

Gel-Pac[®]

Gel-Pac[®] Permits Combining AviPro[®] Megan[®] Egg *Salmonella* Vaccine and Coccidiosis Vaccine for Hatchery Spray Application

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SUMMARY

A principal method of reducing *Salmonellosis* in poultry and the vertical transmission of *Salmonella* to poultry products for humans includes administering a live *Salmonella* vaccine at one day of age in the hatchery. Operators have not been able to successfully combine live *Salmonella* vaccines with coccidiosis vaccine due to the inactivation of *Salmonella* vaccine by antagonistic additives in coccidiosis vaccines. This report details the negative impact of this antagonism and demonstrates a process to overcome it, allowing hatcheries to combine *Salmonella* vaccine with a coccidiosis vaccine in one application of stabilized edible gel beadlets. Gel delivery is proven to be a more effective vehicle for gut-protective vaccines; this process allows the hatchery to vaccinate chicks or poults against both diseases simultaneously, and ensure the birds receive a fully protective dose targeted directly at the gut, where both vaccines initiate immune stimulation.

DESCRIPTION OF PROBLEM

Human populations are growing in number and in affluence; creating a literal hunger for more food, and healthier diets. Improving food safety is one goal that spurs workers to improve animal production practices. The effort is global in scope, sprouting from governments' desires to protect citizens and protect the reputation of their meat exports. Shoulder-to-shoulder with governments, multinational animal health and integrated food companies also work to be valued for their role in protecting animal health and, consequently, food safety. Protection from *Salmonella* tops the agenda, and the list of tactics to reduce the *Salmonella* burden from poultry products includes maintaining biosecurity, monitoring pathogens, selectively using antimicrobial drugs, and defending gut health through probiotics, prebiotics and vaccinations.¹ This research report focuses on making vaccination more convenient as well as more effective.

The weight of more consumers preferring meat from antibiotic-free animals, plus government regulations to reduce animal antibiotic treatments, have tipped a balance; it puts more pressure on *Salmonella* prevention rather than cure. Prevention is the role uniquely suited to vaccines, helping animals to build their own defenses rather than using medications to correct sickness after the fact.

Live *Salmonella* vaccines work. AviPro[®] Megan[®] Egg and AviPro[®] Megan[®] Vac,¹ attenuated live *Salmonella* vaccines, reduce the *Salmonella* load in chickens and turkeys - a benefit that is measured in significantly reduced *Salmonella* in live birds,²⁻⁴ and significantly less contamination in processing plant pre-evisceration, post-chill, and parts rinse when birds are vaccinated at day 1 in the hatchery and again at day 14.⁵ Importantly, the bird's gut on day 1 is at its cleanest. Like a freshly cultivated field, the new gut is virtually free from contamination. It is fertile ground, allowing the *Salmonella* vaccine to seed itself and occupy the gut to defend the young bird. The vaccine locally immunizes the chick or

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poult on the mucosal surface, and initiates cell-mediated systemic immunity for internal organs. These defenses protect the gut tissue and the internal organs from pathogens that are sure to come after the chick or poult arrives at the farm.^{6,7}

The AviPro Megan vaccines' mode of action is to first colonize the gut, then to stimulate cellular, local and systemic immunity. An accepted means of vaccinating with the *Salmonella* vaccines at one day of age in hatcheries is via coarse spray, using cabinets with aerosol spray nozzles similar to those used to vaccinate against bronchitis, Newcastle disease, and coccidiosis.

Coccidiosis vaccines, like *Salmonella* vaccines, are among those that can be sprayed on chicks and poults or consumed by the birds in drinking water or feed. Researchers reported that coccidiosis vaccines administered in sprayed edible gel droplets protected significantly better than the same vaccine in liquid spray.^{8,9} In addition to edible gel being an intuitive way to deliver an oral vaccine or other oral additive, gel has been shown to uniformly suspend vaccine without constant agitation, induce less evaporative heat loss from chicks,¹⁰ improve vaccine uniformity, and reduce vaccine waste.¹¹

Edible gel droplet applicators, either newly installed or retrofitted from existing cabinet sprayers, are placed in-line at the hatchery. Early tests with gel droplets used gels prepared by sequential heating, forming and cooling.⁸ Newer versions of gel require little preparation. A cool-water soluble gel powder is homogenized with approximately 97% hatchery tap water, then the vaccine is uniformly mixed in. Hatchlings, sprayed while being moved through the hatchery, eat the gel droplets within a few minutes, giving the oral additive direct access to the gut before the bird leaves the hatchery. Protection begins before the bird arrives at the farm.

