Acta Veterinaria (Beograd), Vol. 46. No. 2-3, 95–102, 1996.

UDK 619:616.078:616.988:636.5

PRODUCTION OF THE ANTIGEN OF INFECTIOUS BURSAL DISEASE VIRUS (IBDV) FOR THE AGAR-GEL PRECIPITIN TEST AND ITS SENSITIVITY COMPARED TO THE VIRUS-NEUTRALIZATION TEST AND ELISA

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(Received, 17. January 1996.)

An antigen for the agar-gel precipitin test (AGPT) was prepared from an isolated strain of the infectious bursal disease virus (IBDV). In this paper the results obtained by comparative investigation of sera from o ne-day-old chickens and rearing chickens on the presence of antibodies to IBDV by using the methods of AGP, virus-neutralization (VN) and enzyme-liked immunosorbent asaay (ELISA) were described.

The geometric mean value (GMV) of the reciprocal of the VN titers for the AGP-positive sera in group 1 was 638,30 and for the AGP-negative sera 50,80. In group 2 (vaccinated chickens) the GMV of the reciprocal of the VN titers for the AGP-positive sera was 747,30, and for the AGP-negative sera 68,57. In both chicken groups AGP - positive sera had a VN titer of 2^4 or greater, with exception of one serum with the VN titer 2^5.

In 95% of the investigated samples the AGP-positive sera had an antibody titer as determined by ELISA greater than 3. It could be concluded that the obtained antigen is specific in determining precipitin for IBD virus. Because of the appearanceof false negative reactions, the antigen obtained for the AGPT can be recommended with some caution for establishing postvaccional or postinfec5ious antibody titers.

Key words: infectious bursal disease, precipitin antibodies, virus-neutralizing antibodies, serological response, ELISA, chickens

INTRODUCTION

Gumboro disease or infectious bursal disease (IBD) is caused by a virus from the Birnavirid family. Diagnosis of the disease can be made on the basis of characteristic pathoanatomical changes, virus detection in the bursa of Fabricius of infected chickens or by serological tests.

The agar-tel precipitin test (AGPT) has been used for many years to detect antibodies in the sera of infected or vaccinated chickens (Cullen and Wyeth, 1975; Hitchner et al., 1980; Kösters, 1971; Hirai, 1973).

In most laboratories the antigen for the AGPT is prepared from homogenized bursae or allantoic membranes of infected eggs (Schneider and Haass, 1969a, 1969b; Wagner and Kosters, 1968).

Different sensitivity of these antigens compared to the virus-neutralization (VN) test, plaque-reduction or ELISA has been reported (Weisman and Hitcher, 1978; Chettle et al., 1985).

Sera from chickens of different hybrids and from different production cycles were sent to the laboratory for serological testing to IBDV, and were examined in parallel by the AGPT, VN and ELISA.

The aim of these investigations was to determine the sensitivity of the antigen for AGPT. It was prepared from bursae of chickens that had been previously infected with the IBD virus isolated during an outbreak of the disease on a farm.

MATERIAL AND METHODS

The sera used were obtained from one-day-old chickens of light provenience, whose parents had been vaccinated with a live and an oil vaccine (group 1) as well as from chickens of the same category, which had been vaccinated during rearing according to different programs of immunoprophylaxis. These sera were separated in sterile test tubes and inactivated at 56^0C for 30 min.

A total of 111 sera from one-day-old chickens and 84 sera of rearing chickens were examined by the VN, test ELISA and AGPT.

Antigen preparation for the AGPT The IBD virus was extracted from bursae of Fabricius of infected commercial chickens of light provenience with a mortality up to 20%.

The bursae were homogenized in phosphate-buffered saline (PBS) pH 7,2 (1:10). After centrifugation at 3000 rpm for 15 min, the supernatant was poured off and inoculated intracloacally in the quantity of 0,2 ml into receptive 4-week-old chickens of the Harco provenience. The bursae of the infected chickens were collected 72 hours after infection and homogenized. The tissue was then disintegrated by an ultrasound of 20 Kc for 3 minutes, in short periods of 15 sec with breaks of equal length.

