Efficacy study and field application of an inactivated new type gosling viral enteritis virus vaccine for domestic geese

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ABSTRACT New type gosling viral enteritis virus (NGVEV) caused a serious disease in naive juvenile goslings. In the described studies the performance of 2 vaccines was analyzed: a vaccine containing adjuvanted inactivated NGVEV and a vaccine containing adjuvanted inactivated NGVEV and recombinant goose IL-2. Breeder geese were subcutaneously vaccinated at the beginning of the egg production period with the vaccines. Breeder geese sham vaccinated with PBS served as control. The cellular and humoral immune responses of the vaccinated breeder goose, as well as the presence of maternally derived antibody to NGVEV, were investigated by ELISA, virus neutralization test, and lymphocyte proliferation assay, respectively. A significantly higher immunogenicity (P<0.05) was induced by the inactivated NGVEV–recombinant goose IL-2 adjuvant vaccine compared with the inactivated NGVEV vaccine. The offspring of the vaccinated birds were challenged with virulent NGVEV (100 50% lethal dose) and the protective efficacy of the vaccines was determined. Furthermore, in a field trial the efficacy of the inactivated NGVEV vaccine was recorded from years 2003 to 2007. No clinical signs or abnormal health status were observed in the vaccinated breeder goose and the progeny. After a single application, >80% protection was shown in the progeny of geese vaccinated against NGVEV challenge for approximately 5 mo. The extensive field trials further demonstrated that vaccination of breeder goose with the inactivated NGVEV vaccine could be a safe and efficacious means to control NGVE disease. Moreover, the level of maternally derived NGVEV antibody titer in the egg yolk reflected the level of NGVEV antibodies in the breeder goose, suggesting that the egg yolk could be used to monitor the vaccination efficacy in commercial goose breeder flocks.

Key words: new type gosling viral enteritis virus, inactivated vaccine, breeder goose, protective efficacy, laboratory and field trial

INTRODUCTION New type gosling viral enteritis (NGVE) was first reported by Cheng and colleagues (Cheng, 1998, 2000; Cheng et al., 2001; Lu and Wang, 2002). Observed in goslings less than 30 d of age, NGVE was regarded as a new disease and the causative agent was identified as adenovirus, namely new type gosling viral enteritis virus (NGVEV; Wang et al., 2002; Chen et al., 2008a; Saif et al., 2008). In gosling-producing areas where the disease has been reported, NGVE has caused significant economic losses in the offspring of breeder geese as a result of high mortality and disease characterized by digestive, neurological, and respiratory symptoms and sudden death (Cheng, 1998; Cheng et al., 2001; Yu et al., 2003; Zhao and Tang, 2006; Liu et al., 2008). The isolated NGVEV was capable of reproducing the disease identical to natural infection in terms of clinical signs and histopathologic lesions, and the oral route was considered to be the best infection route (Cheng, 2000; Cheng et al., 2001). Catarrhal hemorrhagic fibrinonecrotic enteritis and coagulative embolus in the middle-lower part of the intestine were the typical pathological lesions of the NGVEV-infected goslings (Cheng et al., 2001; Cheng and Wang, 2002). Some diagnostic meth-
ods were reported for NGVE disease (Cheng, 1999a, b, 2000; Cheng et al., 2001; Cheng and Wang, 2002; Zhao and Tang, 2006; Chen et al., 2008a, b, 2009a, b, 2010b; Liu et al., 2008). It has been described that NGVE induced apoptosis (Chen et al., 2008a, c, d, e). In addition, a partial nucleotide sequence was determined (Wei et al., 2004) and the structural protein of NGVEV was previously identified (Wang et al., 2002). Currently, no vaccine is available to prevent disease caused by NGVEV. Therefore, it is imperative to develop a safe and effective vaccine to control and eradicate NGVEV disease.

