Gel-Pac Improves Coccidia Vaccination Uniformity and Permits Combined IBV Vaccination via Gel-Drop

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Primary Audience: Veterinarians, Poultry Flock Supervisors, Hatchery Managers

SUMMARY

This study evaluated the stability of vaccines against infectious bronchitis virus (IBV) and coccidiosis in commercial poultry when combined and administered via aerosolized water spray or gel-drop diluents, both *in-vitro* and *in-vivo.* Diluents were compared for their impact on IBV vaccine thermal stability, IBV vaccine titer stability, coccidiosis vaccine positional stability throughout the application process, hatchling chick body temperature, and coccidia vaccine cycling pattern uniformity. Diluents did not differ in effect on chick thermal response or IBV vaccine stability. Gel-drop diluent provided more stable coccidia oocyst suspension without agitation during vaccination, and improved vaccine oocyst uniformity during post-vaccination cycling. Gel-drop proved at least as effective as traditional water spray for delivering the IBV and coccidia vaccines used in this study, both alone and together in a single vaccine suspension.

Key words: coccidiosis vaccine, infectious bronchitis virus vaccine, gel-drop

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DESCRIPTION OF PROBLEM

Infectious bronchitis virus (IBV) and coccidia vaccines have been traditionally administered by aerosol spray cabinet in the hatchery at one day of age. IBV is an upper respiratory tract pathogen, therefore exposing the IBV vaccine to upper respiratory tissue (conjunctiva, Harderian gland, choanal cleft, and trachea) is critical for proper vaccination and immunization. Application volumes of IBV vaccines vary, but typically range from 7-21 ml per 100 chicks (1 chick box). Unlike IBV, coccidia is an enteric pathogen, making vaccine ingestion critical to deliver the oocysts to the target tissue in the gut. Recently introduced gel-drop vaccination technology has not been thoroughly tested for all vaccine types (respiratory vs. enteric). Gel-drop vaccine diluent is intended to increase coccidia vaccine application efficiency via direct ingestion. The gel-drop mechanisms utilize a highly viscous gel applied under pressure through an application bar with openings of varying size which release the gel. The gel then "streams" out of the openings and forms droplets before reaching the chicks. Ideally the chicks then preen the droplets, ingesting the coccidia oocysts at the same time. With the recent introduction of new coccidia vaccine formulations, designed to permit combining with other vaccines during application (IBV most notably), the question arises whether IBV vaccines can be effectively applied via gel-drop, combined with coccidia vaccine, in the same manner.

MATERIALS AND METHODS

In-vitro Assessment of Gel Effect on Vaccines

IBV vaccine[2] was mixed with Gel-Pac® (GP)[3] in the recommended concentration using room temperature water (21°C) by reconstituting powdered gel, then adding vaccine. A sample of the gel/IBV vaccine mixture was

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then taken immediately after mixing and every thirty minutes for 4 hours. These samples were tittered in embryonated eggs to evaluate the stability of IBV vaccine in the gel over time. A water/IBV vaccine mixture was also tittered in the same way at the same concentration as a control. In a second experiment, chilled water (13°C) was used to reconstitute the GP and the experiment repeated, using a chilled water/IBV vaccine group as a control. It has been previously shown that IBV vaccine needs to be kept cool (<18°C) to maintain titer over time, and this experiment tested any differences from that for the gel.

After the temperature effects for the water to be mixed with the gel were evaluated, the next experiment involved mixing IBV and coccidia[4] vaccines together in a single gel-drop vaccine and repeating the experiment. Previous studies have shown that there is no effect of IBV vaccine on coccidia oocysts, so the only evaluation was to measure the IBV vaccine titer over time.

Lastly, the coccid vaccine was only mixed in the gel and held with no further mixing to establish the positional stability of oocysts in suspension for each diluent. Samples were taken from the top 1/3, the middle 1/3, and the bottom 1/3 layer of the vessel immediately after mixing and then every 30 minutes for at least 2 hours. These samples were then floated and coccidia vaccine oocysts were counted. A coccidia vaccine mixed in water group was prepared in the same way and sampled as a control.

In-vivo Assessment of Gel Effect on Vaccines

Diluent Effect on Chick Body Temperature. Newly-hatched chicks were placed into chick baskets prior to vaccination and allowed to acclimate to the environment of the vaccination room. Temperatures of 25 chicks were measured by rectal thermometer before vaccination to obtain baseline data. After vaccination, the temperatures of 25 chicks from each vaccinated group were measured throughout 60 minutes to evaluate any reductions and/or recoveries in body temperature from the vaccination process.

