Original Research

Comparison of Different Serological Tests in Detection of Antibodies to Marek's Disease Virus

Priya, P. M. [1*], Mini, M.[2], Chintu Ravishankar[3], Leo Joseph[4] and Sunanda, C.[5]

[1]*Assistant Professor, Department of Veterinary Microbiology, CV&AS, Mannuthy, Thrissur-680651, Kerala, INDIA
[2]Professor and Head, Department of Veterinary Microbiology, CV&AS, Mannuthy, Thrissur-680651, Kerala, INDIA
[3]Assistant Professor, Department of Veterinary Microbiology, CV&AS, Pookkode, Wayanad-673 576, Kerala, INDIA
[4]Director (Retd), CAS in Poultry Science, CV&AS, Mannuthy, Thrissur-680651, Kerala, INDIA
[5]Assistant Professor, Department of Statistics, CV&AS, Pookkode, Pookkode, Wayanad-673 576, Kerala, INDIA

***Corresponding author:** priya@kvasu.ac.in

Rec. Date:	Nov 13, 2017 18:14
Accept Date:	Feb 07, 2018 17:37
DOI	10.5455/ijlr.20171113061411

Abstract

Marek's disease (MD) is a highly contagious oncogenic and neuropathic disease of chickens responsible for great economic losses to the poultry industry worldwide. An investigation was done to assess the status of MD in Kerala by serological tests viz., agar gel immunodiffusion (AGID) test, enzyme linked immunosorbant assay (ELISA) and indirect fluorescent antibody (IFA) test. Among the three tests employed for detection of antibodies to MD virus (MDV), out of 1030 sera screened, 157 samples (15.2 per cent) were positive by ELISA, whereas 62 samples were positive by AGID, which represents 6 per cent. The IFA tests detected 95 positive samples from 1030 samples (9.2 per cent). The results were analysed statistically using Cochran's Q and Fischer's exact test and it was found that ELISA was more sensitive than the other two tests compared.

Key words: AGID, ELISA, IFA, Kerala, Marek's Disease

How to cite: Mani, P., Mangattumurupel, M., Ravishankar, C., Joseph, L., & Chirayil, S. (2018). Comparison of Different Serological Tests in Detection of Antibodies to Marek' s Disease Virus. International Journal of Livestock Research, 8(7), 342-347. doi: 10.5455/ijlr.20171113061411

Introduction

Marek's Disease (MD), a highly contagious lymphoproliferative disease affecting chicken caused by Gallid herpes virus-II, an alphaherpesvirus (ICTV, 2013). Lymphomas in visceral organs with mortality among layers have been reported in the year 2010 from a regional poultry farm and other parts of the state. Preliminary investigation confirmed the role of MD which warrants a detailed investigation on the

status of the disease in Kerala. Except few private poultry farms, no MD vaccination has been practiced in Kerala for the past 15 years. Hence, a study was framed to assess the seroprevalence of MD using different serological tests and to evaluate their comparative efficacy.

Materials and Methods

Revival of Positive Viral Isolate

Field isolate of MD virus was obtained from the Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal and were revived by experimental inoculation as per Silva (1992) with modification in the dose of inoculum. The isolate was administered by intra-abdominal route into one-day- old desi chicks at the rate of 10^3 pfu/ bird. Antigens were prepared as per Bulow and Biggs (1975) from tissues of infected birds (tumor masses) and purified by 36 per cent sucrose cushion centrifugation. It was then suspended in TE buffer and stored at -20^{o}C until further use.

Raising of Hyper Immune Serum

Antisera against the purified MD virus were prepared in rabbits as per the standard procedure.

Collection of Sera Samples

A total of 1030 sera from chicken above four weeks of age were collected from nine organized farms of Kerala, which represented two to five per cent of total poultry population of each farm.

Serological Tests

Three tests were employed to detect MD antibodies viz., AGID (OIE, 2010), ELISA (Todd *et al*., 1993) and IFA (Ianconesco, 1977).

Results and Discussion

The MD viral isolate was revived successfully. Barrow *et al.* (2003) also selected the same dose and route and inoculated into two -week-old SPF, anti-MD maternal antibodies- free chicken. Handberg *et al.* (2001) inoculated intramuscularly with 10^3 TCID of CVI 988. Bacon et al. (2001) confirmed the existence of agerelated resistance in older chicken against MD tumour formation. 20 to 22 week's old chicken which were free of earlier infection were more resistant than one- day- old chicks. MDV exposure causes mortality and tumor induction in later. These older chicken were refractory to inoculation with up to 104 chick tumor-inducing doses of MDV and following contact infection, lesion frequencies were 8 to 30 per cent of corresponding responses in chick controls. Hence, in this work, one-day-old chicken were procured locally which were not vaccinated against MD and by history, their parent stock was free of any infection recently. This avoids the interference of maternal antibodies with the viral inoculum. While

DOI 10.5455/ijlr.20171113061411

reviving MDV, six out of ten chickens showed clinical signs of MD with 60 per cent mortality at six to eight weeks P. I. Handberg *et al.* (2001) noticed a mortality rate of 58 to 65 per cent at third week PI, whereas around nine percent mortality at eight to ten weeks of age PI was observed by Karikkathil *et al.* (2008).

