory personnel.

Recent advances in molecular biology together with a newly invented method of producing antigen-specific antibodies in egg yolk (IgY) have created new opportunities to develop a safe, convenient and inexpensive way of manufacturing various immunodiagnosics (2, 3). These methods have already led to the development of orally administered agents for the prevention of enteric colibacillosis (4), dental caries (5) and human rotavirus infection (6). The method of producing IgY antibody has some advantages over the production of antibodies from mammals in that (i) there is no need to bleed the animals, (ii) it is easy to purify a large amount of antibody; and (iii) it is feasible to produce a specific antibody to a small amount of antigen that is poorly immunogenic in mammalian hosts (2,7,8).

In this report, we have developed a method to produce a large amount of rabies virus-specific antibodies by immunizing layer hens with recombinant rabies virus internal

proteins expressed in *Escherichia coli*.

Preparation of the recombinant nucleoprotein (rN) of rabies virus was carried out according to the protocol reported by Inoue et al. (3). The recombinant P protein (rP) was prepared similarly. The primers used for amplification of the P gene were RabiesPV-P5-SalI (designed to include a *SalI* site [CCG TCG ACA TGA GCA AGA TCT TTG]) and RabiesPV-P3-PstI (designed to incorporate *PstI* site [TGG ACT GCA GCG GTT AGC AAG ATG TAT]). The PCR amplicon was inserted into the pQE-9 vector plasmid (QIAGEN, Valencia, Calif., USA) so that six consecutive histidine residues (His-Tag) could be attached. The rN and rP were expressed in *E. coli* DH5 $\alpha$  after transformation by plasmid DNAs. The recombinant proteins were purified using a nickel-nitrilotriacetic acid column (QIAGEN), and the expected sizes of purified rN and rP were confirmed by SDS-PAGE.

Immunization of hens was performed by intramuscularly injecting Goto's MOMIJI laying hens with 0.36 mg each of antigen, rN or rP, emulsified in 1 ml of Freund's complete adjuvant. Booster shots were also given intramuscularly at 2-week intervals after the first injection with the half dose of the antigen emulsified in 0.5 ml Freund's incomplete adjuvant. Eggs were collected every day and egg yolks were isolated. Purification of IgY from egg yolk was carried out according to the protocol of the  $\lambda$ -carrageenan method described by Hatta et al. (9). In brief, the stored egg yolks (200 g) were mixed with 600 ml of 0.5% NaCl and homogenized. The homogenate was mixed with 400 ml of 0.4%  $\lambda$ -carrageenan solution. The mixture was left for 1 h at room temperature followed by centrifugation at 7,000  $\times$  g for 30 min. The supernatant was filtered, precipitated by 15% (w/v) sodium sulfate three times and dialyzed against 10 mM disodium hydrogenphosphate. Purified IgY fractions were stored at  $-30^{\circ}\text{C}$  until use.

For the immunofluorescent assay (IFA), the confluent MNA cells in a 96-well plate were infected with  $2 \times 10^2$  FFU of rabies virus, then incubated for 48 h at  $35^{\circ}\text{C}$ . After fixation with 70% cold acetone, the anti-rN IgY or anti-rP IgY was added. After incubation for 30 min at room temperature, the cells were washed and further incubated with FITC-conju-

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gated anti-chicken IgG (whole molecule) (SIGMA, St. Louis, Mo., USA) for 30 min. The presence of rabies-specific antigen was observed using a fluorescence microscope.

For the Western blotting analysis, the CVS-11 strain of rabies virus ( $10^7$  FFU/ml) in  $20 \mu\text{l}$  of SDS gel-loading buffer (50 mM Tris-HCl pH 6.8, 2% SDS, 0.1% bromophenol blue, 3.75% 2-Mercaptoethanol) was subjected to SDS-PAGE using 12% gel after boiling for 10 min. After electrophoresis, the proteins were transferred onto nitrocellulose membranes. The membranes were immersed in 5% skim milk solution in PBS(-) for 30 min to block nonspecific reaction and then incubated for 45 min with  $3 \mu\text{l}$  of 1 mg/ml of purified anti-rN IgY or 0.2  $\mu\text{g}/\text{ml}$  of anti-rP IgY. The membranes were washed four times in PBS(-) containing 0.05% Tween20 and incubated for 30 min with 3 ml of 1:1,000 diluted alkaline phosphatase-conjugated anti-chicken IgG (SIGMA). After washing, the immune complex was detected by staining with Western Blue (Promega, Madison, Wis., USA).

To ascertain that both IgY could detect rabies virus antigen in the neurons of infected mice, immunohistochemical analysis of formalin-fixed mouse tissue infected with CVS-11 was performed according to the procedure described by Inoue et al. (3), with the exception that anti-rN or rP IgY and horseradish peroxidase-conjugated anti-chicken IgG (ICN/CAPPEL, Aurora, Ohio, USA) were used as the primary and secondary anti-bodies, respectively.

