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Nucleocapsid Protein N of Lelystad Virus: Expression by Recombinant Baculovirus, Immunological Properties, and Suitability for Detection of Serum Antibodies

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The ORF7 gene, encoding the nucleocapsid protein N of Lelystad virus (LV), was inserted downstream of the P10 promoter into *Autographa californica* nuclear polyhedrosis virus (baculovirus). The resulting recombinant baculovirus, designated bac-ORF7, expressed a 15-kDa protein in insect cells. This protein was similar in size to the N protein expressed by LV in CL2621 cells when it was analyzed on sodium dodecyl sulfate-polyacrylamide gels. The N protein expressed by bac-ORF7 was immunoprecipitated with anti-ORF7 peptide serum, porcine convalescent-phase anti-LV serum, and N protein-specific monoclonal antibodies, indicating that this N protein had retained its native antigenic structure. The recombinant N protein was immunogenic in pigs, and the porcine antibodies raised against this protein recognized LV in an immunoperoxidase monolayer assay. However, pigs vaccinated twice with approximately 20 μg of N protein were not protected against a challenge with 10^5 50% tissue culture infective doses of LV. Experimental and field sera directed against various European and North American isolates reacted with the N protein expressed by bac-ORF7 in a blocking enzyme-linked immunosorbent assay. Therefore, the recombinant N protein may be useful for developing diagnostic assays for the detection of serum antibodies directed against different isolates of LV.

Lelystad virus (LV), also called porcine reproductive respiratory syndrome virus, causes respiratory distress in piglets and reproductive problems in sows (4, 28). LV contains a positive-strand RNA genome of 15.1 kb, and during infection of alveolar lung macrophages, a 3′ nested set of six subgenomic RNAs is synthesized (5, 13, 14). LV belongs to a group of small enveloped positive-strand RNA viruses, provisionally designated arteriviruses. This group also includes lactate dehydrogenase-elevating virus, equine arteritis virus, and simian hemorrhagic fever virus (5, 14).

Although the European and U.S. isolates of LV share similar morphological and physicochemical properties, the various isolates from Europe and the United States are antigenically different (6, 19, 26). Open reading frames (ORFs) 2 to 7 of LV share only 55 to 79% identical amino acids with those of U.S. and Canadian strains (11, 12, 18).

Recently, we have further characterized the protein compositions of the virions of LV. Three structural proteins were identified: the *N*-glycosylated envelope protein E of 25 kDa encoded by ORF5, the non-*N*-glycosylated membrane protein M of 18 kDa encoded by ORF6, and the 15-kDa nucleocapsid protein N encoded by ORF7 (15). The N protein appears to be most abundantly present in the virions of LV, and sera from pigs infected with LV are highly reactive with the N protein (2, 15). Furthermore, most of the monoclonal antibodies (MAbs) obtained from mice immunized with semipurified LV specifically recognize the N protein (6, 19).

In the study described in this report we studied the antigenic properties of the N protein in more detail, using a baculovirus recombinant expression product. The recombinant N protein appeared to be a good candidate for use in a diagnostic blocking enzyme-linked immunosorbent assay (ELISA) for detection of serum antibodies from pigs infected with LV.

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MATERIALS AND METHODS

Cells and viruses. *Autographa californica* nuclear polyhedrosis virus (AcNPV) and recombinant viruses were propagated in the *Spodoptera frugiperda* cell line Sf21 (23). Sf21 cells were grown as described by Hulst et al. (7). LV and ATCC VR2332 were grown either on porcine alveolar macrophages or on CL2621 cells (courtesy of Boehringer-Ingelheim, St. Joseph, Mo.). Macrophages were maintained as described before (28). CL2621 cells were maintained in Eagle's basal medium supplemented with 5% fetal bovine serum, 100 IU of penicillin per ml, and 100 μg of streptomycin per ml.

