EVALUATION OF FOWL CHOLERA VACCINE BY MICE VACCINATION AND CHALLENGE INOCULATION SYSTEM

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Abstract

In the present study, 8 lots of commercial fowl cholera vaccines were evaluated by different methods. Results indicated that the lots failed to pass the two stages protection test in chicken. Comparable results were obtained by conducting either active immunization in mice or by the mouse vaccination challenge inoculation system. From the obtained results it can be concluded that mice can be used instead of chickens for evaluating fowl cholera vaccines as they are more cheaper and less susceptible to viral diseases that may complicate the evaluation test procedure in chicken.

INTRODUCTION

Fowl cholera caused by *Pasteurella multocida* is one of the oldest known infectious diseases of poultry. It is contagious and infects most domestic fowl and many wild birds (Heddleston and Watko, 1963). The acute form is characterized by septicaemia with high morbidity and mortality rates, however, chronic form is frequently observed (Dorsey and Harshfield, 1959). Immunization against this disease dates back over 100 years to the experiments of Pasteur, but research continues today to produce a more efficacious immunizing agents.

Fowl cholera bacterins have been produced commercially for over 30 years and methods of evaluating the potency of these bacterins have been in existence for over 25 years. However, a meaningful, standardized assay system that will give reproducible results has remained elusive.

Potency testing of avian *P. multocida* vaccines is not described in either the British or European Veterinary Pharmacopoeia. Most European companies depend on seroconversion of vaccinates as an evaluation procedure. The bioassay procedure was developed by the USA. Veterinary Services Laboratories measure the ability of a fowl cholera bacterin to prevent acute pasteurellosis in chal-

lenged chicken. This assay procedure limits manifestation of disease as an evaluation factor. The interpretation of potency is prevention against death. It follows a statistical model which is designed to accept a bacterin having 75% or greater efficacy (95%) and rejects a bacterin having an efficacy of 50% or less (Heddleston and Reisinger, 1960). In this assay, 12-week-old chickens were used for type A:1 bacterins and on 6-8-week-old turkeys for type A:3 bacterin (Heddleston, 1962).

The main drawbacks of the chicken protection assay procedure is that many viral diseases to which the test chickens are susceptible may complicate the protection results. Also, the source of these chickens may vary from lot to lot which makes standardization procedures difficult. The used chickens must not have a history of fowl cholera infection or vaccination. Chicken carriers for *P. multocida* may interfere with the protection test procedure.

It was the aim of this study to find out if it may be acceptable to perform potency test of fowl cholera bacterins in other small animal models, as mice, based on vaccination and challenge infection. Also, to compare results of mice vaccination challenge inoculation system with the results of potency test in chickens.

MATERIALS AND METHODS

1. Pasteurella multocida strains

The standard A:1 and A:3 strains of *P. multocida* were obtained from National Animal Disease Centre, Ames, Iowa, USA through the courtesy of Dr. S.M. Gergis, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

2. Fowl cholera vaccines

Eight lots of imported and local fowl cholera vaccines were subjected to potency test evaluation.

3. Two stages protection test in chicken

This test was carried out as described in the USA Code of Federal Regulations (1985). Briefly, the assay procedure was interpreted as a 2-stages cumulative death test. A bacterin was accepted if 6 or less of 20 birds died and was rejected if 9 or more birds died on the first stage. If 7 or 8 birds died, the bacterin was assayed by a second stage which had the same test procedure as the first stage. However, the death rate was cumulative and if 15 or less of the 40 birds assayed died, the bacterin was accepted, but if 16 or more of the 40 birds

died, the bacterin was rejected.

