Animal Health Research Institute "Immunity".

DIAGNOSTIC VALUE OF AGAR GEL PRECIPITATION TEST WITH FEATHER TIPS IN DIAGNOSIS OF MAREK'S DISEASE

(With 4 Tables and One Figure)

By

A.M.E. ASWAY

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القيمة التشخيصية لاختبار الأجارجل الترسيبي بإستخدام اطراف الريش في تشخيص مرض الماريك

أبو الخير محمد ابراهيم عيسوي

تشخيص مرض الماريك في معظم الحالات يبنى على تاريخ القطيع والعلامات الإكلينيكية وأيضاً وجود علامات ترى بالعين المجردة والميكروسكوب وفي بعض الأحيان تجرى بعض التجارب الأخرى "السيرولوجية" التي تعتمد على وجود الأجسام المناعية في السيرم. وتلاحظ أن تكاثر ونزول فيروس الماريك يكشف عنه بواسطة اختبار الآجارجل الترسيبي باستخدام نبت الريش أو أطراف الريش كأجسام محدثة "أنتيجين" مع الأجسام المناعية لهذا الفيروس في دم الطيور المصابة. وكذلك تلاحظ أن تفاعل الأجسام المحدثة مع الأجسام المناعية الخاصة بهذا الفيروس يحدث خلال ٢٤ – ٣٦ ساعة في درجة حرارة الغرفة ويتميز بوجود خطوط ترسيبية مميزة.

SUMMARY

In most cases, diagnosis of Marek's disease "MD" can be made on the basis of history, clinical signs and the presence of gross and microscopic lesions (Payne and Venugopal., 2000; Witter and Schat., 2003). However, in certain cases, additional tests e.g. immunohistochemistry using monoclonal antibodies are recommended. Being the replication in and shedding from feather follicle epithelium of complete Marek's disease virus (MDV), simple agar- gel diffusion test is employed, using an homogenate of feather follicles or more simply, the feather tips as antigen against an hyperimmune specific antiserum. The reaction antigen-antibody occurs in 24–36 hours of incubation at room temperature and is characterized by very marked precipitation line.

Key words: *Agar gel precipitation test, marek's disease*

INTRODUCTION

Marek's disease (MD) an economically important lymphoproliferative disease of primarily chickens and a complex neoplastic disease due to herpesvirus.

MD virus (MDV) is classified into 3 serotype 1, 2 & 3 with all virulent or pathogenic strains placed in serotype 1. Currently, virulent strains of MDV are classed into 4 pathogens, mild (mMDV), virulent (vMDV), very virulent (vvMDV), and very virulent plus (vv+MDV) (Witter *et al.*, 2003). Lesions of MD can be expressed in one or more of 4 forms, namely neural, visceral, ocular and cutaneous (Payne and Venugopal, 2000; Witter *et al.*, 2003) one of the most interesting aspects of MD has always been the mechanism of transmission.

It was demonstrated that the cells of feather follicle epithelium (FFE) are the sites of greatest maturation, concentration and shedding of MDV (Calnek *et al.*, 1970; Zanella, 1970). Also, non oncogenic MDV and HVT mature in such tissues (Cho, 1977; Witter *et al.*, 1972). Diagnosis of MD has been based for long period above all on macro– and microscopic lesions in tissues. After the discovery that FFE is the main site of productive MDV replication, also agar-gel diffusion (AGD) test was get ready, using the homogenate of FFE or the feather tips (FT) as antigen and an hyperimmune antiserum as antibody (Heider *et al.*, 1970; Ronnga-Tabbu and Cho, 1982; Davidson *et al.*, 1986). Successively, finer techniques as dot-blot and polymerase chain reaction (PCR), able to reveal viral DNA in feather pulp have been applied. This last techniques are very sensitive, detecting also very small amounts of virus and would permit not only the differentiation among the 3 serotypes of virus, but also between the oncogenic serotype 1 viruses (Becker *et al.*, 1992; Davidson *et al.*, 1986; Davidson and Barenshtain, 2002). Nevertheless, the differentiation between oncogenic and non-oncogenic MDV and HVT resulted possible also with AGD test, because FFE, infected with the last 2 types of virus very rarely or not include positive reaction that is likely due to their relatively low replication and concentration in such tissues (Rannga-Tabbu and Cho, 1982; Zanella *et al.*, 2004).