Plasmid constructions. The specific oligonucleotides LV40 (5′-GATTGGATCCTCGTCAAGTATGGCCGG-3′) and LV41 (5′-CTAAGGATCCTGTCAAATTAACTTGCA-3′), located upstream and downstream of ORF7, respectively, were synthesized. The underlined nucleotides were not derived from the genome sequence of LV but were added in order to create *Bam*HI sites in the primers. These primers were used to amplify ORF7 from cDNA clones by PCR as described earlier (13). The *Bam*HI restriction sites incorporated in the primers were used to clone ORF7 downstream of the p10 promoter of baculovirus transfer vector pACAS3 (24). Recombinant DNA techniques were performed essentially as described by Sambrook et al. (21).

Transfection and selection of baculovirus recombinants expressing N protein. Wild-type AcNPV DNA and pACAS3 ORF7 DNA were cotransfected in Sf21 cells as described by Hulst et al. (7). Recombinant viruses expressing β-galactosidase were plaque purified at least three times and were tested for their expression of the N protein in the immunoperoxidase monolayer assay (IPMA) with porcine anti-LV serum sample 21 (15) and MAb SDOW17, which is specific for the N protein (19), essentially as described by Wensvoort et al. (27).

Viral and cellular DNAs were isolated from Sf21 cells infected with wild-type and recombinant AcNPV as described by Summers and Smith (22). Viral DNA was digested with *Bam*HI and *Xho*I to confirm that the ORF7 gene was correctly inserted in the p10 locus of baculovirus.

Radioactive labeling and analysis of proteins. Confluent monolayers of Sf21 cells were infected with recombinant or wild-type baculovirus at a multiplicity of infection of 5. At 40 h after infection, cells were labeled with L-[^{35}S]methionine, and cell lysates were prepared as described by Hulst et al. (7). The cell lysates were used for immunoprecipitation. Metabolic labeling of CL2621 cells infected with LV was performed as described previously (15).

Immunoprecipitation and gel electrophoresis. Recombinant radiolabeled N protein was immunoprecipitated with porcine anti-LV serum sample 21; N-specific peptide serum sample 713, which is directed to the same peptide as serum sample 714 (15); and MAbs SDOW17 and WBE6 (6). Immune complexes

were bound to protein A-Sepharose CL-4B beads (Pharmacia) essentially by the method of Hulst et al. (7). The protein A-Sepharose pellets were then resuspended in Laemmli sample buffer, and the mixture was heated for 2 min at 100°C before analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on gels containing 12.5% polyacrylamide (9). The gels were soaked for 30 min in Amplify (Amersham) for fluorography and dried, and the immunoprecipitated proteins were visualized by autoradiography. The same procedure was used to immunoprecipitate proteins from lysates of CL2621 cells infected with LV.

Time course of N protein production as measured by ELISA. Nearly confluent monolayers of Sf21 cells were infected with recombinant baculovirus bac-ORF7 at a multiplicity of infection of 5 to 10 50% tissue culture infective doses ($TCID_{50}$s). At 24, 48, 72, and 96 h after infection, the cells were harvested, washed with phosphate-buffered saline (PBS), and lysed on ice in lysis buffer (30 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, and 1% Nonidet-P40; 40×10^6 cells per ml). The lysate was centrifuged at $1,400 \times g$ for 5 min to remove nuclei containing polyhedrin and cellular debris. Aliquots of the bac-ORF7 lysate were stored at −70°C.

The ELISA plates were coated overnight with bac-ORF7 cell lysate diluted 1:125 in coating buffer (50 mM $NaHCO_3$ [pH 9.65]). The plates were washed in PBS containing 0.05% Tween 80 and were incubated for 1 h at 37°C with a 1:500 dilution of ascitic fluid of protein N-specific MAb WBE6 diluted in ELISA buffer (PBS, 0.05% Tween 80, and 4% horse serum). The plates were washed and incubated for 1 h at 37°C with horseradish peroxidase (HRPO)-conjugated rabbit antibodies directed against mouse immunoglobulin diluted 1:1,000 in ELISA buffer. The plates were washed and stained as described previously (7).

