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Table 1. Summary of 35 day mortality and performance (one week after APEC challenges).

Day of hatch vaccine	APEC challenge	Body weight (g)	Weight gain % (28-35d)	Rate of feed conversion	% Mortality
None	None	1,483.3[b]	18.83	2.54	0.0
	O1	1,475.2[b]	14.01	3.40	4.2
	O2	1,540.0[a]	18.66	2.70	4.2
	O78	1,439.1[b]	6.06	3.84	8.3
	3-APEC Average	1,485.4[B]	12.91	3.31	5.6
Poulvac E. coli	None	1,584.6[a]	29.85	2.25	0.0
	O1	1,579.1[a]	21.15	2.21	4.2
	O2	1,524.9[a]	21.18	2.57	0.0
	O78	1,440.0[b]	11.67	3.66	4.2
	3-APEC Average	1,514.7[A]	18.00	2.85	2.8

[a,b,A]Groups having a different letter are not statistically different, based on Duncan's multiple range test for performance values and Chi-square analysis for mortality ($P<0.05$).

GEL DROPLETS FOR THE DELIVERY OF POULTRY VACCINES IN THE BARNS

Eng H. Lee and Majed Al-Attar

Vetech Laboratories Inc.131 Malcolm Road, Guelph, Ontario, Canada N1K 1A8

SUMMARY

Gel droplets for the delivery of coccidiosis vaccines in the hatchery have been used successfully for the past few years. However, to use the same delivery in the poultry barns, it was necessary to add a "sticky" edible gum such as xanthan gum to the existing formulation. This addition helped the suspended vaccine droplets to linger on the back of birds longer and for easier pickups. When this modified gel droplets delivery was used to deliver an IBD vaccine to one-week old SPF chicks, colored tongues were found in over 80% of the treated chicks. Fourteen days post vaccination (PV), seven of 10 vaccinated birds were positive by ELISA tests compared to nine of 10 controls vaccinated by gavage. At 18 days PV, all 10 gel droplet vaccinated birds became positive. Similar results were obtained in the repeated test. When used for the delivery of a *Salmonella* vaccine, 14 of 15 vaccinated birds were found to be positive. A scaled-up version of what described here, most likely, will not require the time consuming withdrawal of

chlorine from the water before vaccination, and take away the worry of the same disinfectant that might cause vaccine failures after the water is restored.

Generally poultry vaccines are either live or inactivated and they may require different routes of administration. The most common methods are: Through drinking water, spraying, subcutaneous, or intramuscular routes (4). Sometimes, consideration for the routes of infection is the most natural for the application of the vaccine to stimulate good immune response. Poxvirus vaccines, for example, must be given in a manner that causes the vaccine to penetrate the skin (2).

The oral route of vaccination via drinking water is a common practice in administering live viral, bacterial, and parasitic vaccines for poultry. Usually, successful drinking water vaccination requires a lengthy preparatory procedure and many precautions to be considered before, during, and after vaccination. All these are needed in addition to the importance of maintaining the quality drinking water for vaccine administration (3). Two main concerns or disadvantages with water vaccination are: The uneven vaccine distribution affecting the amount of intakes and the inactivation of the vaccine before it is ingested. Added to these, the laborious steps that must be followed to achieve successful drinking water vaccination and the large quantity of water that must be used (3).

Vaccines to protect against common poultry diseases such as infectious bursal disease (IBD) and salmonellosis are good examples of orally administered poultry vaccines. Many types of IBD vaccines are now available; the live attenuated, the immune complex vaccine, or the inactivated oil-emulsion adjuvanted vaccines (8,6). Although these IBD vaccines can be administered by subcutaneous or *in ovo* injection or by spray, the most common route of administration is still through drinking water.

The use of gel droplets spray delivery system as a method of vaccinating chicks as well as turkey poults against coccidiosis in hatcheries and barns have been used successfully for many years (5,7). The ease, the uniformity of delivering the coccidial vaccine and the efficacy of the vaccination procedure as shown by the improvement of feed efficiency (7) may reflect the protection obtained against the clinical disease after vaccination. The present work is an extension for the use of the gel droplets spray method to replace the more laborious method of the drinking water vaccination of chicken against IBD and *Salmonella*. This method may be extended to replace the water vaccination for a number of other viral and bacterial diseases in the poultry houses. Therefore the aim of this study is to explore the possibility of replacing water vaccination by the gel droplets delivery system.

