

معهد أمراض الدواجن
الجامعة الحرة ببرلين - ألمانيا الغربية
رئيس المعهد : أ. د. ج. مونريال

تبيان الاجسام المضادة لفيروس التهاب الشعب الهوائي
المعدي في الدجاج باختباري الترسيب في الاجار والاليزا

كمال الزناتي ، ابراهيم سكر

تم اجراء التجارب السيرولوجية وهي اختبار الترسيب في الاجار واختبار الاليزا لتبيان الاجسام
المضادة لفيروس التهاب الشعب الهوائي المعدي . ثم فحص عدد ٢٥٩ عينة سيرم جماعية
(٥ طيور / عينة) بكلا الاختبارين وجد أن ٢١٢ (٨١٫٩%) عينة سيرم ايجابية في الاليزا بينما
٣٣ (١٢٫٧%) عينة سيرم فقط ايجابية في اختبار الترسيب في الاجار ٤٧ (٨١٫١%) عينة سيرم
سالبة في كلا الاختبارين . وعلى هذا وجد أن اختبار الاليزا أكثر حساسية لتبيان الاجسام
المضادة لفيروس التهاب الشعب الهوائي المعدي عن اختبار الترسيب في الاجار .

*Institute of Poultry Diseases,
Free University of Berlin,
Head of Institute Prof. Dr. G. Monreal.*

**MEASUREMENT OF ANTIBODIES TO INFECTIOUS BRONCHITIS
VIRUS BY AGAR GEL PRECIPITATION TEST
AND ENZYME-LINKED IMMUNOSORBENT ASSAY***
(With 3 Tables)

By
K. EL-ZANATY and L. SOKKAR
(Received at 15/11/1987)

SUMMARY

Two serological tests, the agar gel precipitation (AGP) test and the enzyme-linked immunosorbent assay (ELISA), were used to measure antibodies against infectious bronchitis virus (IBV). A total of 259 pooled chicken sera (5 birds/sample) were examined by both tests. 212 (81.9%) sera were positive in ELISA, while only 33 (12.7%) sera had precipitating antibodies and 47 (18.1%) sera were negative in both tests.

It was demonstrated that, based on the higher sensitivity, the ELISA test is more suitable than AGP to measure IBV antibodies.

INTRODUCTION

Avian infectious bronchitis is an acute respiratory disease of chickens and is recognised as one of the more important respiratory syndromes (DARBYSHIRE, 1981). Specific IBV antibodies have been demonstrated by several serological tests, such as the serum-neutralization test (FONTAINE, *et al.* 1963), the AGP test (WITTER, 1962; GOUGH and ALEXANDER, 1978), the HI test (ALEXANDER, *et al.* 1976 and BROWN, *et al.* 1962) and recently an ELISA has been introduced (GARCIA and BANKOWSKI, 1981; HERLING, 1981; MARQUARDT, *et al.* 1981; MOCKETT and DARBYSHIRE, 1981; SOULA and MOREAU, 1981).

The AGP test for detection of antibodies to IBV of fowls has been described by WOERNLE, 1959), WITTER (1962) and CHUBB and CUMMING (1971). As a flock test for antibody recovery, it has been widely adopted by diagnostic laboratories because the reagents are cheap, large numbers of sera can be processed and the time required to set up and read the tests is shorter than for the serum neutralization tests.

The ELISA was able to detect an antibody response to IBV infection earlier than the virus-neutralization test and antibody titers obtained by ELISA were considerably higher than those obtained by virus-neutralization (MARQUARDT, *et al.* 1981).

The present work was designed to examine the chicken flocks located in Upper Egypt by AGP test and ELISA for detection of antibodies against IBV.

* This work was carried out in Institute of Poultry Diseases, F.U. Berlin.

EL-ZANATY and SOKKAR

MATERIAL and METHODS**Viruses:**

IBV (TAD-1 B vacc. II vaccine strain)(Massachusetts type) was first propagated by three chicken embryo passages and then used for the preparation of the ELISA antigen.

IB-Beaudette-strain (222), kindly provided by Prof. Dr. Monreal, Institute of Poultry Diseases, F.U. Berlin, was used to prepare antigen for the AGP test.

All virus strains were grown in 10 to 11-day-old embryonated SPF-VALO eggs (Lohmann, Cuxhaven).

Sera:

Blood samples were taken from laying and breeding chicken farms located in Upper Egypt (Assiut, El Wadi El Gadid, Sohag Provinces). Sera were separated, and each serum sample was pooled from 5 chickens of the same farm. Sera were stored at -20°C. Immediately before use sera were heat inactivated (56°C, 30 min.).

Specific hyperimmune serum against IBV as well as negative control sera were supplied from Institute of Poultry Diseases, FU Berlin.