Recently it has been demonstrated that the feather pulp is considered the most favorable source of subgroup- J of Avian Leucosis Virus (ALV- J).

The concentration of virus was usually found higher than in other tissues (Sung *et al.*, 2001) and PCR, using DNA from FT, resulted

more effective for diagnosis of infected chicken (Davidson and Barenshtain, 2002). Nevertheless, no mention has been done on AGD test so far, it could be not able due insufficient concentration of antigen.

The aim of this work is to evaluate the meaning of the results of AGD test with FT, carried out in pullets at any time, but particularly between 12 and 18 weeks of age not only from diagnostic, but also and mainly from prognostic point of view with regard to MD.

MATERIALS and METHODS

New growing feather from apparently healthy bird were plucked from 3 different parts of each hen. Tips of each feather quill were cut (5mm), they cloud be kept in refrigerator or in freezer for weeks or longer.

Agar-gel diffusion test:

The AGD tests were performed using an agar-gel prepared as described by Davidson *et al.,* (1986). 3 ml of melted agar were overlaid on 76 x 26 mm slides and solidified. Up to 8 wells, 3 mm in diameter were cut on each slide. Some (5-10) FT from each bird were inserted aroum each well, 3-4 mm apart. Then the wells were filled with hyperimmune MDV antiserum and refilled 10 minutes later. The reaction was read 24-36 h. postincubation at room temperature. Formation of precipitation line was observed, exhibiting an identity with line of positive control antigen (one every slide).

Viruses:

Vaccinal viruses:

MDV LCBS 216/68, attenuated by 75 passages on tissue-culture, Rispens and HVT Fc-126 strains (Zanella and Marchi, 1984) were supplied by ISO, Italy.

Challenge viruses:

MDV LCBS 212/65, E 107/81, MD-5 strains (Zanella *et al.,* 2004) were supplied by Vaccine and Sera Institute, Abbasia, Cairo.

Experimental trails:

Trail 1:

120 one-day old commercial chicks, divided into 4 groups, were vaccinated I/M with HVT Fc-126 for the 1st group, MDV LCBS 216/68 for the 2nd group (Zanella and Marchi, 1984), or both for the 3rd group, while the 4th group kept as control "unvaccinated". After 10 days the bird of each group were identified with numbered ring and challenged by contact SPF chicks, previously infected with vvMDV (E-107/81) strain.

At intervals of 2 weeks from challenge, the feathers from birds vaccinated were plucked for AGD test. The birds were controlled for 30 weeks. All birds were necropsid.

Trail 2:

90 one-day old SPF chicks, divided into 3 groups, were vaccinated I/M with monovalent HVT Fc-126 for the 1st group bivalent MDV LCBS 216/68, for the 2nd group, while the 3rd group kept as control "unvaccinated". After 10 days the birds of each group were identified with numbered ring and challenged I/P respectively with LCBS 212/65, E-107/81 or MD-5 strains of MDV (Zanella *et al.*, 2004). At intervals of 2 weeks from challenge, the feathers from number of birds were plucked for AGD test. The birds were controlled for 30 weeks and all were necropised.