Active protection test in mice

The same procedure recommended by USDA for chickens previously mentioned was applied in mice. Challenge inoculation was by inoculation of 100 LD_{50} of respective *P. multocida* strains as recommended by:

Mouse vaccination challenge inoculation system

The method described by Ose and Muenster (1968) was followed. Briefly, fifty White Swiss mice (16-18 g) were each vaccinated subcutaneously with 0.2ml vaccine and again 14 days later. On day 24, they were divided into 10 groups of 5, each group being challenged with respective dilutions of a 24 hour broth culture of respective *P. multocida* strain in the range of -1 to -10 log_{10}. Fifty unvaccinated controls were similarly challenged and all mice observed for 5 days. The median lethal dose (LD_{50}) can then be calculated which was an indication of sufficient protection. A minimum of 2 logs protection was required for a vaccine lot to be accepted.

RESULTS AND DISCUSSION

The development of a standard potency test for evaluating *Pasteurella multocida* vaccines for poultry was crucial. The bioassay procedure developed by the USA Veterinary Services Laboratories for evaluating the potency of fowl cholera vaccines had several distinct advantages over previously used procedures depending on seroconversion of vaccinated birds. The challenge procedure had a greater chance of producing an acute septicaemic disease with a high mortality rate, allowing survival to be used for evaluating protection and thereby, allowing more objectiveness in interpretation of results. When dead birds were considered, the reproducibility of the test increased since the variation of determination of the severity of clinical signs among investigators would not be a factor (Heddleston and Reisinger, 1960; Code of Federal Regulation, 1985).

In the present study, evaluation test procedures in chicken and mice were compared for detecting the validity of different lots of local and imported fowl cholera vaccines. Reference bacterin was used in the test procedure to guard against over-challenging birds and mice vaccinated with the bacterins being evaluated. This reference bacterin allows further accurate measurement of immunity.

Results of the two stages protection test applied on chicken indicated that lots A, B, D, E, F and H passed the first stage of the test as the protection levels

can be considered protective according the USA Code of Federal Regulation. Meanwhile, lots C and G failed to pass the first stage protection test. These two lots were further evaluated by the second stage protection test. The cumulative number of dead chickens after challenge with A:1 and A:3 virulent strains of *P. multocida* further indicated the unvalidity of these two lots. These results gave further evidence to the two stage procedures recommended by the USA Code of Federal Regulation.

Comparable results were obtained by the application of active immunization test in mice, as lots C and G gave 55% and 50% protective respectively.

Similarly, when conducting mouse vaccination challenge inoculation system lots A, B, D, E, F and G gave 3-4 logs protection. Meanwhile, lots C and G gave 1 log protection which can be considered unacceptable according to Ose and Muenster (1968).

From the results of this study, it can be conducted that mice can be used instead of chickens for evaluating the potency of fowl cholera vaccines. The use of mice in the challenge test procedure had several advantages of which larger number of mice can be used allowing more accurate measurements of protection which can be easily calculated. Replication of the test can be performed at the same time for obtaining confirmed results. Using of mice instead of chickens had the privilege that they are not susceptible to many viral, bacterial and parasitic disease of poultry which can interfere with the evaluation procedure in chickens. Furthermore, they are cheaper and more susceptible to the different dilutions of virulent *P. multocida* strain with various degrees, and this procedure would overcome the difficulty of adjusting the challenge dose in chickens which may vary from one strain to another as one challenge dose is only used.

Table 1. Results of two stages protection test for evaluation of fowl cholera bacterins using virulent A:1 *P. multocida* as challenge strain.

Treatment	Challenge strain	First Stage		Second Stage	
		No. of chickens	No. of dead chickens	Cumulative No. of chickens	Cumulative No. of dead chickens
Control	A:1	20	18	-	-
Standard			3	-	-
Lot "A"			4	-	-
Control	A:1	20	17	-	-
Standard			4	-	-
Lot "B"			5	-	-
Control	A:1	20	19	-	-
Standard			3	-	-
Lot "C"			8	40	18
Control	A:1	20	19	-	-
Standard			5	-	-
Lot "D"			6	-	-
Control	A:1	20	18	-	-
Standard			4	-	-
Lot "E"			6	-	-
Control	A:1	20	17	-	-
Standard			4	-	-
Lot "F"			5	-	-
Control	A:1	20	19	-	-
Standard			3	-	-
Lot "G"			7	40	16
Control	A:1	20	18	-	-
Standard			4	-	-
Lot "H"			5	-	-

Table 2. Results of two stages protection test for evaluation of fowl cholera bacterins using virulent A:3 *P. multocida* as challenge strain.