Enzyme linked immunosorbent assay (ELISA): Antigen preparation and the procedure of the ELISA were done as described by HERLING (1981) and HERLING, *et al.* (1981). In brief, allantoic fluid, harvested 24 hours post-infection was clarified by centrifugation (2000 xg, 20 min, 4°C) and further purified by ultracentrifugation (95,000 xg, 1 hour) on to a cushion of a 55% solution of sucrose. flat bottomed micro-ELISA plates (M 129 B, Dynatech) were coated with antigen diluted 1 to 20 in 0.05 M carbonate-bicarbonate buffer (pH 9.6) in 100uL amounts per well. PBS (pH 7.2) containing 0.05% Tween 20 was used for washings. Sera were two serial fold diluted starting with 1:40 in 0.5 M Na CL solution (pH 7.4) with 0.05% Tween 20. Peroxidase conjugated rabbit serum anti-chicken IgG (Cappel laboratories, Inc., Cochranville, USA) and H₂O₂ and 5-amino-2-hydroxy-benzoic acid were used to detect bound IBV antibodies. Reactions were read with an automatic reader (Micro-ELISA-Auto-Reader M 580, Dynatech) at a wavelength of 490 nm.

Following the method of standardisation of the ELISA described by HERLING, *et al.* (1981), the discrimination level between positive and negative sera was set at an optical density (OD) of 0.2. Titres were expressed as-log₂ of the highest serum dilution with an OD \geq 0.2.

Agar gel precipitin test:

The tests were carried out by the micro-method on microscope slides using 1% DIFCO-Noble agar in 8% NaCl solution. The antigen was prepared from chorio-allantoic membranes of embryos infected with IBV (Beaudette-strain B 222) and the tests were read after 24 and 48 hours. Precipitation lines were called strong or weak, or scored +, ++ or +++ according to those described by BULOW and BIGGS, 1975.

RESULTS

Antibodies against IBV could be detected in chicken flocks by AGP-test and ELISA. In all chicken flocks tested other than one flock (flock no. 5) precipitating antibodies were

ANTIBODIES TO IBV

demonstrated, while all flocks were positive by ELISA Table 1. Out of 259 tested sera, 212 (81.9%) sera were positive in ELISA, while only 33 (12.7%) sera had precipitating IBV antibodies and 47 (18.1%) sera were negative in both tests Table 1. Out of 33 sera positive sera in AGP test, 23 sera showed strong or ++, +++ positive reaction and 10 sera were weak or + positive reaction in AGP test. All sera had precipitating antibodies were also positive with different titres in ELISA. In one flock (flock no. 3) as shown in Table 1. all 39 tested sera were positive in ELISA, while only 8 (20.5%) sera had precipitating antibodies.

The antibody titres of positive sera in ELISA could be seen in Table, 2. The highest antibody titre of some sera was 1:1280 with optical density 7 0.2.

As shown in Table, 3. The distribution of ELISA-antibody titres positive sera in relation to the reactions (strong, weak or negative) in AGP test. In general, most sera with low ELISA titres were negative in AGP test-179 sera had no precipitating antibodies but showing different ELISA titres antibody, 10 sera were weak or + positive reacted in AGP test and 23 sera had strong or +++ reactions in AGP test while these 33 AGP-test positive sera showed different antibody titres in ELISA, Table 3. Sometimes, serum samples with low ELISA titres (1:80 or 1:160) strongly or ++, +++ reacted in AGP-test, and few serum samples with higher ELISA titres showed no reactions in AGP test.

Table (1)
Chicken flocks examined for IBV antibodies by AGP test and ELISA

Flock No.	No. of tested sera	No. of Pos. sera in AGP	No. of Pos. sera in ELISA	%
1	44	6	29	65.9
2	62	5	46	74.2
3	39	8	39	100.0
4	28	5	24	85.7
5	35	-	28	80.0
6	51	9	46	90.2
Total no.	259	33	212	81.9

Table (2)
Antibody titres in ELISA positive sera

Total No. of Pos. sera in ELISA	ELISA-titre					
	1:40	1:80	1:160	1:320	1:460	1:1280
212	42	60	53	34	16	7

EL-ZANATY and SOKKAR

Table (3)
Distribution of ELISA-titres positive sera

Reaction in AGP test	No. of sera	1:40	1:80	1:160	1:320	1:640	1:1280
No precipitation	179	42	59	46	28	4	-
Weak precipitation	10	-	1	5	3	1	-
Strong precipitation	23	-	-	2	3	11	7