The rN and rP purified from *E. coli* lysates using the affinity columns was subjected to SDS-PAGE analysis (Fig. 1). The rN was detected as a single band of 52 kDa molecular mass. The rP migrated slightly slower than the P protein derived from virus particles, probably due to the presence of His-tag (Figs. 1, 4). This result indicated that both proteins were successfully purified.

We immunized layer chickens with the respective recombinant proteins, then collected the egg yolks and determined the antibody titers by IFA. As shown in Fig. 2, the anti-rN IFA titer increased and reached a peak (1:4,000) at 4 weeks after immunization, then declined and remained at a titer of 1:500 for 4 weeks. On the other hand, the anti-rP IgY titer increased to 1:4,000 at the end of the second week and reached its peak (1:32,000) at the end of the third week. The titer remained high (1:32,000) for 3 weeks thereafter, then gradually declined. Representative results of the five IFAs performed

using purified IgY obtained from egg yolks of the immunized chickens are shown in Fig. 3. The fluorescent antigens were detected in the cytoplasm of infected cells.

To be sure that these antibodies were directed to rabies virus proteins, Western blotting analysis was carried out. As

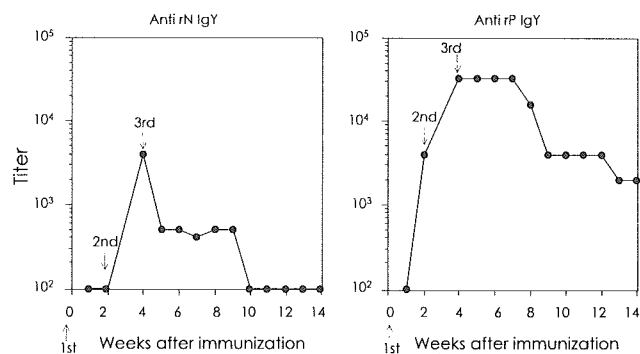


Fig. 2. Antibody titer of IgY obtained from a hen at various days after immunization with rN or rP protein. Antibody titers were measured by immunofluorescence assay. Booster inoculations were given at 2 and 4 weeks after the primary inoculation.

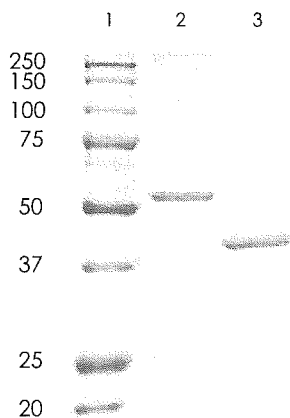


Fig. 1. SDS-PAGE analysis of rN and rP protein expressed in *E. coli*. The 12.5% polyacrylamid gel and tris-Tricine buffer were used for electrophoresis. Lane 1, molecular weight marker; lane 2, purified rN; lane 3, purified rP.

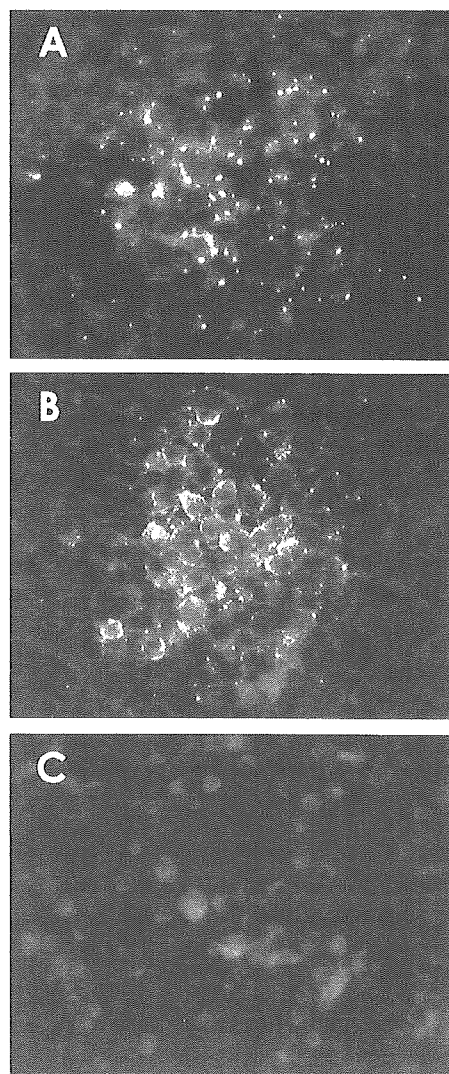


Fig. 3. Detection of viral antigen by immunofluorescence assay. MNA cells infected with CVS-11 were stained with anti-rN IgY, 10  $\mu\text{g}/\text{ml}$  (A), anti-rP IgY, 2.5  $\mu\text{g}/\text{ml}$  (B), non-immunized IgY, 10  $\mu\text{g}/\text{ml}$  (C) followed by FITC-labeled anti chicken IgG.