Diluent Effect on IBV Vaccine Titer. Four groups were vaccinated using commercial hatchery aerosol spray or gel application vaccination equipment.

Group 1 - One hundred broiler chicks were spray vaccinated using dual fan nozzles at a full dose with IBV vaccine alone, mixed in water. All 100 chicks were held in the chick box for 1 hour and then placed in a colony house following vaccination.

Group 2 - One hundred broiler chicks were spray vaccinated using dual fan nozzles at a full dose with IBV and coccidia vaccine combined in water. All 100 chicks were held in the chick box for 1 hour and then placed in a colony house following vaccination.

Group 3 - One hundred broiler chicks were vaccinated using gel at full dose with IBV vaccine alone. Vaccine and gel were mixed according to the manufacturer's instruction. All 100 chicks were held in the chick box for 1-hour post vaccination to allow for preening and monitoring and then placed in a colony house.

Group 4 - One hundred broiler chicks were vaccinated using gel at full dose with IBV and coccidia vaccine combined. Vaccine and gel were mixed according to the manufacturer's instruction. All 100 chicks were held in the chick box for 1-hour post vaccination to allow for preening and monitoring and then placed in a colony house.

IBV vaccine infection rate and viral load were evaluated by swabbing every chick remaining in a colony house from each group on days 5 and 7 post-vaccination. All chicks were euthanized after their respective sampling period.

Diluent Effect on Coccidiosis Vaccine Shedding. For all groups receiving coccidia vaccine, twenty chicks from each group were removed from the colony houses on day 4 and placed individually into isolators. They were held there for 5 days (days 5-10 post-vaccination). Feces from each chick were collected daily and oocysts were counted to evaluate infection rate and oocyst numbers shed per gram of feces.

Vaccines. Commercially available IBV (Ma5) and coccidia vaccine (B-52) from Merck Animal Health.

IBV Vaccine Detection Post-Vaccination. At 5- and 7-days post vaccination, all chicks were swabbed in the intrachoanal cleft, and qRT-PCR were performed on all samples.

[4] B-52, Merck Animal Health, Madison, NJ 07940 USA

Virus Detection. Samples were tested for IBV by quantitative real time RT-PCR and expressed as the relative amount of virus (cycle threshold (Ct) value) in the sample, as well as viral genome copies. Viral RNA was extracted from each sample using the MagMAX-96 RNA Isolation Kit (Ambion Inc., Austin TX) according to the manufacturer's protocol on a KingFisher magnetic particle processor (Thermo Scientific, Waltham, MA) and used as template in the reaction. Real time RT-PCR was conducted using an Applied Biosystems Fast 7500 Real Time PCR Machine (Life Technologies, Carlsbad, CA) and the AgPath-ID™ One-Step RT-PCR kit (Ambion Inc.) according to the manufacturer's recommendations. Primers and probe for the real time RT-PCR were previously published and consist of a forward primer IBV5'GU391 (5'-GCT TTT GAG CCT AGC GTT-3'), a reverse primer IBV5'GL533 (5'-GCC ATG TTG TCA CTG TCT ATT G-3') and a Taqman® dual-labeled probe IBV5'G probe (5' –FAM-CAC CAC CAG AAC CTG TCA CCT C-BHQ1-3'). The primers were obtained from Integrated DNA Technologies (Coralville, IA) and the Taqman® probe was synthesized by BioSearch Technologies (Novato, CA). Real time RT-PCR components and thermocycler parameters were conducted as previously described.

Oocyst Enumeration. Oocysts were enumerated for all parts of the trial utilizing a McMaster's chamber. Feces was collected and saturated in 10-times water and allowed to soak overnight to release oocysts. Fecal slurry was filtered through a double layer of cheese cloth, and the flow through was centrifuged to concentrate oocysts. Sample was then mixed with an appropriate dilution of saturated salt water. The resulting sample was then mixed and pipetted into a McMaster's chamber. The chamber was held for three minutes so oocysts could rise to the top of the chamber, then oocysts were counted using the method of Conway and McKenzie. Oocysts were speciated according to the morphological characteristics of the different species present in the vaccine according to the manufacturer, including size and shape (Conway and McKenzie, 2007).