Using different serological tests used to screen 1030 sera samples for MDV antibodies, number of positive samples detected by AGID, ELISA and IFA were 62 (6 per cent), 157 (15.2 per cent) and 95 (9.2 per cent), respectively. The percent positivity varied between the three tests employed (Table1).

Table 1: Percent positivity of MD viral antibodies by different serological tests

S. No.	Farms/ strength	No. of sera collected	AGID		ELISA		IFA	
			No. positive	% positive	No. positive	% positive	No. positive	% positive
1	Farm-1/3180	114	2	1.8	8	7	5	4.4
2	Farm-2/5500	120	17	14.2	37	30.8	22	18.3
3	Farm-3/5000	120	5	4.2	14	11.7	8	6.7
4	Farm-4/2900	110	4	3.6	13	11.8	7	6.4
5	Farm-5/3050	112	3	2.7	10	8.9	6	5.4
6	Farm-6/2950	110	3	2.7	9	8.2	7	6.4
7	Farm-7/2830	116	9	7.8	20	17.2	15	12.9
8	Farm-8/2640	115	6	5.2	15	13	9	7.8
9	Farm-9/4800	113	13	11.5	31	27.4	16	14.2
Total		**1030**	**62**	**6**	**157**	**15.2**	**95**	**9.2**

The results were analysed statistically as per Snedecor and Cochran, (1994) using Cochran's Q test and it was found to be significant ($p< 0.01$). This indicated variation in the detection of antibodies by three methods. Therefore each method was compared pair- wise by using Fischer's exact test and in all cases it was found to be significant ($p< 0.01$). The statistical values obtained were shown in Table 2.

Table 2: Kappa and cross tabulation values of MD

Tests	MD	
	Kappa Score Value	Cross Tabulation Value (per cent)
ELISA Vs AGID	0.525	9.8
IFA Vs AGID	0.773	3.4
ELISA Vs IFA	0.772	6.6

If Kappa value is zero, it indicates no agreement between the two methods and one indicates perfect agreement. All Kappa score in this study was greater than 0.5 and less than one, which indicates partial agreement between the methods compared. Kappa score was lower in case of comparison between ELISA and AGID, which shows that the agreement was lesser compared to other two comparisons (AGID versus IFA and ELISA versus IFA). It was because ELISA detected more than twice positive

DOI 10.5455/ijlr.20171113061411

samples than AGID (157 versus 62).

The ELISA versus AGID in receiver operating characteristics (ROC) curve indicates sensitivity was more for ELISA and it was confirmed by area under curve (AUC) which was 0.951. Between IFA and AGID, the AUC was 0.983, which indicates IFA was sensitive than AGID. The cross tabulation value of MD revealed that the samples detected as negative by ELISA were also negative by AGID and IFA, whereas among the negative samples by AGID, 9.8 per cent was found to be positive in ELISA and 3.4 per cent was found to be positive in IFA. By comparing the outcome of IFA and ELISA, all positives in IFA was positive in ELISA also, but among all the negatives by IFA, 6.6 per cent was found to be positive by ELISA. This indicates sensitivity was more for ELISA than the other two tests in detecting MD antibodies. The ELISA used in the study was developed indigenously. Though AGID is known for low sensitivity, it is the most common and widely used test to detect MDV antibodies (Sung *et al.*, 1997; Cho *et al.*, 1998; Davidson *et al.*, 2002; Posia, 2003; Gimeno *et al.*, 2005, Vathsala and Mohan, 2006). The AGID is the test recommended by OIE (2010) for detecting MDV antigens/ antibodies. In this study, the specificity of all AGID reactions was ensured by including positive sera in each test. A reaction was recorded only if a line of identity between the test sera and the positive antigen was observed.

The best results concerning the signal-to-noise ratio were obtained in an ELISA assay developed by Lee *et al.* (1999) when using chicken kidney cells (CKC) rather than chicken embryo cells (CEC) for antigen preparation with an optical density (OD) of 492 nm of plasma or serum samples. Hence, in this study the colour reaction developed after the addition of antichicken IgG peroxidase conjugate to the bound chicken sera and 2'-2 azino-di-ethyl benz- thiozoline 6-sulfonic acid (ABTS) as substrate was measured at OD 492 nm. It was clear that ELISA detected more numbers of positive cases than AGID and IFA tests. This was in accordance with Cheng *et al*. (1984), who found out that ELISA was 20 to 40 time's sensitive than IFA. The use of ELISA as an immunologic assay depends on a strict standardization of all reagents and procedures used. The high sensitivity of ELISA requires very pure antigen preparations. Because of the highly cell-associated nature of MDV and because of the fact that little or no virus particles are released into the culture medium, the standard procedures failed to provide antigen pure enough to reduce nonspecific background absorption. No ELISA for MDV antibodies were reported until 1984, owing mainly to the cell-associated nature of the virus and difficulty in obtaining large quantity of purified virus. The picture was changed by the use of infected cells rather than purified virus as convenient and efficient for detecting both chicken and monoclonal antibodies by Cheng *et al*. (1984).