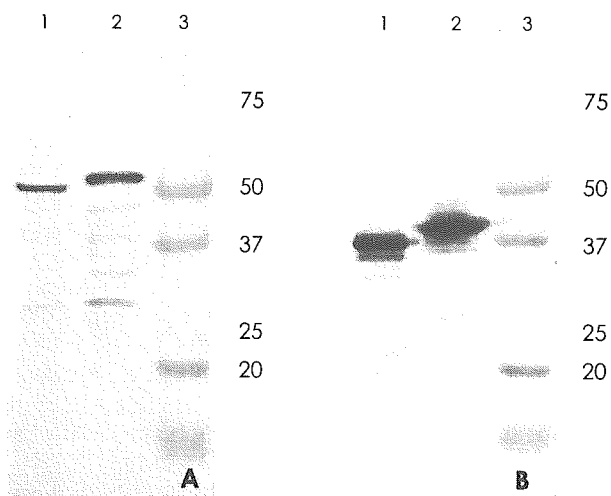


Fig. 4. Western blot detection of the CVS-11 virus protein and recombinant protein with anti-rN IgY (A) or anti-rP IgY (B). (A) Lane 1, CVS-11 virus protein; lane 2, rN protein; lane 3, molecular weight marker. (B) Lane 1, CVS-11 virus protein; lane 2, rP protein; lane 3, molecular weight marker.

shown in Fig. 4, anti-rN IgY and anti-rP IgY specifically bound to the proteins with molecular weights corresponding to the N or P proteins of rabies virus, respectively.

We next investigated whether these antibodies could be applied to immunohistochemical detection of rabies-specific antigens. For this purpose, the trigeminal ganglion obtained from a rabid mouse was subjected to immunohistochemical staining using anti-rN IgY (10  $\mu$ g/ml) and anti-rP IgY (5  $\mu$ g/ml). Paraffin sections of formalin-fixed tissues were deparaffinized and incubated with IgY antibodies. As shown in Fig. 5, viral antigens were detected in the cytoplasm of neurons by either antibody, indicating that anti-rN IgY and anti-rP IgY recognized the authentic N and P proteins of rabies virus, respectively.

Production of rabies virus-specific antibodies requires immunization of animals with inactivated rabies virus. Preparation of inactivated virus must be conducted in a specially designed facility where highly pathogenic viruses can be handled safely. It would thus be ideal if antigens could be prepared without propagating infectious virus. The expression of viral proteins in bacterial cells not only can provide a large amount of antigens for immunization but also can be achieved very cheaply. We observed that the N protein expressed in *E. coli* migrated as the 52 kDa protein in the SDS-polyacrylamide gel slightly more quickly than the N protein from virus particles of the CVS-11 (Figs. 1, 4). The rP also migrated slightly more slowly than the authentic P protein. The difference in mobility in the gel between the recombinant and authentic proteins was probably due to the presence of the His-tag in the recombinant proteins. Antibodies prepared against the rN and rP reacted with the respective viral proteins synthesized in infected cells by either IFA or immunohistochemistry.

Using the  $\lambda$ -carrageenan purification method, we obtained about 1.2 g of purified IgY with a purity of 95% from about 180 g of egg yolk, which corresponded to approximately 14 eggs. It has been reported that about 1.4 g of specific antibody could be obtained from an immunized rabbit (6). In the present study, therefore, we showed that it is possible to obtain a nearly equivalent amount of specific antibody,

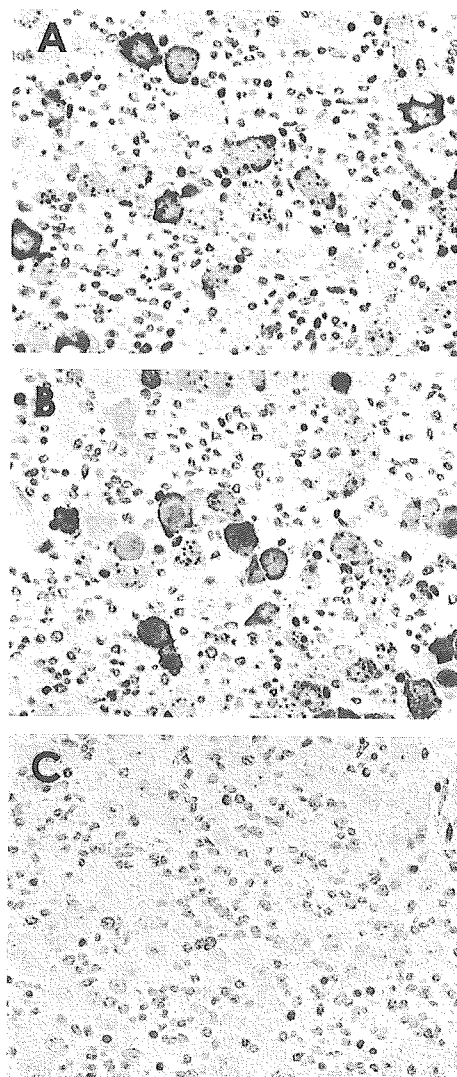


Fig. 5. Immunohistochemical detection of rabies virus in mouse tissue by anti-rN IgY or anti-rP IgY. The trigeminal ganglion of CVS-11 infected mouse was stained using anti-rN IgY (A) or anti-rP IgY (B), non-immunized IgY (C). Representative results of two independent experiments are shown.

without euthanizing animals, by immunization of a hen instead of a rabbit. In this way, by combining the expression of rabies N or P protein and the immunization of hens, it will be possible to supply a plentiful amount of antibodies for easy and inexpensive diagnosis of rabies.