Afterwards, the antigen was conserved with sodium azide and titrated in the gel with the positive control serum. The positive hyperimmune monovalent serum was separated three weeks after chicken infection and the titer determined by the VN test was 2^{11}.

Plates for the AGP test were made with 1% Noble agar in distilled water containing 8% NaCl and 0,1% sodium azide. A template consisting of 6 peripheral holes and a center hole was used to cut wells in the AGP plates. Test sera and a control serum were placed in the peripheral wells, and the antigen was placed in

the center well. For antigen testing, monovalent hyperimmune sera for Marek's disease virus, reovirus, infectious bronchitis virus (strains M41, D247, D212) and fowl influenza virus were also included in the AGP test. Nonspecific reactions estimated.

Virus neutralization test (VN) A cell culture of chicken embryo fibroblasts was prepared from 9-10-day old embryos that originated from eggs of a parent flock of Leghorn provenience reared in our Institute. Cells were grown on a Eagle MEM medium with 10% lamb serum. After 24 hours, the cultures were trypsinized and the cells removed to microneutralization plates (Linbro) to which the Eagle MEM medium with 10% of lamb serum was added. After 24 hours, double dilutions of 50 μl of the serum were made on the plates for serum titration. To sera diluted this way 50 μl of the virus (strain D-78) was added. It had been previously titrated on CEF culture and diluted so that each well on the plate contained 100 $CCID_{50}$. After one hour the mixture of serum and virus was removed to microneutralization plates with cells, which were then covered and incubated at 37^0C for 72 hours. Afterwards, the VN antibody titer was read. Each plate included the positive hyperimmune serum, the virus control and the cell control.

The geometric mean value (GMV) of the reciprocal of the VN titers, in relation to the sera which were positive or negative in the AGPT, was estimated by using the t-test (Student test).

The performance of the enzyme-linked immunosorbent assay ELISA from IDEXX (Portland, Maine) were used. Sera were diluted 1:500 and then aliguots of 100 μl were transfered to plates for the ELISA. At the beginning. 100 μl samples of the positive control serum were placed in one well each and 100 μl of the negative control serum in other wells. (Control sera were prepared by the producer.) After incubating the samples at room temperature for 30 min, the liquid was carefully aspirated using a vacuum-pump and the plates were rinsed several times with distilled water. The plates were dried by strong shaking on absorbent paper cotton. Afterwards, 100 μl of the antichicken conjugate (goat) for the IBD virus was added and after 30 min the plates were rinsed again in the described way. The solution of TMB (3, 3, 5, 5, tetramethylbenzidine) was left on the plates for 15 minutes and after rinsing the stop solution was added. The plates were read in the next 15 min. Data on the sera were fed in to the reader while the assay was being set up using the software Flock Check IDEXX corp., so the computer was ready for reding. The obtained results were expressed in the form of antibody titers calculated according to the mathematics formula from the extinction of the investigated sera as well as the positive and negative control sera included in the test.

According to the instructions of the kit producer, values higher than 390 are positive in the ELISA which means that the bird had had contact with the IBD virus.

Titers were grouped using the following scale (set up by IDEXX): group O (0-499), 1 (500-999), 2 (1000-1999), 3 (2000-2999), 4 (3000-3999), 5 (4000-4999), 6 (5000-5999), 7 (6000-7999), 8 (8000-9999).

RESULTS

Antibody titers were registered in all sera of one-day-old chickens (31), that were investigated using the VN test, 22 (71%) of these sera were positive in the AGP test and 9 (29%) were negative.

The AGP-positive sera showed VN titers from 2^5 to 2^{12}, while the AGP-negative sera had VN titers ranging from 2^2 to 2^{10} (table 1).