It sounds simple, spray an edible gel vaccine in the hatchery to gain the upper hand on *Salmonella*. Even more convenient if the two most commonly-used vaccines to address *Salmonella* and coccidiosis could be mixed and consumed in the same gel. Such mixing is possible, but one must take deliberate care to prevent the antagonistic preservatives in most coccidiosis vaccines from killing the *Salmonella* vaccine and ruining this critical first step in *Salmonella* control. It is a dominant practice among coccidiosis vaccines to include powerful preservatives; these preservatives are lethal to other biologicals, including *Salmonella* and IB/ND vaccines, unless the vaccines are properly stabilized. One edible gel delivery system, Gel-Pac[®], is engineered to protect fragile biologicals from antagonistic coccidiosis vaccine. Gel-Pac has been observed to reduce oxidation attributed to 4 commercial coccidiosis vaccines preserved with potassium dichromate or chloramine,¹² and is reported to stabilize IB vaccine and probiotic bacteria against inactivation by chlorine, iron and nitrate.¹³

**Protection begins
before the bird
arrives at the farm**

MATERIALS AND METHODS

To assess the protective effect of Gel-Pac toward AviPro[®] Megan[®] Egg, an attenuated live *Salmonella* vaccine, researchers¹⁴ prepared 3 vaccine solutions and measured the *Salmonella* vaccine titer retention in each solution throughout 6 hours. The vaccine solutions were:

1. *Salmonella* Vaccine (10^8 CFU/ml) in water, as control
2. *Salmonella* vaccine (10^8 CFU/ml) plus coccidiosis vaccine (100 doses/ml) in water, as a non-stabilized solution
3. *Salmonella* vaccine (10^8 CFU/ml) plus coccidiosis vaccine (100 doses/ml) in water stabilized with Gel-Pac (25g/l), as a gel-stabilized solution

Throughout the 6-hour period, the *Salmonella* vaccine titers were determined in triplicate at 0, 0.5, 1, 2, 4, and 6 hours following mixing.

^aAviPro[®] Megan[®] Egg, Elanco Animal Health, Greenfield, IN 46140 USA

^bHATCHPAK[®] COCCI III, Boehringer Ingelheim, Gainesville, GA 30503, USA

^cGel-Pac,[®] Animal Science Products, Inc., Nacogdoches, TX 75963, USA

RESULTS

There were no significant differences in vaccine concentrations among the treatments at the initial and 30 minute applications. After 1 hour in solution, the gel-stabilized vaccine maintained greater concentrations than both the control ($p < 0.01$) and non-stabilized vaccine ($p < 0.05$), the gel-stabilized vaccine being $10^{8.42}$ CFU/ml, control vaccine $10^{8.11}$ CFU/ml, and non-stabilized vaccine $10^{8.19}$ CFU/ml (Table).

The trend in the decay of non-stabilized vaccine continued, the titer falling to $10^{7.72}$ CFU/ml at 4 hours, remaining significantly less than control at $10^{8.11}$ CFU/ml ($p < 0.05$) and stabilized gel at $10^{8.29}$ CFU/ml ($p < 0.01$). The respective concentrations, ranked and expressed as percentages of the initial control titer for gel-stabilized, control, and non-stabilized vaccines were 118%, 80%, and 32% (Figure).