Vaccines play a major role in preventing of infectious diseases (Rappuoli et al., 2002). Previous experiences indicated that inactivated vaccine was much easier and successful development was more promising (Seto, 1981; Pattison and Cook, 1996). New type gosling viral enteritis is a serious disease in naive juvenile goslings, and control measures are supposed to generate adequate immunity during the first 4 wk of life. Some of the early outbreaks of NGVE occurring in China were controlled by the use of hyperimmune serum to obtain passive protection in newly hatched goslings (Cheng, 1998). However, passive immunization was found to be expensive and time consuming. Our previous study indicated that the inactivated NGVEV vaccine could elicit the humoral and cellular immunity responses in adult geese, and the vaccine in conjunction with 1,000 U of recombinant goose IL-2 (rGoIL-2) adjuvant per goose is the most effective dose (Chen et al., 2010a). Maternally derived antibody is acquired by progeny through the passage of IgG from bird serum to yolk (Brambell, 1970), which is an important part of the protective immunity in the offspring (Horie et al., 1996; Sharma, 2003).

In this study, immunization of breeder geese with the inactivated NGVEV vaccine in conjunction with or without rGoIL-2 adjuvant at the beginning of the egg production period was evaluated, and the protective efficacy of vaccination was determined in laboratory trials. Furthermore, field trials of the inactivated NGVEV vaccine were conducted from 2003 to 2007 in commercial white female breeder goose farms of the Sichuan province. The progeny egg yolk could be used instead of serum in assessing response to vaccination in commercial vaccinated breeders by ELISA or virus neutralization test (VNT), which is less time-consuming.

MATERIALS AND METHODS

Viral Strain, Rabbit Anti-NGVEV Polyclonal Serum, Formalin-Inactivated NGVEV Vaccine, and rGoIL-2 Adjuvant

The highly virulent NGVEV-CN isolate, which has been described previously (Cheng et al., 2001; Chen et al., 2008a, b, c, d), was provided by Avian Disease Research Center of Sichuan Agricultural University (Yaan, Sichuan, China). The rabbit anti-NGVEV polyclonal serum was provided by Avian Disease Research Center of Sichuan Agricultural University. The rabbit anti-NGVEV polyclonal serum had been prepared from male New Zealand rabbit and purified through caprylic acid and ammonium sulfate method and High-Q columns anion exchange chromatography (Bio-Rad, Hercules, CA), which has been described previously (Chen et al., 2009a, b).

The rGoIL-2 and the formalin-inactivated NGVEV vaccine were provided by Avian Disease Research Center of Sichuan Agricultural University. The preparation has been described previously (Chen et al., 2010a).

Animal Immunization

In this study, 150 Sichuan white female breeder geese (Anser cygnoides Linn. var domestica; 7 mo of age) were used at the beginning of the egg production period. All the breeder geese were tested by ELISA before immunization and were negative for antibody to NGVEV. The geese were randomly divided into 3 equal groups (by random number table), which were subcutaneously inoculated with 3 different vaccines as follows (according to Chen et al., 2010a). Group A received inactivated NGVEV vaccine in conjunction with rGoIL-2 adjuvant (1,000 U/gosling; 0.5 mL/gosling). Group B received inactivated NGVEV vaccine (0.5 mL/gosling). Group C received PBS solution (0.15 mol/L; 0.5 mL/gosling). The subcutaneous injection site was the nape of the neck. Vaccination day was referred to as d 0. Three groups of immune geese were maintained in the different isolation units in a biosecure animal building and were fed a commercial diet ad libitum. The health of the inoculated geese was observed daily and their weights were measured weekly.

Blood Collection

Blood samples of the breeder geese were drawn on d 0, 3, 7, 14, 21, 28, 35, 49, 63, 77, 91, 105, 119, 133, 147, 161, and 175 postvaccination (PV). The serum was collected from blood after centrifugation at 3,000 × g for 10 min at 4°C, heat inactivated at 56°C for 30 min, batched, and stored at −20°C. At each time, 30 vaccinated geese from each group were chosen randomly for sampling (by random number table). The blood collected before vaccination was treated as the control sample. All the sera were tested for humoral immune response by ELISA and VNT, and the samples of whole blood (with heparin) collected earlier than 49 d PV were evaluated for cellular immunity response by using lymphocyte proliferative assay.