Table 1. The VN antibody titers and precipitin test results in the sera of one-day-old chickens (group 1)

Serum No.	AGPT	No. of sera with VN Log2 antibody titer												
		%	1	1	2	3	4	5	6	7	8	9	10	11	12
22*	+	71	—	—	—	—	—	1	—	3	5	2	4	3	4
9**	—	29	—	—	1	—	3	—	2	1	1	—	1	—	—

*GMV - 1: 638,30
**GMV - 1: 50,80
P < 0,001

The GMV of the reciprocal of the VN titers (638,30) for the AGP-positive sera was significantly higher (p<0,001) than the GMV for AGP-negative sera (50,80).

All 11 sera of vaccinated chickens which had a VN titer from 2^8 to 2^{12} were also AGP-positive. For the AGP-negative sera the VN values ranged from 2^3 to 2^9 (table 2). The difference in the GMV between AGP-positive and AGP-negative sera in this group of chickens was also statistically highly significant (p<0,001).

Table 2. The VN antibody titers and precipitin test results in the sera of vaccinated chickens (group 2)

Serum No.	AGPT	No. of sera with VN Log2 antibody titer												
		%	1	1	2	3	4	5	6	7	8	9	10	11	12
11*	+	31,42	—	—	—	—	—	—	—	—	1	5	4	—	1
24**	—	68,57	—	—	—	2	4	4	3	4	4	3	—	—	—

* GMV - 1:747,30
**GMV - 1:69,79
P < 0,001%

Out of a total of 80 sera from one-day-old chickens examined by ELISA, 56 sera (70%) were AGP-positive (table 3). The 24 AGP-negative sera (30%) had an ELISA titer up to group 3 (2000-2999).

Table 3. The antibody titers obtained by the ELISA and precipitin method in the sera of one-day-old chickens (group 1)

Serum No.	AGPT	ELISA group antibody titer										
		%	0	1	2	3	4	5	6	7	8	9
56	+	70,00	—	—	—	14	17	12	6	4	3	—
24	—	30,00	—	2	10	11	—	—	—	—	—	—

Out of a total of 49 sera from rearing chickens, 52,02% were AGP-positive and the ELISA titer for these sera ranged from group 1 to group 7. The AGP-negative sera (48,97%) had an ELISA titer up to group 3 (table 4).

Table 4. The antibody titers obtained by the ELISA and precipitin method in the sera of vaccinated chickens (group 2)

Serum No.	AGPT	ELISA group antibody titer										
		%	0	1	2	3	4	5	6	7	8	9
25	+	71,02	—	1	2	6	5	6	4	1	—	—
24	—	48,97	4	12	7	1	—	—	—	—	—	—

DISCUSSION

In the AGP test, 71% of serum samples from one-day-old chickens from the first group and 31,42% from the second group were positive. However, the VN titer was positive in all sera of the first and the second group (maternal and postvaccinal antibodies).

There is an obvious difference in the sensitivity of these two methods, which was also reported in the papers of other authors (Chettle et al., 1985;Weisman and Hitcher, 1978). Similarly to the results of Philips (1981), a statistically significant difference between the VN-positive sera and AGP-positive and AGP-negative sera was found.

All AGP-positive sera from one-day-old chickens had a VN titer 2^7 or higher, except for one sample with the titer 2^5. If we, therefore, consider the VN titer 2^7 or higher, except for one sample with the titer 2^5. If we, therefore, consider the VN titer 2^7 the limit titer, 33,33% of the investigated samples showed false-negative AGP reactions (out of 9 sera 3 had the titer 2^7 or higher). In the group of vaccinated chickens (group 2) all 11 AGP-positive sera had a titer higher than 2^7. If we also consider the titer 2^7 the limit titer this group, out of 24 AGP-negative sera 11 (45,83%) had false-negative AGP reactions. Philips (1981) found that 60,20% of AGP-negative sera had a VN antibody titer of 2^2-2^8. Chettle et al. (1985) regarded the titer 2^6 or lower as the limit titer, because the sera of receptive chickens with this titer were always AGP-negative, and concluded that there were no false-positive AGP values. After artifically infections chickens of different ages these authors also concluded that 1/3 of the chickens were protected at the antibody titer of 2^6. For these chickens there were 52,9% false-negative VN values and 79,4% false-negative AGP-values.