The method reported here will provide rabies-endemic nations or developing countries with an opportunity to produce rabies-specific antibodies for the diagnosis of rabies.

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### 3. 人獣共通感染症

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現在ヒトに感染することが知られている感染症は分類上の種で1415に上る。このうち868種(61%)が人獣共通感染症であるとされている。すなわちヒトが感染する病原体の半数以上はヒト以外の動物にも感染する。また、新興感染症の75%は人獣共通感染症である。人獣共通感染症の病原体のうちの33%がヒトからヒトへ感染しうる。公衆衛生上の問題となる病原体は一端動物からヒトに伝播した後、ヒト間でさらに伝播できるこの33%の病原体であるといえる。

感染症が新たに勃興してくる原因として地球人口の激増、生態系への干渉、航空機輸送の発達に伴うヒト、動物、物の国際間移動の高速化、気候の変化などがあげられる。野生動物が介在する感染症は生態系との関わりが深く、たとえ僅かであっても環境の変化がエコロジカルニッチに与える影響は無視しがたく、ニッチを利用する動物の生態系が攪乱されることにつながる。生態系が攪乱すると例えば捕食動物が減少し、様々な病原体を保有している可能性のある齧歯類などが異常繁殖し、結果的に齧歯類に由来する感染症が発生することになる。

#### I. はじめに

人獣共通感染症は Zoonoses (単数は Zoonosis) の日本語訳であるが、世界保健機関 (WHO) は Zoonoses を「自然の状態、ヒトと脊椎動物の間で伝播する疾病あるいは感染症」と定義している<sup>1)</sup>。この定義が現在最も一般的に受け入れられているが、この定義では節足動物で増殖する病原体による疾患は、ヒト以外の脊椎動物が関わらなければ Zoonoses ではないことになる。例えばヒトに感染するマラリア原虫のうち熱帯熱マラリア (*Plasmodium falciparum*)、三日熱マラリア (*P. vivax*)、卵形マラリア (*P. ovale*) はヒト以外の動物宿主は知られておらず、唯一四日熱マラリア (*P. malariae*) がチンパンジーに感染するとされている<sup>2)</sup>。従って、ヒトのマラリアの場合、四日熱マラリア以外は人獣共通感染症ではない。また、ヒト免疫不全症ウイルス (HIV-1) はチンパンジーに由来する<sup>3)</sup>と考えられているが、自然の条件下では最早動物には感染しないとされており、一般に人獣共通感染症とはいわない。このように

WHO の定義を厳密に解釈するかどうかで、人獣共通感染症の範囲が変わる場合がある。欧州経済共同体は Zoonosis を「動物からヒトへ自然に伝播すると思われるいかなる疾患あるいは感染症」としており<sup>4)</sup>、WHO の定義よりは広範囲の疾患を含むと考えられる。

#### II. 今なぜ人獣共通感染症なのか

動物によって媒介される病気が存在することは紀元前から認識されており<sup>5)</sup>、その重要性についての理解も今に始まったことではない。にもかかわらずここ数年人獣共通感染症に対する関心が高まっているのは何故であろうか。国内的には経済の発展に伴い生活様式が変化し、動物とのつきあい方が変わってきたことが一つの理由と考えられる。高気密住宅において、家族の一員として飼養される愛玩動物との接触の度合いは、かつてなかった程濃厚なものになりつつある。また、社会の高齢化や高度医療の発達の結果免疫的弱者の数が増加し、結果として様々な感染症に対しての感受性ポピュレーションが増加してきている。これらの要因は人獣共通感染症に罹患する機会を増加させ、感染者数の増加に繋がるものと考えられる。一方、世界に目を向けるとウエストナイル熱や重症急性呼吸器症候群 (SARS) のようないわゆる新興感染症の勃興が人々の関心を集めているが、これら新興感染症の多くが動物に由来すると考えられており、結果として、人獣共通感染症が注

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目されるようになってきたものと思われる。細かく見ると、ヒトに感染する病原体は472属1415種（SARS コロナウイルスは除く）とされるが、その61%にあたる313属868種は人獣共通感染症の病原体である<sup>9)</sup>。またこれら1415種のうち新興感染症として登場してきたものは175種であり、その75%が人獣共通感染症であるとされている<sup>9)</sup>。