Egg Yolk Collection

Ten progeny eggs were collected from inoculated breeders on d 0, 3, 7, 14, 21, 28, 35, 49, 63, 77, 91, 105,
Neutralization antibody titers were measured as in our previous study (Chen et al., 2010a). Briefly, peripheral blood mononuclear cells from the obtained blood samples were isolated by Ficoll gradient centrifugation (density 1.077, TBD Sciences, Tianjin, China). The viability of the cells was estimated with trypsin blue dye. Pellets were washed with cold RPMI-1640 medium (Invitrogen) containing penicillin-streptomycin and resuspended in RPMI-1640 medium supplemented with 10% newborn calf serum (Hyclone, Logan, UT). Cells (5 × 10⁶ cells/mL) were seeded in the 96-well flat-bottom plates at 100 μL/well (triplicate wells per sample). Concanavalin A at a working concentration of 25 μg/mL was added to stimulate the cells. The peripheral blood mononuclear cells of nonimmune geese were used as negative controls. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma) was added to each culture well. Then, 100 μL of dimethyl sulfoxide was added in each well to completely dissolve the purple crystal. The optical density (OD) value of each well was measured using a microplate reader (ELX80, BioTek Instruments) with a test wavelength of 490 nm. The lymphocyte proliferation index (LPI) was calculated by subtracting the mean OD of negative controls from the mean OD of the vaccinated group. The results are shown as the arithmetic mean ± the SE of the individual LPI.

Protective Efficacy of Vaccine in Laboratory Trial

Ten goslings (3-d-old) derived from 3 groups of the vaccinated breeders on d 0, 3, 7, 14, 21, 28, 35, 49, 63, 77, 91, 105, 119, 133, 147, 161, and 175 PV were collected for histopathologic and immunohistochemical examination.

Samples were subjected to hematoxylin-eosin (HE) staining for observation of histological changes as described (Cheng et al., 2001). Following challenge, the clinical signs and the mortality were monitored daily for at least 4 wk. The intestinal tracts of the dead and the remaining progeny from vaccinated breeder geese post NGVEV challenge were collected for histopathologic examination (density 1.077, TBD Sciences, Tianjin, China). The viability of the cells was estimated with trypsin blue dye. Pellets were washed with cold RPMI-1640 medium (Invitrogen) containing penicillin-streptomycin and resuspended in RPMI-1640 medium supplemented with 10% newborn calf serum (Hyclone, Logan, UT). Cells (5 × 10⁶ cells/mL) were seeded in the 96-well flat-bottom plates at 100 μL/well (triplicate wells per sample). Concanavalin A at a working concentration of 25 μg/mL was added to stimulate the cells. The peripheral blood mononuclear cells of nonimmune geese were used as negative controls. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma) was added to each culture well. Then, 100 μL of dimethyl sulfoxide was added in each well to completely dissolve the purple crystal. The optical density (OD) value of each well was measured using a microplate reader (ELX80, BioTek Instruments) with a test wavelength of 490 nm. The lymphocyte proliferation index (LPI) was calculated by subtracting the mean OD of negative controls from the mean OD of the vaccinated group. The results are shown as the arithmetic mean ± the SE of the individual LPI.

Virus Neutralization Test

Neutralization antibody titers were measured as in our previous study (Chen et al., 2010a). Briefly, each sample was serially diluted into 2-fold dilution with Eagle’s minimal essential medium (Invitrogen, Carlsbad, CA), then mixed with an equal volume of 100 50% tissue culture infective dose of NGVEV (determined by the Reed-Muench method). After neutralization, each grade of the mixture was successively added into monolayer primary duck embryo fibroblast cells in microplates (100 μL/well). Each dilution was tested in triplicate. A volume of 100 μL of minimal essential medium was added to wells for the duck embryo fibroblast cell control and unneutralized 100 tissue culture infective dose NGVEV was added to wells for the virus control. The specific cytopathic effect (CPE) was monitored every 12 h until all the virus controls showed CPE in comparison with the noninfected control cells. Neutralization antibody titer was defined as the highest dilution of serum that protects 50% of the cultures against CPE, which calculated as the log2. Results are given as the arithmetic mean ± the SE of the individual titer.