The general pattern of *Salmonella* vaccine preservation in gel and inactivation in the non-stabilized solution continued through the 6-hour sampling. Stabilized gel maintained $10^{8.23}$ CFU/ml, significantly more than non-stabilized vaccine at $10^{7.86}$ CFU/ml ($p < 0.01$). Control vaccine titer was intermediate at $10^{8.08}$ CFU/ml. The respective concentrations for gel-stabilized, control, and non-stabilized vaccine were 102%, 80%, and 44%, expressed as percentages of the initial control titer. Throughout the entire study, the *Salmonella* vaccine in the non-stabilized application exhibited a markedly accelerated decay leading to significantly reduced final titer, compared to the gel-stabilized solution (Figure). There was no apparent *Salmonella* vaccine titer loss in the gel-stabilized vaccine solution; the rate of change for gel-stabilized vaccine was +2.7% per hour, the control treatment change rate was -4.5% per hour, and the decay rate in non-stabilized spray was more than twice as fast, -11.2% per hour.

CONCLUSIONS AND APPLICATIONS

The significant deterioration experienced by the *Salmonella* vaccine in the presence of coccidiosis vaccine was completely prevented by Gel-Pac's stabilizing properties. Safely combining the two vaccines in one application offers hatcheries the potential to efficiently vaccinate with one spray. Furthermore, the edible gel droplets facilitate chicks or poults receiving the vaccine directly targeted to the gut, where both vaccines must inhabit before beginning their protective work. Edible gels carrying coccidiosis vaccine have been shown to protect the gut significantly better against coccidiosis challenges than liquid spray.^{8,9} Live *Salmonella* vaccine's mode of action is also to first colonize the gut, before traversing into the reticuloendothelial system, where cellular, local and system immunity are stimulated. This research confirms Gel-Pac rescues live *Salmonella* vaccine from the antagonistic effect of coccidiosis vaccine, offering hatcheries an efficient, convenient way to administer both vaccines.

It is a great time to serve agriculture. Our growing population requires more food, while demanding it to be healthy and safe. Those demands require heavy lifting on our part; in the case of preventing *Salmonellosis* using live vaccines, the hatchery is uniquely positioned to take the critical first step.

Table. *Salmonella* vaccine concentrations in water alone, non-stabilized coccidiosis vaccine solution, and coccidiosis vaccine solution stabilized with Gel-Pac.®