RESULTS AND DISCUSSION

In-vitro Assessment of Gel Effect on IBV Vaccine

IBV vaccine was mixed with GP gel made with room temperature (21°C) water or chilled (13°C) water and titrations were performed on the mixture initially, then every 30 minutes for 4 hours to assess longevity of IBV vaccine over time in gel. In addition, IBV vaccine and coccidia vaccine were mixed with GP at the same two temperatures and IBV titrations were again performed.

Overall, minor variations in individual titers at various timepoints proved transient, resulting in no difference in IBV vaccine titer over the 4-hour period when mixed alone in GP, whether at room temperature or chilled (Table 1a). To account for variability that may have been induced by using separate vaccine vials for each treatment, titer changes from each treatment's initial input were calculated (Figure 1a). When evaluating titers of IBV vaccine mixed with coccidia vaccine, there was a pattern of slight decline in IBV titer from time 0 to 1 hour, then a stabilization of IBV titer from the 1-hour to the 4-hour timepoint in all groups (Table 1b and Figure 1b). The decline from 0 to 1 hour in all IBV plus coccidia groups was not seen in the IBV only groups and therefore may be attributed to mixing with coccidia vaccine. The decline in IBV titer in the presence of coccidiosis vaccine was apparently unrelated to diluent temperature or gel effects, as evidenced in Figure 1a. While some previous research has shown that coccidia vaccine without oxidizing preservatives, such as the coccid vaccine used in this experiment, does not impact IBV vaccine when combined, other research has shown a small impact of coccid vaccine on IBV titer as was seen in this study. It is always recommended to ensure individual IBV and coccidia vaccine compatibility before mixing.

Table 1a. Log 10 EID_{50} titers of IBV vaccine alone in different diluents[1]

	Hours in solution								
	0	.5	1	1.5	2	2.5	3	3.5	4
RTW	4.83	5.48	4.63	4.57	5.50	5.50	4.83	5.32	5.32
RTG	4.57	4.63	4.50	4.63	3.50	4.32	4.83	4.50	4.50
CW	4.75	4.57	5.32	4.68	5.38	5.50	4.57	5.35	4.63
CG	3.67	4.50	4.65	4.53	4.65	-	4.78	-	3.58

Table 1b. Log 10 EID_{50} titers of IBV vaccine combined with coccidiosis vaccine in different diluents[1]

	Hours in solution								
	0	.5	1	1.5	2	2.5	3	3.5	4
RTW	5.32	5.17	4.57	4.50	4.83	4.83	4.32	4.43	4.84
RTG	3.60	2.57	2.83	2.83	2.67	2.57	2.83	2.60	2.83
CW	4.17	4.20	3.32	3.63	3.17	2.50	2.50	2.50	2.56
CG	3.19	2.17	2.50	2.31	2.70	2.63	2.48	2.32	2.31

[1]RTW = room temperature (21°C) water; RTG = room temperature gel
CW = chilled (13°C) water; CG = chilled gel

Positional Stability of Coccidiosis Vaccine in Water and Gel Suspension

One drawback to using water to dilute and apply coccidia vaccine is the constant mixing required to keep oocysts in proper suspension; this need for constant