Blocking ELISA for detection of N protein-specific antibodies. Lysates of Sf21 cells, harvested 2 days after infection with recombinant baculovirus, were used to coat ELISA plates as described above. The wells were washed and incubated with 100 μl of test serum diluted 1:4 in ELISA buffer. Each serum sample was tested in triplicate. As a control, serial dilutions (1:4 to 1:2,048) of porcine anti-LV serum sample 21 and three negative serum samples were tested on each plate. After a 1-h incubation at 37°C, 25 μl of culture supernatant of MAb SDOW17 (immunoglobulin concentration, approximately 20 μg/ml) was added to each well, and the incubation was continued for 1 h at 37°C. Plates were washed and incubated for 1 h at 37°C with HRPO-conjugated rat MAb (RM19ME) to mouse kappa chain diluted 1:750 (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). The plates were washed and stained as described above. When the binding of MAb SDOW17 to recombinant N protein is blocked by pig immunoglobulins, the anti-mouse MAb RM19ME is not able to bind, indicating that the test serum is positive for LV. Blocking was calculated by the following formula: % blocking = 100 − (mean OD of test serum/mean OD of negative pig sera) × 100, where OD is optical density.

Immunization of pigs with bac-ORF7 and challenge with LV. Two 2-week-old specific-pathogen-free pigs were immunized intramuscularly with 2 ml of bac-ORF7 lysate (approximately 20 μg of N protein) in a double water-oil emulsion (1) on days 0 and 28. Two control pigs were immunized similarly with 2 ml of wild-type baculovirus lysate. On day 42 all four pigs were challenged intranasally with 1 ml of 10^5 $TCID_{50}$s of LV. Serum samples were collected on days 0, 21, 28, 42, 45, 52, 58, and 63. Titers on macrophages infected with LV were determined in all serum samples by IPMA as described by Wensvoort et al. (28). The blocking ELISA for N protein was performed with serum samples collected on days 0, 21, 42, and 58. LV titers were also determined in sera collected after challenge as described by Wensvoort et al. (28). All animals were observed daily for signs of disease, that is, fever or respiratory distress. Pigs were killed on day 63 after immunization, and tissue sections of lungs were analyzed for lesions. Viral antigens were visualized by an immunoperoxidase staining method with MAb SDOW17 and HRPO-conjugated rabbit antibodies directed against mouse immunoglobulin as described earlier by Pol et al. (20).

RESULTS

Expression and antigenicity of the ORF7-encoded N protein of LV in insect cells. The ORF7 gene was amplified from a cDNA clone by PCR as described in Materials and Methods and was cloned downstream of the p10 promoter of AcNPV in the transfer vector pACAS3 (24). Sf21 cells were cotransfected with the transfer vector pACAS3-ORF7 and wild-type AcNPV DNA. Polyhedrin-positive plaques expressing β-galactosidase were isolated and analyzed for their expression of the N protein in an IPMA with porcine anti-LV serum sample 21 or MAb SDOW17, both of which recognized the N protein of LV (15, 19). One purified recombinant virus that reacted positively in this IPMA was designated bac-ORF7 and was used for further studies.

The recombinant expression product of bac-ORF7 was further characterized by radioimmunoprecipitation. A 15-kDa