ELISA

The ELISA assay was performed as in our previous study (Chen et al., 2010a). Briefly, polystyrene 96-well plates were coated with purified, diluted, and inactivated NGVEV (Chen et al., 2009a,b; 100 μL/well, contained 6 μg of virus protein). The samples were tested at a dilution of 1:100 (100 μL/well). Each dilution was assayed in triplicate. After washing the plates, the horseradish peroxidase-conjugated rabbit antigoose IgG antibody at a dilution of 1:2,000 (100 μL/well) and the 3,3',5,5'-tetramethylbenzidine substrate (Sigma, St. Louis, MO; 100 μL/well) were sequentially added to each well. After stopping the colorimetric reaction with 0.5 mol/L of sulfuric acid (100 μL/well), the absorbance value at 450 nm was measured using a microplate reader (ELX80, BioTek Instruments, Winooski, VT). The results are presented as the arithmetic mean ± the SE of individual absorbance values.

Lymphocyte Proliferative Response

Lymphocyte proliferative response was performed as in our previous study (Chen et al., 2010a). Briefly, peripheral blood mononuclear cells from the obtained blood samples were isolated by Ficoll gradient centrifugation (density 1.077, TBD Sciences, Tianjin, China). The viability of the cells was estimated with trypsin blue dye. Pellets were washed with cold RPMI-1640 medium (Invitrogen) containing penicillin-streptomycin and suspended in RPMI-1640 medium supplemented with 10% newborn calf serum (Hyclone, Logan, UT). Cells (5 × 10⁶ cells/mL) were seeded in the 96-well flat-bottom plates at 100 μL/well (triplicate wells per sample). Concanavalin A at a working concentration of 25 μg/mL was added to stimulate the cells. The peripheral blood mononuclear cells of nonimmune geese were used as negative controls. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma) was added to each culture well. Then, 100 μL of dimethyl sulfoxide was added in each well to completely dissolve the purple crystal. The optical density (OD) value of each well was measured using a microplate reader (ELX80, BioTek Instruments) with a test wavelength of 490 nm. The lymphocyte proliferation index (LPI) was calculated by subtracting the mean OD of negative controls from the mean OD of the vaccinated group. The results are shown as the arithmetic mean ± the SE of the individual LPI.

Ten goslings (3-d-old) derived from 3 groups of the vaccinated breeders on d 0, 3, 7, 14, 21, 28, 35, 49, 63, 77, 91, 105, 119, 133, 147, 161, and 175 PV were collected for histopathologic and immunohistochemical examination.
The surviving birds that showed no clinical signs, pathological change, and immunostaining cell were recorded as being NGVE-negative. The protection percentage was calculated by dividing the number of NGVE-negative goslings by the total number of challenged goslings.

**Protective Efficacy of Vaccine in Field Trial**

The inactivated NGVEV vaccine, which was safe and efficacious in the laboratory trials, was further applied for field trials from 2003 to 2007 in several commercial goose farms of the Sichuan province selected on the basis of prevalence of NGVEV infection. Prior to vaccination, to investigate whether the geese were negative for antibody to NGVEV, 50 serum samples were randomly obtained from each farm and tested by indirect ELISA as described above. All breeder geese were subcutaneously inoculated with the inactivated NGVEV vaccine (10³ 50% egg infectious dose/mL; 0.5 mL) before the laying period; these geese were recorded as being from the vaccination farms. Other commercial goose farms, far from the farms in which the geese were vaccinated, were used as controls. All progeny goslings from the vaccinated or control breeders were observed daily and mortality was recorded for 4 wk. Dead goslings (30%/d) were randomly taken from both the vaccinated and nonvaccinated control breeders. The cause of death was analyzed by the laboratory diagnosis using histopathological and immunohistochemical methods.