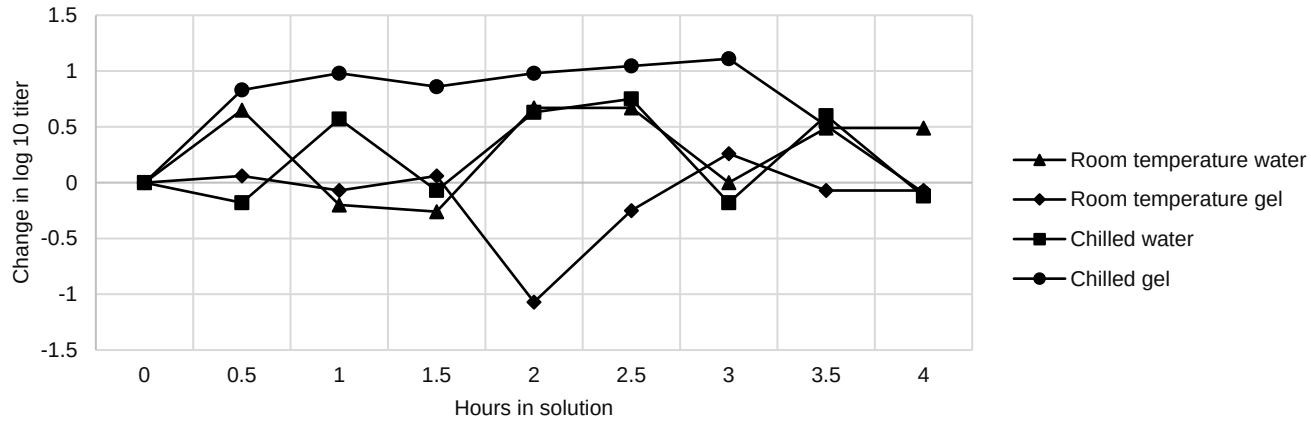

Figure 1a. Change in IBV vaccine EID_{50} titers (+ gain and - loss from initial time 0) in water or gel diluent at room temperature (21°C) and chilled (13°C). Vaccines were prepared from separate vials at time 0 and held for 4 hours, with samples extracted for titration each 30 minutes.

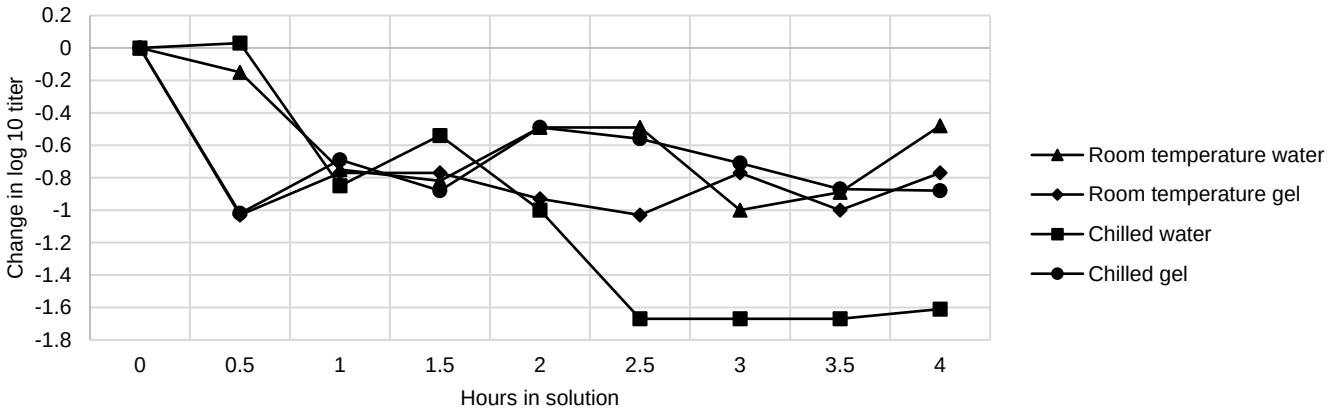

Figure 1b. Change in IBV vaccine EID_{50} titers (+ gain and - loss from initial time 0) when combined with coccidiosis vaccine in water or gel diluent at room temperature (21°C) and chilled (13°C). Vaccines were prepared from separate vials at time 0 and held for 4 hours, with samples extracted for titration each 30 minutes.

mixing may be overcome by using a stable gel suspension. When mixed with water and not continually stirred, the oocysts from the coccidia vaccine settled to the bottom of the vessel almost immediately and stayed there. The oocyst distribution in the vessel was uniform at time 0 immediately after mixing, but by the end of 30 minutes without agitation the top and middle fractions were devoid of oocysts. Concurrently, oocysts concentrated in the bottom fraction of the mixture increased (Figure 2a). This matches previous data from this lab and concurs with the manufacturer's recommendations to constantly mix coccidia vaccine in water diluent.

Conversely, coccidia vaccine mixed with GP experienced very little settling over time. There was a passing decline in oocyst numbers in the top third of the vaccine solution between 0 and 30 minutes, and a correlated increase in the middle fraction at this time point, followed by increasingly uniform oocyst distribution throughout 120 minutes in all fractions of the mixture. Despite the slight initial shift in distribution, over the 2-hour duration the concentration of oocysts remained more uniform in GP than water (Figure 2b).

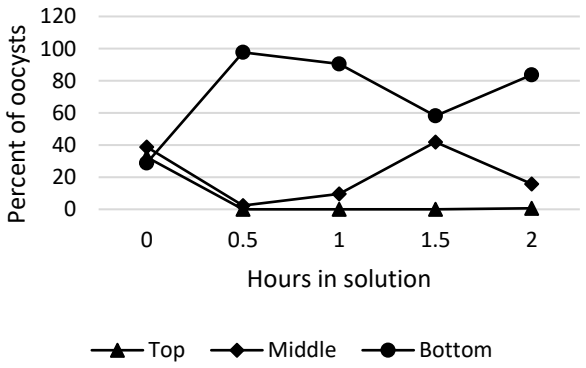

Figure 2a. Percent of coccidiosis vaccine oocysts distributed throughout a water spray solution. Coccidiosis vaccine was stirred in solution at time 0 and held undisturbed for 2 hours.