RESULTS

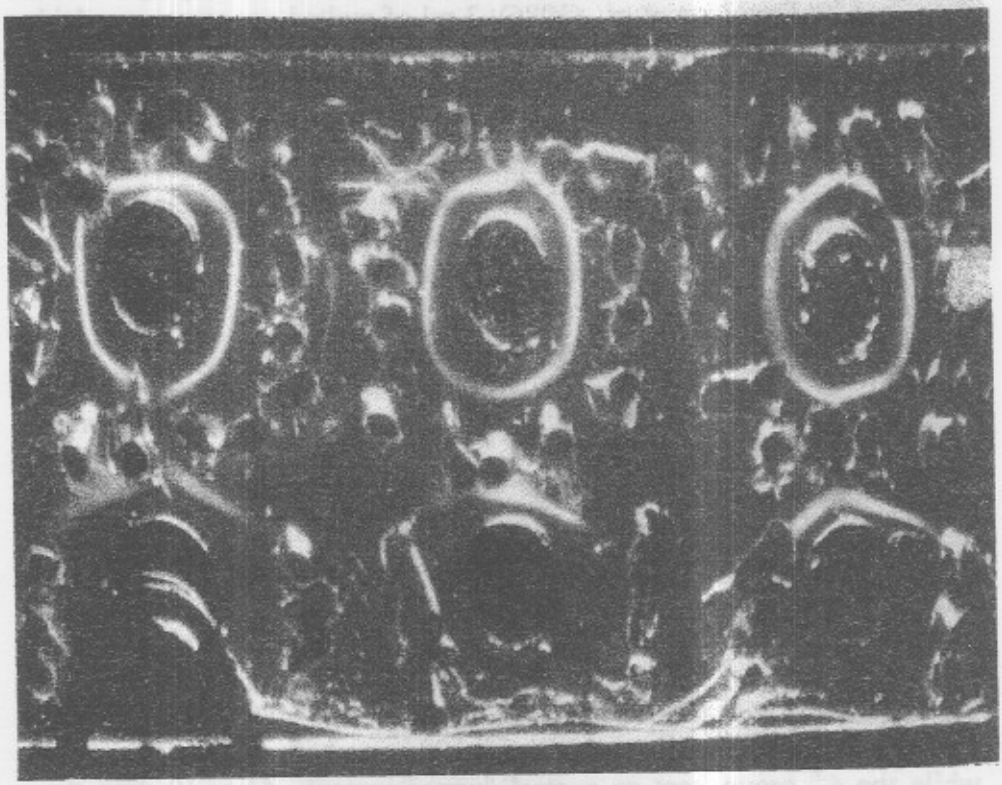

Figure 1: AGD reaction to evidence MD antigen in feather tips.

Table 1: Detection of MDV antigen in FT of commercial chickens vaccinated as one-day old with HVT or attenuated MDV (LCBS 216/68) or bivalent vaccine via I/P and challenged after 10 days by contact with chickens previously infected with vvMDV E-107/81 isolate

Vaccine	AGD with FT at weeks of age									MD mortality or lesions at 30 weeks	
	With challenge										
	2	4	6	8	10	11	12	14		No.	%
								No	%		
HVT	0/20	1/20	3/20	5/20	5/20	3/20	3/20	3/20	15.0	4/20	20.00
MDV-1	0/20	0/20	1/20	2/20	4/20	6/20	6/20	6/20	30.0	5/20	25.0
HVT+MDV 1	0/20	0/20	1/20	1/20	0/20	0/20	0/20	0/20	0.0	1/20	5.0
Control	0/20	9/20	18/20	18/20	18/20	16/20	15/20	14/20	70.0	12/20	60.0

Table 2: Presence at different time of MDV antigen in FT of SPF chickens vaccinated as one-day old, with HVT or bivalent (HVT + MDV LCBS 216/68) vaccine via I/M and challenged after 10 days with 3 different isolates of MDV via I/P

Isolate for challenge	AGD test with FT at weeks after challenge											
	2	4	6	8	2	4	6	8	2	4	6	8
LCBS 212/65 (v)	4	7	3	1	0	5	1	1	6	6	5	5
E-107/8 (vv)	4	9	5	4	0	4	0	0	6	6	6	6
MD-5 (vv)	5	7	6	2	2	2	1	0	6	6	6	5

N.B. All birds with persisting FT positive died with MD lesion.