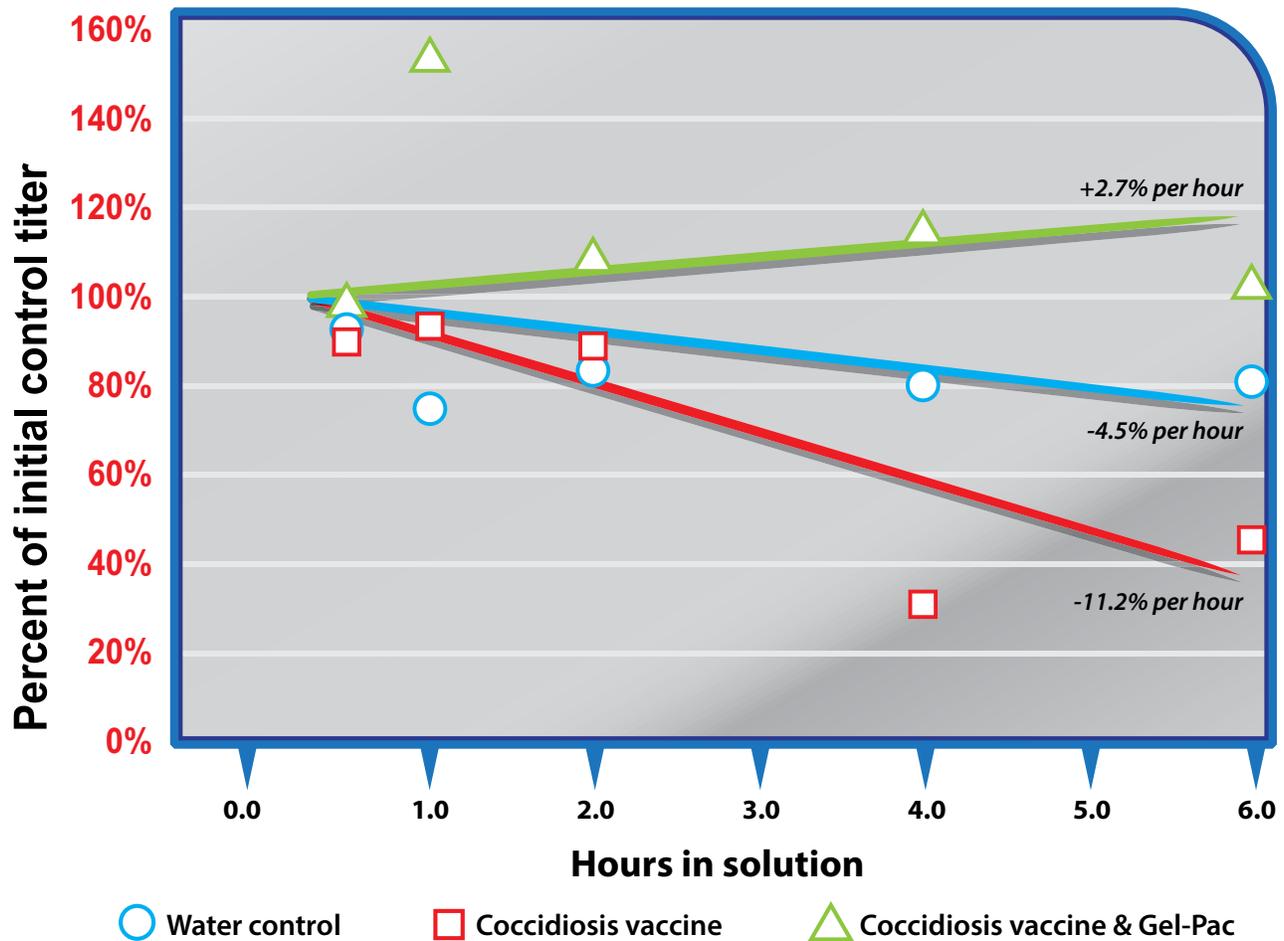
Time, Hours	<i>Salmonella</i> vaccine in water alone	<i>Salmonella</i> vaccine in non-stabilized coccidiosis vaccine solution	<i>Salmonella</i> vaccine in gel-stabilized coccidiosis vaccine solution
0	$10^{8.22}$ a	$10^{8.19}$ a	$10^{8.33}$ a
0.5	$10^{8.17}$ a	$10^{8.16}$ a	$10^{8.21}$ a
1	$10^{8.11}$ b**	$10^{8.19}$ b*	$10^{8.42}$ a*, **
2	$10^{8.14}$ a	$10^{8.15}$ a	$10^{8.25}$ a
4	$10^{8.11}$ a*	$10^{7.72}$ b*, **	$10^{8.29}$ a**
6	$10^{8.08}$ ab	$10^{7.86}$ b**	$10^{8.23}$ a**

Concentration (CFU/ml) of *Salmonella* vaccine (initial dose 10^8 CFU/ml) after being held in solutions of water as control, water with coccidiosis vaccine (100 doses/ml), and water with coccidiosis vaccine (100 doses/ml) stabilized with Gel-Pac (25g/l). Mean of 3 replicates per time point.

^{ab} Within sampling time, means sharing lowercase letters are not significantly different.

*, ** Different lowercase letters indicate significance using * ($p < 0.05$) and ** ($p < 0.01$).

Figure. Live *Salmonella* vaccine survival in water alone, non-stabilized coccidiosis vaccine solution, and coccidiosis vaccine solution stabilized with Gel-Pac®.



Concentration of *Salmonella* vaccine as a percent of initial control titer ($10^{8.22}$ CFU/ml) after being held in solutions of water as control, water with coccidiosis vaccine (100 doses/ml), and water with coccidiosis vaccine (100 doses/ml) stabilized with Gel-Pac (25g/l). Mean of 3 replicates per time point.

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Gel-Pac



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