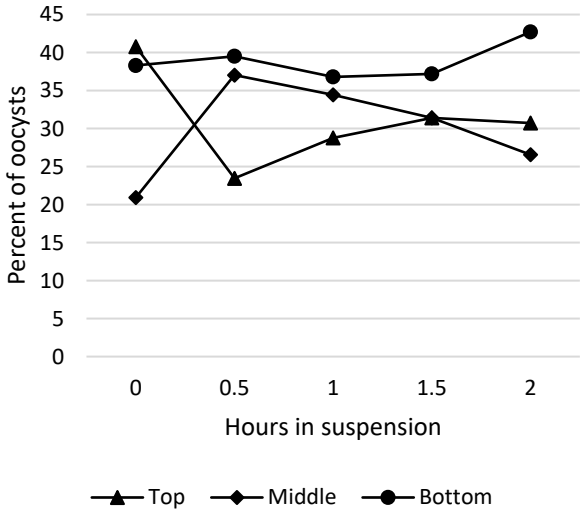

Figure 2b. Percent of coccidiosis vaccine oocysts distributed throughout a gel suspension. Coccidiosis vaccine was stirred in suspension at time 0 and held undisturbed for 2 hours.

Table 2. IBV vaccine assimilation and replication in chickens vaccinated using different solutions[1]

	PCR Ct values			
	Group 1	Group 2	Group 3	Group 4
Day 5 Ct	27	26	27	29
n	94	95	93	95
SD	3.32	2.95	2.60	3.87
Day 7 Ct	26	25	25	26
n	94	86	91	87
SD	2.23	1.79	2.09	2.08

[1] IBV qRT-PCR values from chicks swabbed in the intrachoanal cleft at days 5 and 7 post-vaccination. Group 1 - IBV vaccine alone via water spray; Group 2 - IBV and coccidia vaccines mixed together via water spray; Group 3 - IBV vaccine alone via gel drop; and Group 4 - IBV and coccidia vaccine mixed together via gel drop.

In-vivo Diluent Effect on IBV Vaccine Titer

Evaluating the *in-vivo effect of* GP on the vaccines used four different groups of chicks: Group 1 - IBV vaccine alone via water spray; Group 2 - IBV and coccidia vaccines mixed together via water spray; Group 3 - IBV vaccine alone via gel drop; and Group 4 - IBV and coccidia vaccine mixed together via gel drop. IBV infection and replication rates were determined from post vaccination swabs at 5 and 7 days. Coccidia vaccine oocyst shedding number and patterns were evaluated in 20 chicks from Groups 2 and 4 that were placed in isolators for fecal collections.

All IBV vaccination methods proved successful. At 5 days post-vaccination, the mean PCR Ct values for all treatments, a measure of viral load, was 29 and below (Table 2). This range of low Ct values is indicative of abundant, efficient vaccine RNA replication, commensurate with of fully protective vaccination. Additionally, there was no difference in the percent of chicks positive for IBV between any groups (IBV alone in water 94%; IBV plus coccidia in water 95%, IBV alone in gel 93%, IBV plus coccidia in gel 95%) indicating successful vaccination across all treatment methods. Ultimately at 7 days post vaccination the difference in mean Ct values improved further, reducing the average Ct range to 25-26, with no difference between groups in percent of chicks infected at this timepoint. Furthermore, day 7 Ct variability was less than on day 5, indicating IBV vaccine uniformity via replication continued to improve.

In-vivo Diluent Effect on Coccidiosis Vaccine Oocyst Number and Shedding Pattern

The overall oocyst pattern for coccidia vaccine shedding in feces after vaccination via water spray and gel were similar, with peaks of shedding at 7 days post vaccination (Figures 3a, 3b). The major difference between the two groups was the uniformity of the oocyst counts. Oocysts per gram of feces in the GP group increased steadily from day 5 to 7 (SD ranging 501 to 11,356), whereas those in the water spray group spiked on day 7 post vaccination (SD ranging 5100 to 33,464). Examination of the raw data revealed two birds vaccinated by water spray were shedding many more oocysts at 77,000 and 91,000 per gram on day 7, which skewed the data dramatically and introduced much larger variation at that time point compared to other days and the GP group. For both applications, 90% of birds shed oocysts during the 5 days evaluated.