Treatment	Challenge strain	First Stage		Second Stage	
		No. of chickens	No. of dead chickens	Cumulative No. of chickens	Cumulative No. of dead chickens
Control	A:3	20	17	-	-
Standard			4	-	-
Lot "A"			5	-	-
Control	A:3	20	19	-	-
Standard			3	-	-
Lot "B"			6	-	-
Control	A:3	20	19	-	-
Standard			4	-	-
Lot "C"			7	40	17
Control	A:3	20	18	-	-
Standard			3	-	-
Lot "D"			4	-	-
Control	A:3	20	19	-	-
Standard			5	-	-
Lot "E"			5	-	-
Control	A:3	20	18	-	-
Standard			3	-	-
Lot "F"			4	-	-
Control	A:3	20	19	-	-
Standard			5	-	-
Lot "G"			8	40	16
Control	A:3	20	19	-	-
Standard			3	-	-
Lot "H"			4	-	-

Table 3. Mice vaccination and challenge test results of different lots of fowl cholera vaccine using 100 LD$_{50}$ of virulent A:1 *P. multocida* strain.

	No. of mice	Challenge strain	Dead	Survival	Protection %
Control	20	A:1	20	-	-
Standard			4	16	80
Lot "A"			5	15	75
Control	20	A:1	20	-	-
Standard			3	17	85
Lot "B"			3	17	85
Control	20	A:1	20	-	-
Standard			4	16	80
Lot "C"			9	11	55
Control	20	A:1	20	-	-
Standard			3	17	85
Lot "D"			5	15	75
Control	20	A:1	20	-	-
Standard			4	16	80
Lot "E"			4	16	80
Control	20	A:1	20	-	-
Standard			3	17	85
Lot "F"			4	16	80
Control	20	A:1	20	-	-
Standard			4	16	80
Lot "G"			10	10	50
Control	20	A:1	20	-	-
Standard			3	17	85
Lot "H"			4	16	80

Table 4. Active vaccination and challenge test results of mice vaccinated with different lots of fowl cholera vaccine using 100 LD_{50} of virulent A:3 *P. multocida.*

	No. of mice	Challenge strain	Dead	Survival	Protection %
Control	20	A:3	20	-	-
Standard			3	17	85
Lot "A"			4	16	80
Control	20	A:3	20	-	-
Standard			4	16	80
Lot "B"			4	16	80
Control	20	A:3	20	-	-
Standard			3	17	85
Lot "C"			10	10	50
Control	20	A:3	20	-	-
Standard			3	17	85
Lot "D"			3	17	85
Control	20	A:3	20	-	-
Standard			4	16	80
Lot "E"			5	15	75
Control	20	A:3	20	-	-
Standard			3	17	85
Lot "F"			4	16	80
Control	20	A:3	20	-	-
Standard			4	16	80
Lot "G"			9	11	55
Control	20	A:3	20	-	-
Standard			3	17	85
Lot "H"			4	16	80

Table 5. Result of potency test of lots of fowl cholera vaccine using mouse vaccination challenge inoculation system with replication by A:3 strain of *P. multocida*.