### Ⅲ. 人獣共通感染症の分類

Schwabe<sup>7)</sup>は病原体の自然界における存続の様式から人獣共通感染症を以下の4型に類型化している。この分類の仕方は人獣共通感染症が環境要因即ち生態系との関わりが深いことを理解するためには有用だと考えられる。

#### Direct zoonoses (単純型)

一種類の脊椎動物で病原体の存続が維持される。感染動物から接触、無生物媒体、機械的媒体を通じて感染する(狂犬病、ブルセラ)

#### Cyclozoonoses (循環型)

病原体の存続に複数の脊椎動物を必要とする。無脊椎動物は含まれない(胞虫症)

#### Metazoonoses (異形型)

脊椎動物へ伝達する以前に無脊椎動物体内で増殖する(アルボウイルス、ペスト)

#### Saprozoonoses (腐生型)

病原体の存続に脊椎動物と土壌などの無生物を必要とする。環境中で増殖し、ヒトに経口、経皮、経気道感染する(ボツリヌス毒素、エルシニア症)

### Ⅳ. 新興感染症と人獣共通感染症

新たに登場した感染症、あるいはかつて知られていたが近年その報告数が増加している感染症、またはこれまで存在しなかった地域に登場した感染症のことを新興再興感染症 (Emerging diseases) と呼ぶ<sup>9)</sup>。米国の医学研究所 (Institute of Medicine) は表1に挙げた要因が新興感染症の出現を促すとしている<sup>9)</sup>。

新興人獣共通感染症の多くは特定の地域で、特定の動物と特定の病原体が感染環を形成していたものがそこへヒトが侵入したため、接触の頻度・程度が増し新たな感染症として勃興したものと思われる。即ち安定していた感染環に何らかの干渉が行われることにより、その流行様式が変化したものと考えられる。従って、生態系の変化を引き起こす②、⑧は特に人獣共通感染症の発生に大きな影響を与えらると思われる。

### Ⅴ. 土地利用と人獣共通感染症

ダム建設や森林開発のような大型プロジェクトは人獣共通感染症の発生に大きな影響を与えらる考えられている。住血吸虫症は世界の6億人が流行地域に暮らしており、2

表1 新興感染症の登場に関する因子

①病原体の適応・変異
②経済発展と土地利用
③人口動態とヒトの行動様式
④国際的なヒトと物資の移動
⑤テクノロジーと産業
⑥公衆衛生基盤の破綻
⑦ヒトの感染症に対する感受性
⑧天候と気候
⑨生態系の変化
⑩貧困と社会的不平等
⑪戦争と飢饉
⑫政治的意志の欠如
⑬意図的危害

億人が感染していると言われている寄生虫疾患である<sup>10)</sup>。住血吸虫は淡水産の巻き貝を中間宿主、ヒト、イヌ、ウシなどの動物を終宿主としており、ヒトは中間宿主から泳ぎ出したセルカリアで汚染した水に接触することにより経皮的に感染するとされている。アフリカのセネガル川流域での住血吸虫症の発生数増加やエジプトにおける発生増加はダム建設によって上昇した水位と流れの緩慢化によって貝と寄生虫との出会いのチャンスが増したことや環境変化が中間宿主である巻き貝の繁殖を増進したことに起因すると考えられている。このような環境変化、即ち生態系の攪乱はダム建設だけでなく灌漑施設や運河の建設などによってももたらせられることがある<sup>10)</sup>。

ベネズエラ出血熱はアレナウイルス属のグアナリトウイルスによる出血熱だが、このウイルスはトウマウス (Cane mouse; *Zygodontomys brevicauda*) と呼ばれるネズミに持続感染しており、ウイルスは尿中あるいは唾液中に排泄される。ベネズエラ出血熱は1989年まで殆ど気づかれていなかった疾患だが、森林の伐採と牧草地の開墾により、トウマウスの個体数が激増し、また開墾に伴う人口の流入の結果、人々がグアナリトウイルスに曝される機会が増すことになった。その結果1989年以降250例の患者発生が報告されるようになったと考えられている<sup>11)</sup>。

一方、いったん開墾された土地が再び森林化する(再森林化)ことも人獣共通感染症の発生に大きな影響を及ぼす場合が知られている。アメリカ北東部においては再森林化によってオジロジカの個体数増加と生息域の拡大が進んだ。このシカは雌の成虫マダニの吸血源となっている。マダニは幼生時代に野生げっ歯類から吸血するが、これらのげっ歯類がライム病の病原であるボレリアを保有している。従って再森林化によるシカの個体数の増加とともにこれらの地域で繁殖したマダニはライム病ボレリアを保有することになり、住宅建設やアウトドア活動と一致してヒト