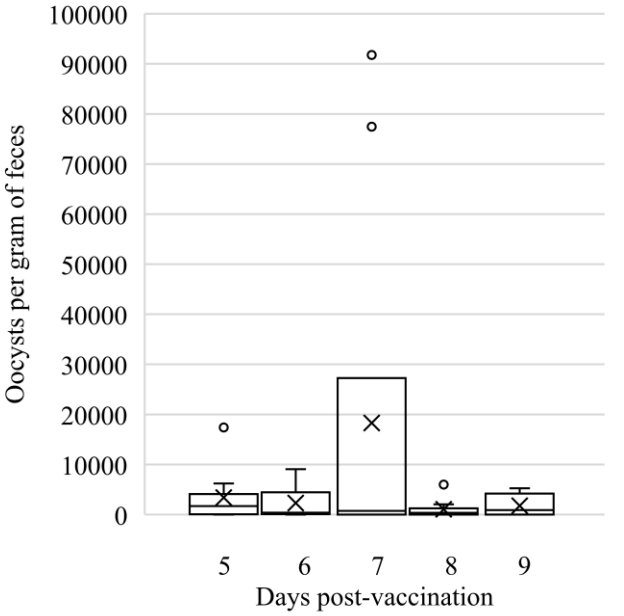

Figure 3a. Water spray coccidia vaccination shedding number and pattern in chick fecal samples. Symbol X represents mean of data, upper and lower limits of box envelop 50% of data, whiskers envelop the range of included data, symbol o represents individual outliers.

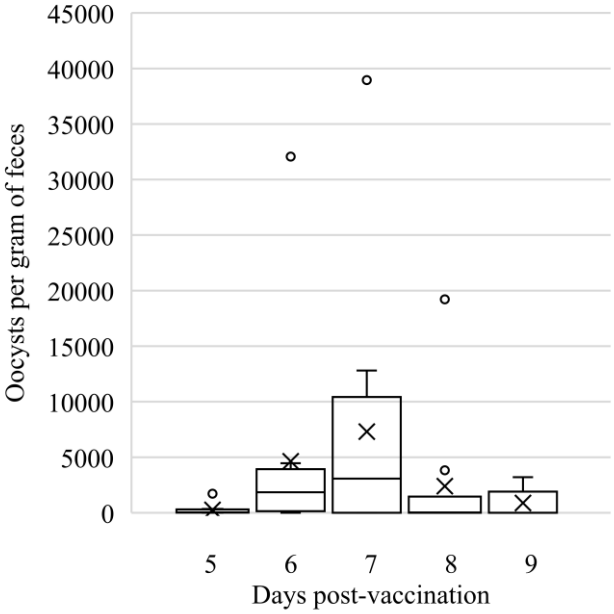

Figure 3b. Gel drop coccidia vaccination shedding number and pattern in chick fecal samples. Symbol X represents mean of data, upper and lower limits of box envelop 50% of data, whiskers envelop the range of included data, symbol o represents individual outliers.

Chick thermal regulation

Overall, the chicks were warm when coming out of the hatchers and had slightly elevated chick temperatures. There was a decline in chick temperatures for both water and gel application groups, and the pattern of decline and subsequent stabilization was very similar, with no differences between the groups (Figure 4).

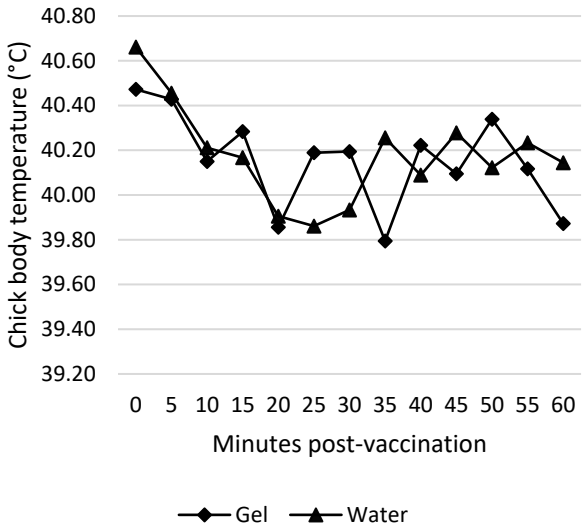

Figure 4. Impact of vaccination on chick post-hatch body temperatures in the controlled environment did not differ between diluent types.