The AGID test was not usually capable of detecting the maximum number of antigens/antibodies present. The resolution was not always great enough as concluded by Ianconesco (1977). Because IFA was labor-

DOI 10.5455/ijlr.20171113061411

intensive, ELISA was preferable for large scale screening. Hence, ELISA could be recommended for routine screening of antibodies against MDV.

Conclusion

Among the three different serological tests used in the present study to detect antibodies against MD viz. AGID, IFA and ELISA, statistically it was found that ELISA was more sensitive than the other two tests. Hence, ELISA could be recommended for routine screening of antibodies against MD as it is simple, sensitive and reliable.

References

1. Bacon L D, Witter R L and Silva R F. 2001. Characterization and experimental reproduction of peripheral neuropathy in White Leghorn chickens. Avian Pathology. 30: 487-499.
2. Barrow AD, Burgess SC, Howes K and Nair V K. 2003. Monocytosis is associated with the onset of leukocyte and viral infiltration of the brain in chickens infected with the very virulent Marek's disease virus strain C 12/130. Avian Pathology. 32: 183-191.
3. Bulow VV and Biggs PM. 1975. Precipitating antigens associated with Marek's disease viruses and a herpesvirus of turkey. Avian Pathology, 4: 147-162.
4. Cheng Y, Lee LF, Smith EJ and Witter RL. 1984. An enzyme- linked immunosorbant assay for the detection of antibodies to Marek's disease virus. Avian Diseases, 28 (4): 900-911.
5. Cho, KO, Endoh D, Quian JF. Ochiai K, Onuma M and ltakura C. 1998. Central nervous system lesions induced experimentally by a very virulent strain of Marek's disease virus in Marek's disease-resistant chickens. Avian Diseases, 21: 512-517.
6. Davidson I, Malkinson, M and Weismann Y. 2002. Marek's disease in Turkeys. II. Characterization of the viral glycoprotein B gene and antigen of a turkey strain of Marek's disease virus. Avian Diseases, 46: 322-333.
7. Gimeno IM., Witter RL, Fadly AM and Silva RF. 2005. Novel criteria for the diagnosis of Marek's diasease virus-induced lymphomas. Avian Pathology. 34: 332-340.
8. Handberg KJ, Nielsen OL and Jergensen PH. 2001. The use of serotype 1 and serotype 3 specific polymerase chain reaction for the detection of Marek's disease virus in chickens. Avian Pathology**.** 30 243-249.
9. Ianconesco M. 1977. Reticuloendotheliosis antigen for the agar gel precipitation test. Avian Pathology, 6 (3): 259- 267.
10. ICTV, 2013. Virus taxonomy: 203 Release (current). http://ictvonline.org/virusTaxonomyasp/version/2011 & bhcpl.
11. Karikkathil SS, Majee SB, Karmaker DB, Gaikwad RE and Khurud VB. 2008. Detection and characterization of virulent Marek' s disease virus in cell culture. Indian Journal of Comparative Microbiology Immunology and Infectious diseases. 29 (1&2): 9- 11.
12. Lee SL, Ohashi K, Morimura T, Sugimoto C and Onuma M. 1999. Re- isolation of Marek's disease virus from T cell subsets of vaccinated and non-vaccinated chickens. Archives of Virology, 144: 45-54.
13. Office International des Epizooties. OIE terrestrial manual. 2010. (7th Ed.) Paris, France, 586 p.
14. Posia RU. 2003. Status of Marek's disease and chicken infectious anemia in commercial broiler birds- Serological and pathological study. M.V.Sc thesis, Anand Agricultural University, Anand. 189

DOI 10.5455/ijlr.20171113061411

p.
15. Silva R F. 1992. Differentiation of pathogenic and non-pathogenic serotype Marek's disease viruses (MDV) by the polymerase chain reaction amplification of the tandem direct repeats within the MDV genome. Avian Diseases, 36: 521-528.
16. Snedecor GM and Cochran WG. 1994. Statistical Methods (8th Ed.). The Iowa State University Press, Ames, Iowa, U.S.A., 564p.
17. Sung HW, Kim SJ, Song CS, Lee YJ, Lee YJ, Lee CW, Kim KS and Kim SJ. 1997. Detection of pathogenic Marek's disease MD virus and survey on MD in broiler farms by the polymerase chain reaction. RDA Journal of Veterinary Science, 39: 38-44.
18. Todd D, Mawhinney KA, McAlinden VA and Douglas AJ. 1993. Development of enzyme linked immunosorbent assay for the serological diagnosis of big liver and spleen disease. Avian diseases, 37: 811-816.
19. Vathsala M and Mohan P. 2006. Agar gel immuno diffusion test for Marek's disease under field condition. Indian Veterinary Journal, 83: 1005-1006.

DOI 10.5455/ijlr.20171113061411