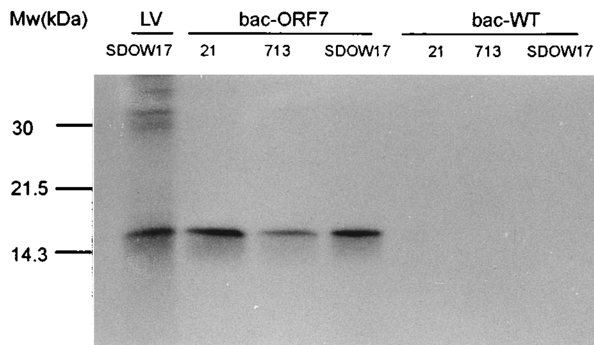

FIG. 1. Immunoprecipitation of the N protein from Sf21 cells infected with bac-ORF7 or CL2621 cells infected with LV. Sf21 cells were infected with bac-ORF7 or wild-type baculovirus (bac-WT) at a multiplicity of infection of 5 to 10, and at 40 h after infection the cells were labeled with L-[^{35}S]methionine as described by Hulst et al. (7). Lysates of Sf21 cells infected with bac-ORF7 or bac-WT were immunoprecipitated with porcine anti-LV serum sample 21, N protein-specific anti-peptide serum sample 713, and N protein-specific MAb SDOW17. The authentic N protein of LV was immunoprecipitated with MAb SDOW17 from L-[^{35}S]methionine-labeled CL2621 cells infected with LV as described by Meulenberg et al. (15); this is shown in the first lane. The immunoprecipitated L-[^{35}S]methionine-labeled proteins were analyzed by SDS-PAGE under reducing conditions and were visualized by autoradiography. The positions and the sizes of the marker proteins analyzed in the same gel are indicated at the left.

protein was immunoprecipitated by porcine anti-LV serum sample 21 from lysates of Sf21 cells infected with bac-ORF7 (Fig. 1). This 15-kDa expression product was also recognized by anti-ORF7 peptide serum sample 713 and by N-specific MAbs SDOW17 (Fig. 1) and WBE6 (6) (data not shown). The recombinant N protein was similar in size to the wild-type N protein immunoprecipitated by SDOW17 from CL2621 cells infected with LV, but no such expression product was immunoprecipitated from cells infected with wild-type baculovirus (Fig. 1). Only very small amounts of N protein could be immunoprecipitated from the culture supernatant of Sf21 cells infected with bac-ORF7, which was probably due to partial lysis of the infected Sf21 cells (data not shown).

The antigenic properties of the recombinant N protein expressed by bac-ORF7 were further investigated in an IPMA with a panel of anti-N MAbs. MAbs SDOW12, SDOW17 (19), and WBE1, -4, -5, and -6 (6) all reacted positively with cells infected with bac-ORF7. MAbs EP147 and VO17 (19), which specifically recognize the nucleocapsid protein of U.S. LV isolates but not that of European LV isolates, did not react positively with cells infected with bac-ORF7 (data not shown).

To ascertain the optimal time for production of the N protein, a time course study was performed. Sf21 cells were infected with bac-ORF7, and at 24, 48, 72, and 96 h after infection, cell lysates were harvested. The lysates were tested for N protein in an ELISA with MAb WBE6. This indicated that maximum amounts of N protein were produced in Sf21 cells at 48 h after infection (data not shown).

No distinctive N protein could be detected in lysates of Sf21 cells analyzed by SDS-PAGE and Coomassie blue staining at 48 h after infection with bac-ORF7 (data not shown). The yield of recombinant N protein was estimated to be 0.3 μg/10^6 cells on the basis of preliminary immunoaffinity purification of the N protein with MAb SDOW17 (data not shown).

Reactivities of experimental and field sera with recombinant N protein. A blocking ELISA was developed to determine the usefulness of the baculovirus-expressed N protein for analyzing the immune responses of pigs infected with LV. Recombinant N protein was used to coat ELISA plates, and the

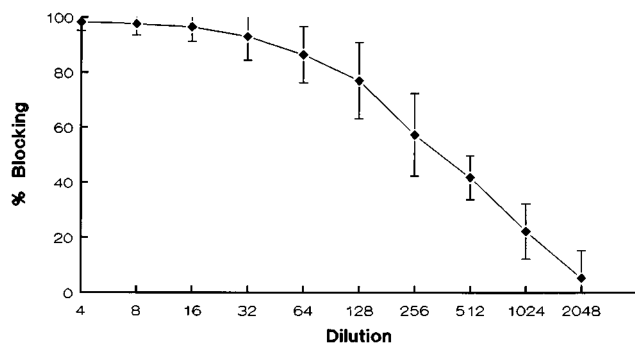

FIG. 2. Inhibition of binding of MAb SDOW17 to recombinant N protein by serial dilutions of porcine anti-LV serum sample 21 in a blocking ELISA. Mean blocking percentages were based on four independent assays and were calculated as described in Materials and Methods. The space between the error bars indicates two times the standard deviation.