DISCUSSION

For serological survey, sera from different chicken flocks were screened for antibodies against IBV by the AGP test and ELISA. The results obtained showed that IBV antibodies were widespread. The incidence of IBV-precipitating antibodies varied between 0.0% and 20.5% and averaged 12.7% among the flocks. Similar results previously reported by AHMED, *et al.* 1968, on the incidence of IBV-precipitating antibodies which varied between 2.0 and 35.0% and averaged 11%. The results of ELISA showed that the ELISA is more sensitive than the AGP test for detection of IBV-antibodies, MONREAL, *et al.* 1985 reported on the sensitivity of ELISA than AGP-test. All positive sera in AGP test were also positive in ELISA with different antibody titres. Few some sera negative or weak reacted in AGP test had high ELISA-antibody titres (1:640) Table 3. indicating that there is no correlation between the type of reaction (negative, weak, strong) in AGP-test and the ELISA-antibody titres.

REFERENCES

- Ahmed, A.A.S.; Saber, M.S.; El Sisi, M.A.; Reda, I.M. and Abbasi, K.H. (1968): Further studies on respiratory infections of poultry in Egypt. *J. Vet. Sci. U.A.R.*, 5, No. 1, 85-104.
- Alexander, D.J.; Bracewell, C.D. and George, R.E. (1976): Preliminary evaluation of the hemagglutination and hemagglutination inhibition test for avian infectious bronchitis virus. *Avian Pathol.* 5: 125-134.
- Brown, W.E.; Schmittle, S.C. and Foster, J.W. (1962): A tannic acid modified hemagglutination test for infectious bronchitis of chickens. *Avian Dis.* 6: 99-106.
- Bülow, V.V. and Biggs, P.M. (1975): Precipitating antigens associated with Marek's disease viruses and a herpes virus of turkeys. *Avian Pathol.* 4: 147-162.
- Chubb, R.C. and Cumming, R.B. (1971): The use of the gel diffusion precipitin technique with avian infectious bronchitis nephritis virus. *Aust. Vet. J.* 47: 496-499.
- Darbyshire, J.H. (1981): Immunity to avian infectious bronchitis virus. In *Avian Immunology*, 1981, 205-226. British Poultry Science Ltd.
- Fontaine, M.P.; Chabas, D.; Fontaine, M. and Brion, A.J. (1963): A simplified technique for the serum neutralization test in infectious bronchitis. *Avian Dis.* 7: 203-206.
- Garcia, Z. and Bankowski, R.A. (1981): Comparison of a tissue culture virus-neutralization test and the enzyme-linked immunosorbent assay for measurement of antibodies to infectious bronchitis. *Avian Dis.*, 25: 121-130.

ANTIBODIES TO IBV

- Gough, R.E. and Alexander, D.J. (1978): Comparison of serological tests for the measurement of the primary immune response to avian infectious bronchitis virus vaccines. *Vet. Microbiology*, 2: 289-301.
- Herling, A. (1981): Indirekter Enzyme-linked immunosorbent Assay (ELISA) zum Nachweis von Antikörpern gegen das virus der infektiösen bronchitis der Hühner, *Vet. Med. Dissertation*, FU Berlin.
- Herling, A.; Monreal, G. and Von Bülow, V. (1981): Standardisierung eines indirekten Enzymelinked immunosorbent-assay (ELISA) zum Nachweis von Antikörpern gegen Hühner Bronchitis-virus. *Zentralblatt für Veterinärmedizin B*, 28: 693-703.
- Marquardt, W.W.; Snyder, D.B. and Schlotthober, B.A. (1981): Detection and quantification of antibodies to infectious bronchitis virus by Enzyme-linked Immunosorbent Assay. *Avian Dis.*, 25: 713-722.
- Mockett, A.P.A. and Darbyshire, J.H. (1981): Comparative studies with an enzyme-linked immunosorbent assay (ELISA) for antibodies to avian infectious bronchitis virus, *Avian Pathol.*, 10: 1-10.
- Monreal, G.; Bauer, H.J.; and Wiegmann, J. (1985): Comparison of the enzyme-linked immunosorbent assay (ELISA), haemagglutination inhibition test and agar gel precipitation test for detection of antibodies to avian bronchitis virus. *Avian pathol.*, 14: 421-434.
- Soula, A. and Moreau, Y. (1981): Antigen requirements and specificity of a microplate enzyme-linked immunosorbent assay (ELISA) for detecting infectious bronchitis viral antibodies in chicken serum. *Archives of Virology*, 67: 283-295.
- Witter, R.L. (1962): The diagnosis of infectious bronchitis of chickens by the agar gel precipitin test. *Avian Dis.*, 6: 478-492.
- Woernle, H. (1959): Diagnose der infektiösen Bronchitis der Hühner mit Hilfe der Präzipitationsreaktion im festen Agarmedium, *Monatshefte für Tierheilkunde*.