**Statistical Analysis**

The data of ELISA, VNT, and LPI from all groups were statistically compared by the statistical software program Systat 11.0 (SPSS 11.0, SPSS Inc., Chicago, IL). The differences between the groups were analyzed by 2-way ANOVA and Tukey’s multiple comparison test. The data were considered to be significantly different if \( P < 0.05 \).

**RESULTS**

**Health Status of the Breeder Geese Postvaccination**

No change was observed in the health status and no clinical sign was observed in 3 groups of the vaccinated breeder geese in the experimental trials as well as in the field trials. No harmful effect on the weight gain of vaccinated geese was detected.

**Absorbance Values of the Specific Anti-NGVEV Antibody**

The time-course analysis of specific anti-NGVEV antibody in the serum and the yolk of the appropriate eggs by an indirect ELISA is shown in Figure 1. No NGVEV-specific antibodies were detected in the samples from birds belonging to the PBS sham-vaccinated group. The specific anti-NGVEV antibody titers in the breeder serum and the egg yolk were increased on d 7 PV and showed the highest values on d 28 PV. This was followed by steadily decreasing titer values until d 175 PV. The maximum specific anti-NGVEV antibody titer in the breeder serum was higher than that in the egg yolk obtained from the appropriate group. A significantly higher \( (P < 0.05) \) value was elicited by the group vaccinated with inactivated NGVEV–rGoIL-2 vaccine compared with the group vaccinated with inactivated NGVEV vaccine from d 7 PV onward. The vaccine-induced specific anti-NGVEV antibody titer value in the serum correlated with the value observed in egg yolk of the appropriate eggs, indicating a fast transfer of maternally derived antibodies to the egg yolk.

**Serum Neutralization Antibody Titer**

The time-course analysis of NGVEV neutralization antibody titers in the serum and the yolk of the appropriate eggs is shown in Figure 2. In the group sham vaccinated with PBS, no NGVEV-specific neutralization antibodies were observed. The neutralization antibody titer levels in the breeder serum and in the egg yolk were remarkably increased starting with d 7 PV and reached the highest level on d 28 PV, and then decreased over time (Figure 2). A significantly higher \( (P < 0.05) \) neutralization antibody titer was elicited by the group vaccinated with inactivated NGVEV–rGoIL-2 vaccine compared with the group vaccinated with inactivated NGVEV vaccine beginning from d 7 until the end of the study. The vaccine-induced neutralization antibody titer in the serum correlated with the titer observed in egg yolk of the eggs obtained from the appropriate group (Figure 2).

**Lymphocyte Proliferative Index**

Lymphocyte proliferation assay showed that inoculation with the inactivated NGVEV vaccine could induce a lymphocyte proliferative response as measured by the determination of the LPI (Figure 3). Hardly any increasing LPI was detected in the PBS group during the experiment. The LPI of birds from both groups vaccinated with either inactivated NGVEV–rGoIL-2 adjuvanted vaccine or the inactivated NGVEV vaccine was increased from 7 d PV and reached the highest value on d 28 PV. This was followed by a decrease of the LPI until the end of the study. The results indicated that LPI of the inactivated NGVEV–rGoIL-2 adjuvant vaccinated group was significantly higher \( (P < 0.05) \) than that of the inactivated NGVEV vaccine from d 3 PV onwards.
Figure 1. Dynamic changes of the anti-new type gosling viral enteritis virus (NGVEV) antibody postvaccination with different vaccines. Significant differences (indicated by asterisk; \( P < 0.05 \)) were observed from 7 d over time. A, B, and C were the serum of the breeders and a, b, and c were the egg yolks of the progeny goslings. A and a: inactivated NGVEV vaccine in conjunction with recombinant goose IL-2 adjuvant. B and b: inactivated NGVEV vaccine. C and c: PBS solution. Data are the mean ± SE of individual absorbance values.