Mice	Challenge strain of *P. multocida*	LD20 on 7th day after challenge	Logs Protection
Control	A:3	$10^{-7.10}$	-
Standard		$10^{-1.82}$	$10^{-5.28}$
Lot "A"		$10^{-2.35}$	$10^{-4.75}$
Control	A:3	$10^{-6.89}$	-
Standard		$10^{-2.11}$	$10^{-4.78}$
Lot "B"		$10^{-2.57}$	$10^{-4.32}$
Control	A:3	$10^{-7.28}$	-
Standard		$10^{-1.97}$	$10^{-5.31}$
Lot "C"		$10^{-6.0}$	$10^{-1.28}$
Control	A:3	$10^{-6.73}$	-
Standard		$10^{-2.10}$	$10^{-4.63}$
Lot "D"		$10^{-3.00}$	$10^{-3.73}$
Control	A:3	$10^{-6.90}$	-
Standard		$10^{-2.0}$	$10^{-4.90}$
Lot "E"		$10^{-2.98}$	$10^{-4.15}$
Control	A:3	$10^{-7.0}$	-
Standard		$10^{-2.8}$	$10^{-4.20}$
Lot "F"		$10^{-2.75}$	$10^{-4.25}$
Control	A:3	$10^{-6.83}$	-
Standard		$10^{-2.55}$	$10^{-4.28}$
Lot "G"		$10^{-5.22}$	$10^{-1.61}$
Control	A:3	$10^{-7.12}$	-
Standard		$10^{-2.63}$	$10^{-4.49}$
Lot "H"		$10^{-3.00}$	$10^{-4.12}$

Table 6. Result of potency test of lots of fowl cholera vaccine using mouse vaccination challenge inoculation system with replication by A:1 strain of *P. multocida*.

Mice	Challenge strain of *P. multocida*	LD20 on 7th day after challenge	Logs Protection
Control	A:1	$10^{-6.32}$	-
Standard		$10^{-1.00}$	$10^{-5.32}$
Lot "A"		$10^{-1.53}$	$10^{-4.79}$
Control	A:1	$10^{-5.63}$	-
Standard		$10^{-1.72}$	$10^{-3.91}$
Lot "B"		$10^{-1.88}$	$10^{-3.75}$
Control	A:1	$10^{-6.12}$	-
Standard		$10^{-1.58}$	$10^{-4.54}$
Lot "C"		$10^{-5.88}$	$10^{-0.24}$
Control	A:1	$10^{-5.90}$	-
Standard		$10^{-1.20}$	$10^{-4.70}$
Lot "D"		$10^{-2.41}$	$10^{-3.49}$
Control	A:1	$10^{-5.20}$	-
Standard		$10^{-1.34}$	$10^{-3.86}$
Lot "E"		$10^{-1.55}$	$10^{-3.65}$
Control	A:1	$10^{-6.31}$	-
Standard		$10^{-1.0}$	$10^{-5.31}$
Lot "F"		$10^{-1.72}$	$10^{-4.59}$
Control	A:1	$10^{-5.70}$	-
Standard		$10^{-1.20}$	$10^{-4.20}$
Lot "G"		$10^{-4.31}$	$10^{-1.39}$
Control	A:1	$10^{-6.00}$	-
Standard		$10^{-1.33}$	$10^{-4.67}$
Lot "H"		$10^{-2.03}$	$10^{-3.97}$

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تقييم لقاح كوليرا الطيور بإجراء اختبارات تحصين الفئران وتحدي المناعة

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تم في هذه الدراسة تقييم عدد ٨ دفعات من لقاح كوليرا الدواجن باستخدام الطرق المختلفة للتقييم وقد أوضحت الدراسة أن دفعتين فشلتا في اجتياز اختبار المناعة ذي المرحلتين في الدجاج. وقد تم الحصول على نفس النتائج سواء باستخدام التحصين الإيجابي للفئران أو اختبار التحصين وتحدي المناعة. ومن هذه النتائج أن الفئران يمكن استخدامها كبديل للدجاج لتقييم لقاح كوليرا الدواجن حيث إنها تتميز بأنها أرخص ثمناً وأقل تعرضاً للأمراض الفيروسية التي قد تتداخل مع إجراء اختبار التحدي في الدجاج.