におけるライム病の発生が目立つようになったと考えられる<sup>19)</sup>。

## VI. 気候・天候の変化と人獣共通感染症

エルニーニョはペルー及びエクアドル沖の海水温が上昇することによって起きる世界的な気候の変動だが、この期間中は平均気温が0.5℃上昇すると言われている。また地域によっては降水量の増加が認められるが、別の地域では早魃が起りやすくなったりもする。エルニーニョと病気の発生の関連を科学的に証明するのはなかなか困難とされているが、いくつかの疾病、例えばマラリアあるいはコレラではその発生数の増加がエルニーニョと関連することが証明されている<sup>20)</sup>。1993年アメリカ合衆国南西部にシンノブルウイルスと呼ばれるハンタウイルスによる急性呼吸促進症候群のアウトブレイクがあった。このウイルスはシカネズミ (*Peromyscus maniculatus*) に持続感染しており尿中に排泄されるが、ニューメキシコ州におけるシカネズミの個体数は1990年代の初頭から増加し始め、1993年に最大になったことが知られている。これは1987年から1992年にかけての早魃でネズミの捕食動物が激減したことと、引き続き発生したエルニーニョの影響で降水量が増加し、冬期の気温上昇と相まってシカネズミの餌になる松の実が豊作であったことによると考えられている<sup>21)</sup>。

気候変動に関する政府間パネル (IPCC) によれば二酸化炭素の排出による地球規模の温暖化は2100年には平均気温を1.5-5.8℃押し上げると推測されている<sup>22)</sup>。地球の温暖化と感染症の発生についてははっきりとした証拠はないが、節足動物、特に蚊によって媒介される感染症の拡大が懸念されている。

## VII. どのような人獣共通感染症が公衆衛生上問題となるか

868種の病原体によって引き起こされる200を超える人獣共通感染症の全てが公衆衛生上問題視されねばならないかという点必ずしもそうではない。種の壁を超えてヒトに感染することに成功しても多く (66%) の人獣共通感染症の病原体は次のヒトに感染を拡大することもなく消滅してしまう<sup>6)</sup>。従ってこれらの感染はよほど致死率が高い場合や、重篤な後遺症を残す場合を除けば公衆衛生に与えるインパクトはさほど大きくないといえる。では公衆衛生上問題にすべき人獣共通感染症はどのようなものであろうか。

### ヒト-ヒト感染に至る人獣共通感染症

ヒトの社会に侵入した後ヒトの間で感染が拡大する人獣共通感染症については警戒が必要である。ペストが代表的であるが、感染げっ歯類のノミを介して感染した後肺ペストを発症するとヒトからヒトへと感染が伝播する<sup>10)</sup>。昨年世界を震撼させた SARS もヒトからヒトへの感染が拡大した<sup>17)</sup>。エボラ出血熱の場合患者の体液に曝露されるなど

かなり濃厚な接触がなければヒトからヒトへの感染の効率は悪いと考えられる<sup>18)</sup>。高病原性ニワトリインフルエンザウイルスに関してはこれまでのところ家族内での小規模なものを除けばヒト-ヒト感染は起きていない<sup>19)</sup>。しかし、ヒト型のレセプターを利用できる新型インフルエンザウイルスが遺伝子再集合あるいは1918年のスペイン風邪ウイルスのように変異の蓄積により生じた<sup>20)</sup>場合、新型ウイルスがヒト-ヒト感染を起こし世界中に拡大し、大きな被害をもたらす可能性も考えられる。

### 感染動物の個体数が多くヒトが罹患する機会が多い人獣共通感染症

狂犬病が日本から駆逐され50年近くになるが、世界的には年間35000人から50000人が狂犬病で死亡するとされている<sup>23)</sup>。アジアを中心とした発生国の多くでは未だにイヌが狂犬病ウイルスの伝播に重要な役割を演じている。イヌの個体数も多く、狂犬病の疑いのあるイヌへの曝露の機会が多いため、狂犬病発症阻止を目的とした曝露後ワクチン接種は年間1千万人に上るとされている。しかし各国からWHOに報告される狂犬病の発生数は実際よりも遙かに少なく見積もられているという。タンザニア共和国で行われた調査では実際の狂犬病発生数は公式な発生数の100倍に及ぶと推定されている<sup>24)</sup>。

1999年にニューヨークに登場したウエストナイルウイルス (WNV) は138種の鳥類<sup>25)</sup>を初めとし、ウマ、インドゾウ、インドサイ、ワオキツネザル、レッサーパンダ、ユキヒョウ、バビルーサ、コウモリ、シマリス、ハイイロリス、シマスカンク、ウサギ、ネコ、イヌ、ヒツジ、シロイワヤギ、オオカミ (幼獣)、ラマ、アルパカ、アリゲータなどの感染も報告されている。媒介するカに関しては43種<sup>26)</sup>で感染が確認されている。アメリカ大陸における WNV の流行がヨーロッパなどより激烈である理由として、アメリカ大陸の主要なカであるイエカ属のカが、実はヒトに対して嗜好性の高いカとトリに対する嗜好性の高いカとのハイブリッドであるからであることがごく最近明らかとなった<sup>25)</sup>。即ちこのハイブリッドのカの個体数が多いため、トリからヒトへの感染の拡大がヨーロッパなどより高率に起きると考えられるのである。

