AVIAN RESPIRATORY MYCOPLASMOSIS IN CHICKENS : STUDIES ON STANDARDISATION, KEEPING-QUALITY AND FIELD TRIALS WITH AN INDIGENOUSLY PRODUCED SERUM PLATE PPLO ANTIGEN

by K. K. AGARWAL, M. P. MALL *and* B. B. MALLICK,
All India Coordinated Research Project on Respiratory Diseases of Poultry, Indian Veterinary Research Institute, Izatnagar, U.P.

The serum plate PPLO antigen previously produced in this laboratory was standardised and was comparable to Brown's opacity tube No. 10. The antigen could be kept for 80 weeks at 4°C and at 20°C –40°C without any loss of titre. Nine hundred and fifty-one sera were tested at 14 Veterinary Colleges/ Institutes with indigenously produced and imported (C. S. L., Australia) antigen, out of which 435 (45.7 per cent) and 448 (47.1 per cent) samples were found to be positive, 477 (50.1 per cent) and 466 (49 per cent) negative and 39 (4.1 per cent) and 37 (3.8 per cent) doubtful with the indigenously produced and imported antigens respectively. Six hundred and seven sera were tested in the states of Uttar Pradesh, Himachal Pradesh, Kerala, Assam and Maharashtra with the indigenous antigen alone. Of these, 368 (60.6 per cent) samples were found to be positive, 198 (32.6 per cent) negative and 41 (68 per cent) doubtful for PPLO agglutinins.

INTRODUCTION

Agarwal *et al.* (1972) developed a serum plate colored antigen for the diagnosis of avian respiratory mycoplasmosis (A.R.M.) in chickens. This paper describes the studies carried out to standardise this antigen, to study its keeping quality, and to evaluate it under field conditions.

MATERIALS AND METHODS

PPLO antigen — The antigen was prepared according to the method of Agarwal *et al.* (1972). For comparative studies, PPLO antigen imported from Commonwealth Serum Laboratories (C.S.L.), Australia, was used. The antigen was stored at 4°C and at room temperature ranging from 20°C to 40°C.

Chicken sera—These were collected from clinically healthy and diseased birds and tested soon after collection or after storage at 4°C.

Standardisation of the antigen—This was done against Brown's opacity tubes.

Keeping-quality of the antigen—This was assessed by periodically testing the same sera with the antigen stored in the refrigerator and at room temperature ranging from 20°C to 40°C.

Field trials with the antigen—A vial of indigenously prepared PPLO antigen and another vial of the freshly imported antigen from C.S.L., Australia was supplied to various Veterinary Colleges. The Scientists at these colleges tested chicken sera with both the antigens simultaneously i.e. each sample was tested with both the

indigenous and Australian antigen. Similar tests were also conducted at this Institute. A total of 951 sera were tested at 14 different places (Table II).

In addition, sera samples were tested with the indigenously prepared antigen alone in tests conducted in the states of H.P., Maharashtra, Kerala, Assam and U.P. (Table III).

Results

It was observed that the antigen with an opacity comparable to Brown's opacity tube 10 gave satisfactory agglutination and was comparable to the standard antigen produced at the I.V.R.I., Izatnagar and C.S.L., Australia.

The serum plate PPLO antigen stored for 80 weeks at 40–50°C and 20-40°C gave similar agglutination reaction (Table I).

Table I

Keeping quality of serum plate P.P.L.O. antigen

Period of storage (weeks)	Number of serum samples tested	Number of serum samples positive with antigen stored at	
		Room temperature (Average : 20—40°C)	Refrigerator temperature (Average : 4°–5°C)
18	10	10	10
36	18	8(2)	8 (2)
58	5	5	5
60	10	10	10
80	10	10	10

Figures in parentheses represent doubtful agglutination reaction.

Out of the 951 sera tested with the imported and indigenously produced PPLO antigen, 435 (45.7 per cent) and 448 (47.1 per cent) were found to be positive, 477 (50.1 per cent) and 466 (49 per cent) negative, and 39 (4.1 per cent) and 37 (3.8 per cent) doubtful results with the two antigens respectively (Table II).