Spray vaccination of chicks in the hatchery has long been a standard production practice for the poultry industry. For respiratory vaccines like IBV, this route of administration, which mists aerosolized vaccine mixed in water onto chicks, seemed appropriate because the vaccine targets respiratory tissues such as the eyes and nares. For enteric vaccines like coccidia, which must be ingested to be protective, spray vaccination was not a natural fit but was adapted to work. As technology evolved in the poultry industry, so have vaccination methods and with that has come the introduction of edible gels for application of poultry vaccines. Unlike spray vaccination, gel vaccination utilizes droplets of gel containing vaccine “dropped” down onto chicks. Naturally, this edible gel containing vaccine seems like a very appropriate way to apply enteric biologicals, including coccidia. Hatcheries sometimes desire to combine coccidia vaccine with respiratory vaccines in a single gel application, but the practice does not intuitively result in respiratory vaccine contact with eyes and nares of a chick. Instead,

gel-delivered respiratory vaccine protection relies on oropharyngeal contact to get into respiratory tissues, not unlike vaccinating via drinking water. The advent of gel application compels researchers to evaluate gel products' delivery of vaccines in comparison to previously used methods.

In this study, GP was evaluated for stable delivery of IBV and coccidia vaccines to commercial poultry, both *in-vitro* and *in-vivo.* The *in-vitro* study tested the stability of IBV vaccine mixed in GP over time, with temperature being the confounding variable. The present study used a commercial IBV vaccine mixed in GP gel made with room temperature water and compared that to IBV vaccine mixed in room temperature water only. The experiment was repeated with GP gel made with chilled water compared to IBV vaccine in chilled water alone.

Overall, there was no difference in titer at the initial timepoint or in the trend over the 4-hour test period between any group tested regardless of diluent (GP gel vs water) or temperature. Any differences in titer at individual timepoints were within ranges previously encountered when working with a gel diluted IBV vaccine. The data indicates that GP gel does not negatively impact IBV vaccine livability or infection rate *in-vitro*.

Gel diluents provide a seemingly more natural route of administration for enteric pathogens or vaccines that need to be ingested (such as coccidia) than a water solution that is aerosolized. Additionally, water-based dilutions of coccidia vaccines must be continually mixed to prevent settling of the heavier, denser coccidia oocysts in the vaccine over time. It is expected that a gel diluted coccidia vaccine solution, being more viscous than water, would have the capability to keep oocysts in suspension over time. Testing the capability of GP to maintain a uniform coccidia suspension, coccidia vaccine was mixed with water or GP gel and held undisturbed, except for sampling, for 2 hours. Samples collected from the top 1/3, middle 1/3, and bottom 1/3 of each solution every 30 minutes highlighted that nearly all oocysts in the water solution migrated from the top and middle portions of the vaccine solution and settled to the bottom of the flask within 30 minutes. A large portion of the oocysts that settled to the bottom of the flask were not retrievable via pipette. Any apparent increase in oocyst numbers in the bottom and middle fractions over time corresponds to oocysts being jarred from the bottom during handling for sample collection and equilibrating to the diluent concentration. Conversely, oocyst counts in the GP solution, while experiencing variation at the 30-minute timepoint, were ultimately well distributed, with oocyst counts being more uniform throughout the top, middle, and bottom of the gel suspension for 2 hours. This leads to the conclusion that, provided the coccidia vaccine is evenly mixed into the GP gel initially, the gel suspends the oocysts without settling over the course of normal vaccination times.

Noting the *in-vitro* studies in this series showing that IBV vaccine is stable in GP gel, and the GP gel keeps coccidia oocysts in suspension over time without continued mixing, another investigation mixed the IBV and coccidia vaccines together in GP gel to examine the possibility of any interactive effect. Like the IBV only experiment, IBV and coccidia vaccines were mixed into water or GP gel solutions made with different temperature water and samples were taken for IBV titrations over 4 hours. All groups behaved the same in terms of titer stability over time. A slight IBV vaccine titer decline over time is consistent with other reports of mixing IBV and coccidia vaccines, and this is not unexpected. This data shows that combining the specific IBV and coccidia vaccines used in this study in GP gel is feasible; GP did not compromise the IBV vaccine stability when combined with coccidia vaccine.