MATERIALS AND METHODS

Experimental chickens. Broiler chicks were hatched in our laboratory from specific pathogen free (SPF) eggs obtained from Sunrise Farm Inc. and were used throughout the experiments. The chicks were placed in single-use cardboard boxes and housed in a disinfected isolated quarter. Feed and water were supplied *ad libitum*.

The gel diluent. The Gel-sprayed vaccines were delivered with 1.3% of the 60/40 gel diluent of Vetech Laboratories Inc. and 0.1% of xanthan gum was added (Lee, USA Patent pending).

IBD virus (IBDV) vaccine preparation. Vaccine stabilizer was prepared by suspending 0.7 g of skim milk powder (Bermudez and Stewart-Brown, 2003) in 250 mL of distilled water. The lyophilized 2500 doses IBDV vaccine (S-706, Merial, Canada) was first dissolved in 5 mL distilled water and 2 mL of this reconstituted vaccine were added to the 250 mL of stabilizer to make a total of 1,000 doses. To this mixture a suspension of *E. acervulina* was added as markers for vaccine take at 300 oocysts per bird for Experiment 1. Inoculation of control birds was performed by using two 1 mL syringes to withdraw 0.75 mL of each of this vaccine suspension with constant agitation. For the gel droplets, about 6.2 g of gel diluents and 0.2 g of food color were then added and all mixed into a suspension and transferred to a 500 mL hand sprayer.

Experiment 1. IBD gel-spray vaccination. A total of 25 SPF chicks were used in this experiment. Control blood samples of 0.5 to 0.7 mL were collected from the jugular vein of 10 randomly selected chicks a day before vaccination. At seven days of age the 25 birds were divided into three groups and vaccinated as follow. Chickens in group 1 (six birds) were vaccinated by water gavage of reconstituted IBDV vaccine. Chickens in group 2 (six birds) were vaccinated by gavage of the gel-spray containing IBDV vaccine. Chickens in group 3 (13 birds) were vaccinated by droplets spraying of gel containing the IBDV vaccine.

Vaccine Take. The presence of coccidial infection was used as an early indicator for possible IBDV vaccine take in Experiment 1. One bird each from groups 1 and 2 and two birds from group 3 were examined on Day 5 post inoculation (PI) for the present of lesions in the duodenum. The rest of the birds were examined for the present of oocysts in their fecal samples collected on Day 6 PI.

Experiment 2. IBD gel-spray vaccination. The IBDV vaccine was prepared as in Experiment 1 except Immucox® (Vetech Laboratories Inc. Guelph Ontario, Canada) was added to the gel of group four. This experiment was done with 25 birds, divided into five groups. Group 1 of four chickens served as non-

vaccinated controls. Chickens in group 2 and 3 of five chickens each were vaccinated by water gavage and gel gavage respectively. Groups 4 were vaccinated by gel gavage containing Immucox and group 5 of six chickens were vaccinated by gel spray.

Antibody response to IBD vaccination. All sera collected before and after vaccination in Experiments 1 and 2 were tested for presence or absence of antibody to IBDV by ELISA test. This was done by the Laboratory Services of the Animal Health Laboratory of the University of Guelph, Guelph, Ontario.

***Salmonella* vaccine.** A live mutant of *Salmonella* Typhimurium vaccine (Salmune®) was used (CEVA, Lenexa, KS, USA).

***Salmonella* gel spray vaccination.** This experiment was done to determine if commercially available live *Salmonella* vaccine can be uniformly delivered by the gel-spray method and if it can be used as an alternative method to water vaccination without being affected by coccidiosis vaccine (Immucox) when mixed. Before vaccination, cloacal swabs as negative controls were randomly collected from 10 birds, four days before any of the 12-day old SPF birds were vaccinated. Then, the 25 birds were vaccinated and divided into two groups of 15 birds each in the sprayed groups and 10 in the gavage group. The prepared Gel-spray that mixed with the vaccines was plated for *Salmonella* identification. Gel-sprayed birds were sprayed at a rate of one spray per three birds which is equal to about one recommended dose (0.25 mL/bird). The positive control birds were inoculated by gavage with the recommended dose of Salmune (0.25mL) through a 1-mL syringe.