Table 3: The percentage of protection and MD immortality or lesions at 30 weeks of age

Isolates for challenge	Vaccines		Control	Protection %	
	HVT	HVT + MDV		HVT	HVT + MDV
LCBS 212/65	3/15 (20.0)	0/15 (0)	11/15 (73.3)	80.0	100
E-107/8	4/15 (26.7)	0/15 (0)	15/15 (100)	73.3	100
MD-5	3/15 (20.0)	1/15 (6.7)	15/15 (100)	80.0	93.3

Table 4: Relationship between presence of precipitating antigen in FT and mortality due to MD in chickens vaccinated with HVT, presenting early break of immunity

Bird No.	Age of feather sampling (in weeks)													Results	
	3	5	7	9	11	13	15	17	18	20	22	24	26	Death age (in weeks)	MD Lesion
1	+	+	+	+	+									12	G
2	+	+	+	+	+									12	G
3	+	+	+	+	+	+								13	G
4	-	-	-	Paralysis										13	N
5	-	-	-	Paralysis										13	N
6	-	-	+	+	+	+	-							13	G
7	+	+	+	+	+	+	+							15	G
8	+	+	+	+	+	+	-							15	G
9	+	+	+	+	+	+	+							15	G
10	+	+	+	+	+	+	+							16	G
11	+	+	+	+	+	+	+							16	G
12	+	+	+	+	+	+	+	+	+	+	-	-	-	20	G
13	+	+	+	+	+	+	+	+	+	+	+	-	-	22	G
14	+	+	+	+	+	+	+	+	+	+	+	-	-	22	G
15	+	±	+	-	-	-	-	-	-	-	-	-	-	-	G
16	±	-	-	-	-	-	-	-	-	-	-	-	-	-	G
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G
19/30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G

G: Generalized. N: Nerves.

DISCUSSION

The positive reaction by AGD test was characterized by Marked, usually continous precipitation line between antigen (FT) and antiserum (Figure 1).

In trail 1, with exposure to vvMDV by contact, the percentage of positivity to AGD test at 11 weeks of age resulted in the different groups, rather similar to the incidence of MD at 30 weeks of age (Table 1).

In trail 2, part of vaccinated birds resulted temporarily positive to AGD test in the 1st week after challenge via I/P with 3 different isolates of v- or vvMDV, becoming negative after 8 weeks in case of bivalent vaccine such effect resulted less evident in case of HVT vaccine (Tables 2 & 3) only for few birds the reaction resulted partial or doutful, but mainly to disappear. Only in 2 birds it was possible to observe recovery

after many weeks of malaise and residual vestige of positive reaction, regression of lesions in proventriculus was observed. The FT of birds (about 10) affected only by peripheral neuropathy resulted always negative.

It has been demonstrated that there is significant relationship between the percentage of positively by AGD test and the cumulative MD mortality, at least up to 50 weeks of life, sometimes to the end of period. The result of some representative trails are reported in Table (4).

The testing of FT for MDV antigen by AGD test may be very simple and useful tool for monitoring oncogenic MDV in flock of chickens, also because of very rare or no occurrence of detectable antigens in FT of chickens infected with non-oncogenic MDV or HVT, more likely due to lower replication of these last viruses in FFE (Rannga-Tabbu and Cho, 1982; Zanella *et al.*, 2004).

High percentage of chicken inoculated with bivalent vaccine (HVT + MDV serovar 1) show negative or temporary and weak positive AGD reaction after challenge, also with vvMDV strains. Such effect was considerably less evident in case of vaccination only with HVT.

Nearly, all birds showing persistently AGD positive reaction were doomed to die, recovery was observed very seldom. The FT of birds affected only by neural lesions, probably related to MD were always negative (at least 30 observation), the reason of that is unknown. However, a syndrome with enlargement of nerves of peripheral neuropathy has been recently reported, but almost exclusively in white leghorn pullets, probably of immunomediate origin (Bacon *et al.*, 2001; Massi *et al.*, 2003; Rampin *et al.*, 2003).

On the contrary, the use of dot-blot and PCR tests would allow only to point out the presence of virus, which persists also in the healthy birds, they have some diagnostic, but no prognostic value.

In conclusion, the use of AGD test with FT deserves particular attention for simple, rapid, accurate and not expensive diagnosis, feasible in all laboratories, but also for its sure prognostic value on future MD incidence in flock of chickens. So, it could be of value, at least indicative if not legal, in the sale of pullets, rather frequent practice in several countries.

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