### 食品媒介性人獣共通感染症

食品の流通規模の拡大、加工食品需要の増大、また医療の進歩や急速な社会の高齢化に伴う免疫機能不全を有するヒトの増加といった近年の状況を背景に、食品媒介性感染症の発生は著しく増加している。また、30年前には知られていなかった食品媒介性感染症が新興感染症として登場してきているが、やはりその多くは人獣共通感染症である。WHOによれば2001年に下痢症で死亡した数は世界全体でおよそ200万人と推計されている<sup>26)</sup>。このうちの殆どが食

品あるいは水を介しての感染によるものと考えられている。アメリカ合衆国では毎年7600万件の食品由来感染症が発生しており、325000件が入院を要し、うち5000人が死亡しているといわれている<sup>27)</sup>。日本においても2002年には27629人の食中毒患者の発生を見ている<sup>28)</sup>。サルモネラ症、カンピロバクター症、コレラ、腸管出血性大腸菌症、リステリア症、エルシニア症などが主なものである。食品を介した人獣共通感染症の場合は直接的な健康被害の他に経済的被害も大きくなる。

## Ⅷ. 人獣共通感染症対策

感染症は感染源、感染経路、感受性宿主の3者が揃って成立する。即ちこの3者に対して適切な対処ができれば感染症の蔓延を防止することができる。一方、感染症は基本増殖率 (Basic reproduction ratio,  $R_0$ ) が1より大きい時に拡大する。 $R_0$ が1未満であれば感染の拡大はない。人獣共通感染症の場合には最も複雑な場合動物間での $R_0$ 、動物からヒトへの伝播における $R_0$ 、ヒトからヒトへの感染における $R_0$ を考える必要がある。通常 $R_0$ は $R_0 = \beta CD$ で表される。ここで、 $\beta$ は伝播率、 $C$ は一定時間における感受性ポピュレーションと感染ポピュレーションの接触頻度、 $D$ は感染期間である<sup>29)</sup>。従って、伝播の確率を下げ、接触機会を減らし、感染期間を短縮できれば $R_0$ を小さくすることが可能である。人獣共通感染症の多くは既に述べたようにヒト-ヒトでの感染は起きにくいと考えられるので、ヒトでの $R_0 < 1$ の感染症では動物における $R_0$ と、動物からヒトへの $R_0$ を1以下に納めるようにすればよい。即ち、感染巣動物の個体数を減らし、感受性動物数を減らすことにより、ヒトとの接触の機会を減らすことができると考えられる。

産業動物における感染症発生の場合にはその公衆衛生上の問題だけでなく経済的打撃の大きさも考慮し、安楽殺が重要な感染症対策とされる場合がある。マレーシアにおけるニパウイルス感染症の発生時には110万頭のブタが殺処分された<sup>30)</sup>。1983年から1984年にかけてペンシルバニア州で発生した高病原性ニワトリインフルエンザウイルスの場合はその制圧のために1700万羽が<sup>31)</sup>、1999年から2001年にかけてのイタリアにおける発生では1300万羽のトリが死亡あるいは殺処分されている<sup>32)</sup>。中国においてはSARSの元凶とされたハクビシンが大量に殺処分されたという。産業動物の場合はその経済性と効果を検討し、殺処分という選択肢を採る方が有効な場合も多く存在するが、感染巣動物が野生動物である場合には同様の効果が期待できるとは限らない。また動物が希少種のような場合には殺処分という選択肢はあり得ない場合もある。

エキノコックス症は日本ではキタキツネやイヌを終宿主とする多包条虫による感染症であるが、北海道ではエゾヤチネズミ (*Clethrionomys rufocanus bedfordiae*) が中間宿

主である。ヒトは終宿主から排泄される虫卵を経口的に摂取することによって感染する。エキノコックスのヒトへの感染を防止するためには動物からの $R_0$ を1以下にする、即ち経口感染の機会を減らすことが重要である。汚染した食品、水を避けることはもちろんであるが、もう一つ有効な手段は感染巣動物の個体数を減らすことである。といっても動物を駆除するのではなく駆虫薬をペイトに仕掛け、感染動物を治療するわけである。これによって動物における $R_0$ も動物からヒトへの $R_0$ も小さくすることができ、エキノコックス症の感染拡大を阻止できるわけである<sup>33)</sup>。

既に述べたように世界の多くの発展途上国では狂犬病の脅威は未だに現実のものである。発症すれば確実に死亡するのが狂犬病の恐ろしさであるが、一方でこの病原体には極めて有効なワクチンが開発されている。従って感染巣動物をなくすためにイヌのワクチン接種のみならず野生動物へのワクチン投与が有効な対策となると考えられる。実際に欧州各国ではキツネの狂犬病をペイトに仕込んだ経口ワクチンの撒布で激減させることに成功している<sup>34)</sup>。