Figure 2. Titers of neutralization antibody postvaccination with different vaccines. Significant differences (indicated by asterisk; \( P < 0.05 \)) were observed from 7 d over time. No neutralization antibody to new type gosling viral enteritis virus (NGVEV) was detected in the PBS group. A, B, and C were the serum of the breeders and a, b, and c were the egg yolks of the progeny goslings. A and a: inactivated NGVEV vaccine in conjunction with recombinant goose IL-2 adjuvant. B and b: inactivated NGVEV vaccine. C and c: PBS solution. Data are the mean ± SE of individual titers.
Protective Efficacy of Vaccine in Laboratory and Field Trials

In the laboratory trial all progeny goslings of the PBS-vaccinated group died following the NGVEV challenge (Figure 4). Of the progeny goslings from breeders vaccinated with the inactivated NGVEV, 70% were protected against NGVEV challenge when eggs were taken 14 d PV. At the same time, 100% protection from virus challenge was observed in the offspring of geese vaccinated with the inactivated NGVEV–rGoIL-2 adjuvant vaccine. Complete protection was observed in the group vaccinated with the inactivated NGVEV vaccine when eggs were taken on 28 d PV. This level of protection was observed until d 133 (inactivated NGVEV vaccine) and d 161 (inactivated NGVEV–rGoIL-2 adjuvant vaccine) after vaccination. This was followed by a decreasing level of protection in both groups. In general, an earlier and longer lasting protective efficacy was detected in the inactivated NGVEV–rGoIL-2 adjuvant vaccine group compared with progeny of geese vaccinated with the inactivated NGVEV vaccine.

Microscopical examination of HE-stained gut samples of NGVEV challenged and subsequently dead goslings showed that most of the villus epithelia and lamina propria were necrotic and some of this debris was partly or completely sloughed into the lumen of the intestine, exposing the lamina propria to the gut lumen (Figure 4, panel L). This resulted in a catarrhal hemorrhagic fibrinonecrotic enteritis of the small intestine (Figure 4, panel L), which was considered as the typical pathological lesion of NGVE infection. Immunohistochemical examination further confirmed that NGVEV antigens were mainly located in the intestine (arrow 1 in Figure 4, right panel) and the intestinal coagulative embolus (arrow 2 in Figure 4, right panel) of dead goslings.

In field trials performed from 2003 to 2007 the number of vaccinated breeder geese and mortality of the progeny is shown in Table 1. The data clearly show that the inactivated NGVEV vaccine was able to induce protection of goslings from NGVE as indicated by the low number of dead goslings obtained from vaccinated breeders. The NGVEV was confirmed to be the pathogen of dead goslings by random sampling through the preliminary epizootiologic investigation, clinical signs observation, causal agent isolation, and histopathologic and immunohistochemical examinations.

DISCUSSION

It is well known that birds transfer their serum immunoglobulins to the egg yolk, which transfers humoral immunity to the offspring (Seto, 1981; Sharma, 2003). The increase of antibody level to antigen in the breeder could result in a higher level of maternally derived antibody in the progeny. Consequently, the progeny could be better protected from the disease (Yuasa et al., 1980; Seto, 1981; Sharma, 1991). Maternally derived antibodies protect progeny from infection through immunizing the breeder animals with a commercial vaccine, for example, against avian infectious bronchitis, infectious bursal disease, chicken anemia, and hemorrhagic enteri-
tis of turkey (Jungherr and Terrell, 1948; Patterson et al., 1962; Wyeth and Cullen, 1976; Fadly and Nazerian, 1989; Otaki et al., 1992).