inhibition of the binding of MAbs by pig sera positive for LV antibodies was measured. First, two MAbs specific for the N protein, SDOW17 and WBE6, were tested for their abilities to be used in this blocking ELISA. SDOW17 has been shown to recognize the N protein of both North American and European isolates of LV (19), whereas WBE6 has been shown to recognize the N protein of European isolates of LV only (6). A panel of seven high-positive porcine anti-LV serum samples blocked the binding of MAb SDOW17 to recombinant N protein much more efficiently than it blocked the binding of MAb WBE6 (data not shown). These findings might indicate that either the epitope recognized by SDOW17 is more immunodominant or conserved than the epitope recognized by WBE6 or that the affinity of SDOW17 for ORF7 is less than the affinity of WBE6 for ORF7. Therefore, SDOW17 was used for further experiments. The blocking ELISA was then used to screen sera from pigs experimentally infected with LV or ATCC VR2332 (a U.S. isolate of LV [3]) and field sera from different countries. Serum sample 21 was used to optimize MAb and antigen concentrations in the blocking ELISA. Figure 2 shows the mean blocking percentages of serial dilutions of convalescent-phase serum sample 21. These percentages are based on four independent experiments. In each ELISA, serum sample 21 and three negative serum samples were used as positive and negative controls, respectively.

Sera from two specific-pathogen-free pigs experimentally infected with 10^5 $TCID_{50}$s of LV, representing the European prototype, and from two pigs experimentally infected with 10^5 $TCID_{50}$s of ATCC VR2332, representing the U.S. prototype, were tested for seroconversion to N protein in the blocking ELISA. Antibodies to N protein could be detected in sera from day 7 on after infection with either prototype (Fig. 3). The percentage of blocking by the anti-LV sera was much higher than the percentage of blocking by the anti-ATCC VR2332 sera. This is probably because antibodies generated in pigs infected with the North American LV isolate ATCC VR2332 cross-react with the N protein of the European LV isolate only partially. Anti-LV antibodies binding in close proximity to the SDOW17 epitope may also inhibit binding of SDOW17 to the N protein. If this vicinity is different in the ATCC VR2332 N protein, the anti-ATCC VR2332 antibodies directed to this region will not be able to bind to the N protein of LV, resulting in a lower blocking percentage in the ELISA. The titers of these experimental serum samples determined by IPMA on macrophages infected with LV or ATCC VR2332 also reflected this antigenic difference. Whereas the titers of the anti-ATCC VR2332 sera by IPMA increased to levels (dilutions of 1/2,560 to 1/10,240) as high as the titers of anti-LV sera in the homologous IPMA, the anti-ATCC VR2332 sera had maximum titers of 1/160 to 1/640 in IPMA with macrophages infected with LV and the anti-LV sera had maximum titers of 1/640 in IPMA with macrophages infected with VR2332.

Finally, a panel of 31 field serum samples from different European, North American, and Asian countries were tested, and the titers were compared with the titers determined by IPMA on alveolar macrophages infected with LV or ATCC VR2332. The field sera reacting highly positive in the IPMA with macrophages infected with LV ($n = 26$; titer, $\geq$1/160) also showed high percentages of blocking in the ELISA ($\geq$29% blocking; Table 1). Four serum samples from North America and one serum sample from Hong Kong, all five containing titers of $\leq$1/10 in the IPMA with macrophages infected with LV, did not inhibit the binding of MAb SDOW17 in the ELISA, even though they contained high IPMA titers ($\geq$1/640) on macrophages infected with ATCC VR2332 (Table 1). This further shows the antigenic diversity between European and North American isolates. Although MAb SDOW17 recognized both European and North American isolates, the bac-ORF7 antigen used in the ELISA is of European origin. Therefore, the ELISA seems to be more sensitive for detecting serum antibodies against the European type of LV than for detecting antibodies directed against the North American type of LV.