Out of 607 sera samples tested with the indigenous antigen alone 368 (60.6 per cent) were found to be positive, 198 (32.6 per cent) negative and 41 (6.8 per cent) doubtful for PPLO agglutinins.

Discussion

Agarwal and co-workers (1972) had produced a plate PPLO antigen. The antigen was standardised by testing it against a set of positive and negative sera samples using imported (C.S.L.) antigen as control. Since continuous importation of the antigen or maintenance of serum samples would have posed a problem in due course of time, standardisation of antigen against Brown's opacity tubes would provide a better standard for preparation of the antigen.

TABLE II

Comparative results of testing chicken serum samples with indigenously produced (IVRI) and imported (C.S.L.) serum plate PPLO antigen

Place of testing	Source of serum samples	Maximum interval between collection of serum and its testing (days)	No. of samples tested	Results of plate testing					
				No. of serum samples					
				Positive with		Negative with		Doubletful with	
				I.V.R.I.	CSL	I.V.R.I.	CSL	I.V.R.I.	CSL
I.V.R.I., Izatnagar	U.P.	144	119	28	28	90	90	1	1
	Chandigarh	78	34	6	6	28	28	0	0
	Punjab	3	48	48	48	0	0	0	0
	Maharashtra	73	23	4	4	19	19	0	0
Veterinary	Trichur	21	39	23	23	15	15	1	1
College	Pondicherry	65	14	14	14	0	0	0	0
Madras	Ranipet	15	12	7	7	5	5	0	0
Bhubaneshwar	Bhubaneshwar	1½	120	45	41	60	64	15	15
	Bhubaneshwar	1½	100	16	27	71	61	13	12
Ludhiana	Ludhiana	1	106	9	9	94	94	3	3
Mathura	Mathura	2	39	20	24	13	10	6	5
	Mathura	7	93	81	85	12	8	0	0
Jabalpur	Jabalpur	—	63	62	62	1	1	0	0
Bangalore	Bangalore		80	21	21	59	59	0	0
Patna	Bihar	—	61	51	49	10	12	0	0
Total			951	435	448	477	466	39	37

TABLE III

Results of serum plate agglutination tests with indigenously produced PPLO antigen

Place of testing	Number of the serum samples				Per cent positive
	Tested	Positive	Negative	Doubtful	
Sarol, Chamba	50	0	40	10	0
Poona	85	72	4	9	84.7
Ernakulam	105	0	89	16	0
	55	33	20	2	60
Izatnagar	252	221	27	4	87.7
Khanpara, Gauhati	60	42	18	0	70
Total	607	368	198	41	
Percentage		60.6	32.6	6.8	

It is known that the imported PPLO antigen can be kept for about one year in the refrigerator at 4–5°C. The finding that indigenous PPLO antigen can also be stored for at least 80 weeks in refrigerator or room temperature should go a long way in the diagnosis and control of A.R.M.

The indigenously produced PPLO antigen when tested by various scientists gave very encouraging results, which were virtually similar to the results obtained with S.S.L. antigen. The slight variation in the results is rather insignificant. Further in the control programme of A.R.M. the percentage of reactors is not as important, as to know whether the flock is infected or not. Even a single reactor on a farm may spread the infection in the whole farm.

It thus appears that the indigenously produced PPLO antigen is very stable in character and can be safely used under field conditions.

ACKNOWLEDGEMENTS

The authors are thankful to Dr S. Kumar, the then Project Coordinator, Respiratory Diseases of Poultry for his interest in this work, and to the Director and Joint Director, IVRI, for the facilities provided for work. Thanks are also due to the scientists working in various Veterinary Colleges and State Departments for testing the sera samples.

REFERENCES

Agarwal, K. K., Prasad, A. K. and Kumar, S. (1972). A note on the development of a serum plate coloured antigen for the diagnosis of avian respiratory mycoplasmosis. *Indian J. anim. Sci.*, **42**, 142–143.