New type gosling viral enteritis is a highly contagious and economically devastating disease of goslings (Cheng, 1998, 2000; Cheng et al., 2001), and no commercial vaccine was available. Our previous research indicated that the inactivated NGVEV vaccine (10^5 50% egg infectious dose/mL; 0.5 mL/goose) could elicit strong humoral and cellular responses in vaccinated adult geese, and rGoIL-2 (1,000 U/goose) as adjuvant injected in combination with the vaccine was considered as the most effective dose (Chen et al., 2010a). Here, for a vaccine development for breeders, a preliminary evaluation of a candidate vaccine in breeder geese and progeny was performed by analysis of the efficacy in a laboratory trial and field trials.

Our results clearly indicated that the inactivated NGVEV vaccine was highly immunogenic in the breeder geese and the induced antibodies were transferred to the offspring via the egg yolk. After a single vaccination, a substantial increase in ELISA antibody titers and virus neutralization antibody titers were observed. These test values maintained a stable high level for the duration of the experiment. The vaccination could transfer the protection to progeny goslings. In efficacy trials, over 80% protection was recorded in the progeny of geese vaccinated against NGVEV challenge for approximately 5 mo. A summary of 5 yr of field application further demonstrated that the inactivated NGVEV

<table>
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<th>Year</th>
<th>Vaccinated breeders (n)</th>
<th>Mortality of progeny (n/n)</th>
<th>Vaccinated breeders (n)</th>
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<tr>
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<td>1,312</td>
<td>17/78,720</td>
<td>1,288</td>
<td>9,543/77,280</td>
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1The progeny goslings from the vaccinated or control breeders were observed daily and the mortality was recorded at each farm for 4 wk. Cause of death was investigated by laboratory examinations (histopathological and immunohistochemical assays) by random sampling of dead goslings. The mortality of progeny is shown by the annual number of dead goslings per total number of progeny.

Figure 4. Protective efficacy of inactivated new type gosling viral enteritis virus (NGVEV)–recombinant goose IL-2 adjuvant vaccine (A), inactivated NGVEV vaccine (B), and PBS (C). The surviving birds showed no clinical sign or pathological change and immunostaining cell was recorded as NGVE negative. Protection percentage was calculated by dividing the number of NGVE-negative goslings by the total number of challenged goslings. Color version available in the online PDF. Panel L: catarrhal hemorrhagic fibrinonecrotic enteritis of the small intestine was observed in the dead gosling (8 d of age) post–NGVEV challenge by hematoxylin-eosin staining. Bar = 100 μm. Panel R: NGVEV antigens were widely detected in the intestine (arrow 1) and the intestinal coagulative embolus (arrow 2) of the dead gosling (8 d of age). The avidin-biotin-peroxidase complex method of immunoperoxidase staining is shown. Bar = 100 μm.
vaccine was safe, efficacious, and long lasting. The vaccine could protect the progeny of vaccinated breeders from clinical NGVE disease.

The yolk proved to be a suitable fluid for the detection of specific IgY antibody. Many reports indicated that yolk samples could be used to monitor the antibody of the breeder flock instead of serum (Rose and Mockett, 1983; Silim and Venne, 1989; Trampel et al., 2006). Bar-Joseph and Malkinson (1980) suggested that the egg yolk can provide a ready source of antibodies to various antigens.

The vaccine-induced anti-NGVEV antibody titers in the breeder geese serum and in the egg yolk of the progeny increased and declined in similar trends, indicating an expected dependence. Moreover, the maternally derived antibodies have a known protective effect against NGVE diseases (Cheng, 1998). It is suggested that the detection of maternally derived NGVEV antibody titers in the progeny yolk could be used for the specific antibody surveillance in layers, instead of serologic surveillance program, which would be less time consuming but have the same sensitivity and specificity. The latter will be confirmed by further additional experiments.

In conclusion, the laboratory and field trials unequivocally affirmed that the inactivated NGVEV vaccine is satisfactory. One routine annual flock vaccination could provide antibodies to cover the entire laying period. This seems to be the most effective method of controlling NGVE disease in goslings before a new alternative vaccine is developed.

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