Immunization and challenge of pigs. To determine whether the recombinant N protein induces an immune response in pigs as strong as that of the native N protein expressed by LV during infection, we immunized two specific-pathogen-free pigs twice with cell lysates of Sf21 cells infected with bac-ORF7. Two control pigs were immunized with cell lysates of Sf21 cells infected with wild-type baculovirus. Anti-LV antibodies were detected in sera by IPMA on LV-infected alveolar macrophages at 21 days after immunization with bac-ORF7 lysate (Table 2). The sera were also tested for their reactivities against the N protein in a blocking ELISA. The sera from the two pigs vaccinated with bac-ORF7 lysates strongly recognized the recombinant N protein in the blocking ELISA, whereas the sera from control pigs did not (Table 2). All pigs were challenged 42 days after immunization with 10^5 $TCID_{50}$s of LV. The pigs immunized with lysates containing recombinant bac-ORF7 or wild-type baculovirus proteins showed no differences in clinical signs, histological alterations due to LV infection of

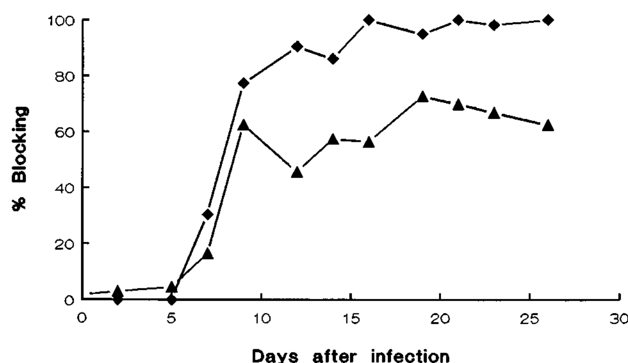

FIG. 3. Seroconversion of specific-pathogen-free pigs experimentally infected with 10^5 $TCID_{50}$s of LV (◆) or 10^5 $TCID_{50}$s of ATCC VR2332 (▲), a U.S. isolate of LV. Sera collected at different time points after infection were tested in the blocking ELISA. The percentage of blocking represents the average of one determination for each of the two animals in the group.

TABLE 1. Reactivities of field sera in the blocking ELISA

Serum source and sample	% Blocking in bac-ORF7 ELISA[a]	IPMA titers[b]	
		On LV	On ATCC VR2332
The Netherlands			
TH-187	93	>640	40
TO-36	96	>640	160
Belgium, PE-1960	73	>640	40
Germany			
BE-352	92	>640	40
BE-392	86	>640	10
NI-f2	36	160	10
Greece			
234-1	90	>640	160
234-2	96	>640	160
234-3	94	>640	160
Italy			
213-1	98	>640	160
213-2	85	160	160
213-3	98	>640	40
Luxembourg, 479	86	>640	40
Russia			
332-1	100	>640	>640
332-2	85	>640	10
332-16	72	>640	<10
Spain			
097-1	93	>640	10
097-2	82	>640	<10
097-4	57	160	<10
United States			
SL-441	97	>640	>640
SL-451	76	>640	>640
AL-P10814/33	0	10	>640
AL-4094A	0	10	640
AL-7525	0	10	40
JC-MN41	61	160	>640
Canada			
RB-16	100	>640	>640
RB-19	0	10	640
RB-22	29	160	>640
RB-23	90	>640	>640
Hong Kong			
324-8	48	160	>640
324-9	0	<10	>640

[a] Percentages of blocking in the bac-ORF7 blocking ELISA were compared with titers in the IPMA.
[b] Expressed as the reciprocal of the highest serum dilution that gave positive staining.

the lungs, or the presence of viral antigens in the lungs. By day 63 after immunization the anti-LV antibody response observed after challenge of the control pigs had increased to titers as high as the antibody titers in the sera of the pigs immunized with bac-ORF7 lysate (Table 2). The bac-ORF7-immunized pigs and the control pigs were viremic at day 45 after immunization, and the virus titers remained similar over a period of 2 weeks (Table 3). This indicated that the immune response against the recombinant N protein is not protective against viremia.