Because of the relatively high percentage of false-negative AGP-values, an insufficiently accurate interpretation of the results concerning chicken protection against the infection with IBDV can occur (Winterfield and Tacker, 1978).

In the chicken group with maternal antibodies (group 1) 97,50% sera were ELISA-positive and 70,00% AGP-positive. In group 2 91,84% sera were ELISA-positive and 51,02% AGP-positive.

Comparing these two methods, it is obvious that ELISA is a much more sensitive method. The AGP-negative sera never exceeded the values of group 3 in the ELISA, thus the antibody titer for these sera did not exceed 2999.

If we consider group 3 the limit group, we can see that out of 81 AGP-positive sera from both chicken groups only 3,7% had an ELISA titer below group 3. On the other hand, out of 48 AGP-negative sera from both chicken groups 25% had an ELISA titer within group 3. It can be concluded that over 95% of sera with an ELISA titer above group 3 (higher that 2999) will be AGP-positive.

The antigen obtained from an isolated virus strain, that was used in the AGPT, gave false-positive values in a very low percentage. According to these results, this antigen is specific but can be recommended for determining the postvaccinal response of chickens with some caution, or for determining IBDV infection in the flock. An answer could not be given to the question whether the sera which are positive in the AGPT as well as in other test are protective at the same time or not. It would be useful to estimate in our conditions the resistance of chickens to the IBDV infection at different levels of antibodies using different laboratory methods.

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PROIZVODNJA ANTIGENA VIRUSA INFEKTIVNOG BURZITA ŽIVINE ZA AGAR-GEL PRECIPITACIONI TEST I UPOREDNO ISPITIVANJE NJEGOVE OSETLJIVOSTI I SPECIFIČNOSTI U ODNOSU NA VIRUS NEUTRALIZACIONI I ELISA TEST

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SADRŽAJ

Antigen za agar-gel precipitacioni test (AGPT) proizveden je od izolovanog virusa infektivnog burzita (VIB) živine. Izloženi rezultati dobijeni su komparativnim ispitivanjem prisustva specifičnih antitela protiv VIB u serumima jednodnevnih pilića i podmlatka, primenom AGP, virus serum-neutralizacije (VSN) i ELISA metode.

Komparativnim ispitivanjem krvnih seruma jednodnevnih pilića VSN i AGP testom, dobijena srednja geometrijska vrednost (SGV) virus neutralizationih titara kod AGP-test pozitivnih uzoraka iznosila je 638,30, a kod AGP-test negativnih uzoraka 50,80. Srednja geometrijska vrednost odgovarajućih titara VSN antitela kod vakcinisanog podmlatka iznosila je u AGP-test pozitivnih uzoraka 747,30, a u AGP-test negativnih 68,57. Obe grupe pilića koje su bile u AGP-testu pozitivne VSN titar iznosio je 2^7 ili veći sa izuzetkom jednog seruma čiji je VN titar iznosio 2^5.

Kod 95% ispitanih uzoraka seruma AGP test pozitivnih, titar specifičnih antitela otkriven ELISA metodom bio je viši od 3. Dobijeni rezultati ukazuju da je proizvedeni antigen specifičan za otkrivanje antitela - precipitina protiv virusa infektivnog burzita živine. Međutim zbog pojave lažno negativnih reakcija pripremljeni antigen za AGP test može se preporučiti sa određenim oprezom kod procene postvakcionalnih i postinfektivnih nivoa antitela.