腸管出血性大腸菌はウシに定着しており、牛肉などを介してヒトに感染し、時には重篤な溶血性尿毒症症候群、脳症、血栓性血小板減少性紫斑病を合併することが知られている。食肉の完全な調理、食肉からの2次汚染の防止、汚染生水の喫飲防止に努めれば感染は防げるものの、リザバーであるウシを減らすことも重要である。そのためにはワクチンの開発が望まれるが、Ⅲ型分泌タンパクでウシを免疫することによってO157:H7の定着率を著しく低下させることができることが最近報告されている<sup>35)</sup>。

スエーデンでは徹底したサルモネラの制御により、食肉と、卵のサルモネラ汚染を殆ど皆無とした。このためには現時点でも血清型の如何を問わず、ヒトであれ、動物であれ、飼料から食品に至るまで、サルモネラが検出された場合には衛生当局へ報告することになっている。ウシやブタでサルモネラ汚染が明らかになった場合は、感染が収まるまで動物の移動禁止、慢性感染動物の廃棄等を含む制限が課せられる。ニワトリの場合は殺処分される。このような厳しい処置により、サルモネラによる健康被害を減らそうとしているのである<sup>36)</sup>。

人獣共通感染症に有効な対策を講じるためには個々の疾患に関する疫学を十分に理解しておく必要がある。即ち各疾患の原因となる病原体の生態を詳細に理解することが重要である。そのためには適当なモニタリング、場合によってはサーベイランスを実施し、対策立案に必要な情報の収集・解析を行う必要がある。

また食品を介して伝播する人獣共通感染症に関しては「農場から食卓まで」一貫した衛生管理を行い、健康被害を最小限にする努力が払われる必要がある。Hazard Analysis and Critical Control Point (HACCP) は食品が元来有する危害による健康被害のリスクを最小にするよう、生産



表 2

輸入動物	個体数 (匹)
哺乳動物	854,202
霊長類	5,171
イヌ	4,948
フェレット	27,418
プレーリードッグ	11,473
ハムスター	678,793
リス	57,540
その他げっ歯類	51,373
鳥類	168,313
爬虫	879,157
両生類	11,587
その他	487,723,860
合計	489,637,119

工程の様々な部分でそのリスクを評価し、重要な管理点を明らかにし、モニター可能な方法で、衛生管理を徹底させることである。

人獣共通感染症対策として輸入動物対策も極めて重要である。表 2 は平成14年度わが国に輸入された動物のリストである (財務省貿易統計より)。

これらの動物のうち何らかの輸入規制のあるものは家畜伝染病予防法及び狂犬病予防法、感染症法で決められた動物のみ (偶蹄類、ウマ、ニワトリ、アヒル、ウズラ、シチメンチョウ、ガチョウ、ウサギ、ミツバチ、イヌ、ネコ、キツネ、アライグマ、スカンク、サル、ハクビシン、プレーリードッグ、ヤワゲネズミ (マストミス)、イタチアナグマ、コウモリ、タヌキ) であり、他の動物はフリーパスである。2003年世界中の関心が SARS に向けられていたアメリカで、サル痘の発生が報じられた<sup>27)</sup>。これはアフリカオニズミから感染したプレーリードッグからヒトが感染したわけだが、このアフリカオニズミと同居していたアフリカヤマネが日本に再輸出されていた。幸いなことに日本でサル痘の発生はなかったが、アフリカの奥地にいた動物がその翌日には家庭のペットとして、居間や食堂をうろうろしているという状況があり得るのである。

#### おわりに

人獣共通感染症はそれ自体決して目新しいものでも、珍しいものでもない。しかし、感染源となる動物が多くの場合野生動物であり、生態系と密接に関連しているという特徴がある。また、家畜などの場合は産業と直結しているし、愛玩動物の場合はヒトとの絆が深い。こういった事情から、対策を講じるにあたっては個々の疾患についての疫学的情報、病原体の生態等を十分に知る必要があるばかりか、実際の対策においては様々な関係者の利害を調整する必要がある場合もある。有効な動物由来感染症対策を立案実施する (リスクマネージメント) ためには科学的根拠に

基づいたリスクアセスメントが欠かせないだけでなく、関係者における円滑なリスクコミュニケーションも重要である。更にリスクアセスメントには獣医師、医師だけでなく、生態学、動物学、医動物学、鳥類学、気象学など多くの専門家との協力が必要になる場合もある。対策の実施にも多くの行政機関の円滑な協力関係が極めて重要である。

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## Zoonoses

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The numbers of microbial species that can infect human beings are shown to be 1415, of which 868 species (61%) are zoonotic. Since most of the emerging pathogens (75%) are originated from other animals, public health sectors should be vigilant against the emergence of new zoonotic diseases. Only 33% of zoonoses can spread from human to human after introduction into human population. Various factors such as human demography, ecological change, global transportation and climate change are responsible for the emergence of zoonoses. Even a slight change in the ecological niche where pathogenic organisms thrive would result in the increase of the incidence of the disease.