TABLE 2. Titers determined by IPMA and reactivities in a blocking ELISA of sera collected from pigs immunized with bac-ORF7 and wild-type baculovirus on days 0 and 28 and challenged with LV on day 42

Inoculum[a]	Pig	Titers by IPMA on day[b]:				% Blocking on day:			
		0	21	42[c]	63	0	21	42[c]	63
bac-ORF7	522	0	640	2,560	40,960	0	64	98	100
bac-ORF7	523	0	640	10,240	40,960	0	53	85	100
Wild type	524	0	0	0	10,240	19	0	19	81
Wild type	538	0	0	0	40,960	0	0	0	83

[a] Lysates of Sf21 cells infected with recombinant bac-ORF7 or wild-type baculovirus.
[b] The titer is expressed as the reciprocal of the highest serum dilution that gave positive staining. Days indicate days after immunization.
[c] Day of challenge.

DISCUSSION

Although the development of the CL2621 cell line, which is permissive for LV infection, has greatly increased the ability to study this virus, the yield of viral proteins is still low, which makes further characterization of these proteins time-consuming. The goal of the present study was to determine whether the baculovirus system would produce the nucleocapsid protein N of LV in an authentic form and in sufficient quantities for antigenic and immunological analysis and application in diagnosis. The maximum quantity of the N protein that we were able to produce, about 0.3 μg/10^6 Sf21 cells at 48 h after infection, was less than others have been able to obtain by using the baculovirus expression system (1 to 60 μg of recombinant protein per 10^6 Sf21 cells [8, 10, 16]). However, it was at least 10-fold more than the amount obtained from LV-infected CL2621 cells. By SDS-gel electrophoresis and immunoprecipitation with polyclonal porcine anti-LV serum and N protein-specific MAbs we have shown that the N protein expressed by recombinant baculovirus bac-ORF7 was indistinguishable from the authentic N protein of LV. Immunization of pigs with the recombinant N protein resulted in high titers of antibodies reacting with the N protein, but the antibody response was not protective against a challenge with LV. This was not surprising since the nucleocapsid protein N is an internal structural protein. The sera of pigs infected with LV generally contain low titers of neutralizing antibodies (17), and thus far, no neutralizing LV-specific MAbs have been isolated. LV causes a long-lasting infection in pigs and seems to evade the immune system (25).

We have demonstrated that the N protein produced by recombinant baculovirus provides a source of antigen that can be used in a blocking ELISA to detect serum antibodies against

TABLE 3. Virus titers in sera of pigs immunized with bac-ORF7 and wild-type baculovirus on days 0 and 28 and challenged with LV on day 42

Inoculum[a]	Pig	Titer (log $TCID_{50}$/ml) on day[b]:				
		42	45	52	58	63
bac-ORF7	522	0	1.05	3.05	2.55	2.55
bac-ORF7	523	0	1.05	1.55	1.55	2.05
Wild type	524	0	1.55	1.55	1.55	1.05
Wild type	538	0	1.55	2.55	1.55	1.55

[a] Lysates of Sf21 cells infected with recombinant bac-ORF7 or wild-type baculovirus.
[b] Days after immunization.

LV. In this blocking ELISA we used MAb SDOW17, which recognized an epitope present on both European and North American isolates of LV. We compared the blocking percentages of experimental and field sera in this ELISA and the titers of these sera in an IPMA with LV- or ATCC VR2332-infected macrophages, an assay which is regularly used for the diagnosis of LV infection. The results indicated that the blocking ELISA is more sensitive in detecting antibodies against the European antigenic type of LV than in detecting antibodies against the North American antigenic type of LV. The antigenic difference between the European and North American isolates of LV has been established earlier by serologic testing with polyclonal pig sera as well as mouse MAbs (6, 19, 26). The detection of antibodies against North American strains of LV might be improved by using a baculovirus ORF7 expression product based on a North American strain.

The serological diagnosis of LV infection has mostly been performed by IPMA (28) or immunofluorescence assay (29). Our results show that the blocking ELISA based on the N protein produced by recombinant baculovirus might be a good alternative for diagnosing LV infection, particularly because the antigen, the baculovirus-expressed N protein, can routinely be produced in quantities large enough to allow standardization for large-scale testing. In addition, this does not require the laborious preparation of alveolar lung macrophages needed for IPMA and the immunofluorescence assay.

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