RESULTS

Serum antibodies response to IBD vaccination. In Experiment 1, the mean ELISA titers of chickens vaccinated by water or gavage and droplets spraying are shown in Table (1). Chickens responded well to the different method of vaccination especially at day 14 and 18 post vaccination, also, good response was obtained at day 11 post vaccination in the group which was vaccinated by the gel gavage route. The mean titers were 2946 and 2850 at 14 and 18 days post vaccination in water gavage respectively. Mean titers of 1805 and 2517 were recorded in the gel gavage group at 14 and 18 days post vaccination respectively (Table 1). The mean titer of chickens vaccinated by the gel droplets at 11 days post vaccination was low, but at 14 days post vaccination the mean ELISA was 3610 which is the highest titer compared to all the groups and to those titers at all periods post vaccination (Table 1). At 18 days post vaccination the ELISA titers were comparable for all groups (Table 1). The percentages of positive antibody titers in all groups were gradually increased and reached 100% at 18 days post vaccination (Table 1).

Vaccine take as shown by coccidial infection. In Experiment 1, duodenal lesions were detected in one of one chick examined and oocysts were detected in four fecal samples out of five examined chickens from the group vaccinated by water gavage. In the chickens vaccinated by the gel gavage duodenal lesions were detected in one of one chick examined and oocysts were detected in all. In the group vaccinated by gel droplets spraying, duodenal lesions were detected in two of two chickens examined and oocysts were detected in six fecal samples out of 10 (Table 1).

In Experiment 2 mean ELISA antibody titers at 11 days post vaccination were generally low except in the group vaccinated by the gel gavage route it reached 2380. The highest mean ELISA titers at 18 days post vaccination reached 2595 in the group vaccinated by the gel droplets spraying that mixed with Immucox method compared to the other groups. The mean ELISA titers of all vaccinated groups were gradually increased with time, but the mean titers of the groups vaccinated by droplets gel spray method, with and without Immucox vaccine, were more uniformly increased (Table 1).

***Salmonella* gel spray vaccination.** All the 10 chickens sampled before vaccination were negative for *Salmonella*. Direct plating of the vaccine revealed pure culture of the vaccine *Salmonella*. All chickens, except one, that were vaccinated by the gel droplets vaccination method including the chickens that had received Immucox and Salmune vaccines mixture; were positive for vaccine *Salmonella* when swabbed from cloaca at two days post vaccination.

DISCUSSION

Finding an alternative to drinking water in the delivery of live poultry vaccines, may be necessary partly because of the tedious procedure needed for the avoidance of chlorine before and after vaccine is applied. In addition to that, the stress of water deprivation to the birds, as well as the increase in the use of closed watering system, all of which makes water vaccination more difficult (1,2).

An alternative with coarse-water spray for IBD vaccination had recently been shown to be successful (1). The modified gel droplets application, reported here, may be another option for this application of IBD vaccination. This is supported by comparable antibody responses of these sprayed chicks to the control birds (Table1).

Similarly, the recovery of the vaccine *Salmonella* from cloacal swabs, were almost the same from the control group vaccinated by water gavage and the group vaccinated by the droplets spraying method.

Therefore, the application of gel droplets method to vaccinate against *Salmonella* was as effective as water vaccination.

This application of the gel droplets vaccination in barn to vaccinate chickens against IBD and *Salmonella* likely can be extended to deliver other poultry vaccines such as hemorrhagic enteritis for turkey and Newcastle disease for chickens.

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Table 1. Infectious bursal disease antibodies detected by ELISA test at different intervals post vaccination using gel droplets spraying vaccination method compared to oral gavage vaccination (Experiment 1).

Groups	Treatments	Coccidia		IBD (ELISA) 1-day	IBD (ELISA) 11-days	IBD (ELISA) 14-days	IBD (ELISA) 18-days
		Lesions	Oocysts				
All groups	10*/25 No treatment	ND**	ND	**0**/10***	-	-	-
1	Water (gavage)	1/1	4/5	-	ND	4/5 (2946)****	4/5 (2850)
2	Gel spray (gavage)	1/1	5/5	-	4/5 (2081)	5/5 (1805)	5/5 (2517)
3	Gel spray (Sprayed)	2/2	6/10	-	2/5 (704)	7/10 (3610)	10/10 (2094)

*Number of chickens tested

** Not done

***Number positive/Number tested.

( ) **** Means ELISA titers.