The final experiments in the series tested the GP gel diluent in chickens to collect live animal data. Four groups of chicks were vaccinated with either IBV vaccine alone in a water diluent by spray, IBV vaccine alone in the GP gel via gel-drop, IBV plus coccidia vaccines together in water diluent by spray, or IBV plus coccidia vaccines together in GP gel via gel-drop. Swabs were collected from all chicks in every group at 5- and 7-days post-vaccination to assess IBV vaccine infection and replication. A subset of chicks from the coccidia vaccinated groups were housed individually from days 5-10 post-vaccination for fecal collection and oocyst enumeration.

Assessing IBV viral load by real-time PCR in chicks after vaccination, overall vaccine uptake and replication in chicks was excellent for all application methods and vaccine combinations, with all groups well above 90% chicks positive. All vaccination methods produced abundant vaccine virus replication. Relying on the 7-day post-vaccination PCR IBV vaccine detection, the standard practice in this laboratory, revealed all groups were nearly identical with an amply protective viral load regardless of application method or vaccine combination.

When evaluated for coccidia vaccine infection, replication, and shedding, a very characteristic shedding pattern was found in both coccidia vaccinated groups with a peak at 7 days post-vaccination. The OPG being shed in feces from the water spray group was much more variable on day 7, influenced by high oocyst counts from two individuals. In the poultry industry, consistency in shedding is critical. Coccidia vaccines used in the US are not attenuated and immunity is achieved through controlled dosing and repeat exposure. Vaccines are administered in low-doses, and these doses are amplified through replication in the bird. When administered properly, the amount shed in the first 7-8 days (first cycle of coccidia replication) is ideally low and evenly distributed among birds. This facilitates chicks re-ingesting the oocysts from the litter and obtaining a second small (albeit bigger than what was applied in the hatchery), uniform dose for a second round of infection and replication. When vaccine is applied in a less efficient manner, the chicks may be dosed non-uniformly which will lead to variable shedding after the first replication cycle of oocysts. Chicks that then re-ingest a very small number of oocysts from the litter will have a smaller second round of infection, but chicks that ingest an extremely large number of oocysts from the first shedding cycle will subsequently have an extremely large second round of infection and replication. When vaccines are not attenuated, the ingestion of a large bolus of oocysts (known in the industry as a cocci bomb) can lead to clinical disease caused by the vaccine. The two chicks in the water spray application group shedding over 70,000 oocysts per gram each represent this potential scenario.

Lastly, when evaluating the body temperature reduction experienced by chicks after vaccination, no difference was observed between the spray or gel applied groups. When chicks hatch, their internal temperature is higher than their equilibrium temperature as they have been in a hatcher at an elevated temperature for 12-36 hours. The longer they are out of the hatcher in lower environmental temperatures there is a natural decline in body temperature to equilibrium state (approximately 40°C). Chicks are vaccinated very soon after being pulled from hatchers, and the process of applying liquid to the chicks to deliver the vaccines accelerates this process to some degree. Often body temperatures following vaccination fall below normal equilibrium state but rebound to normal in most cases. This pattern was observed in this experiment as well. The drop in temperature is not the major issue with thermal stress, the more concerning issue is how long it takes for chick temperatures to return to equilibrium. This is much more influenced by the environment than anything else, with temperature, humidity, air movement, etc. being prime factors. These environmental parameters directly affect how quickly chicks can dry, thus impacting evaporative cooling. In this study, the chicks were held in the same environment for the duration of temperature collection with no direct air blowing on them. In this controlled environment, the chicks began to return to equilibrium temperature after 25 minutes. By this time, chicks began to settle down and compact within the box. This behavior facilitates body temperature increase. In a well-managed hatchery where temperatures and humidity are controlled for chick welfare, this would not be an issue. In areas or hatcheries where environmental control is difficult or external weather is extreme (hot or cold), excessively wetting chicks could pose an issue to chick health.

CONCLUSIONS AND APPLICATIONS

The objective of this study was to compare a gel vaccine delivery product, Gel-Pac, by Animal Science Products, to the established method of water spray vaccine application in commercial poultry. The experiments used the two most common vaccines applied in the hatchery, infectious bronchitis virus and coccidia, in multiple *in-vitro* stability studies and an *in-vivo* application, infection, replication, and thermal regulation study.

1. GP proved capable of providing uniform coccidia dosing with less variability than the same vaccine administered in water spray, with no negative effect on chick thermal regulation.
2. GP demonstrated the ability to maintain IBV vaccine stability and attain abundant vaccine assimilation and replication when administered alone, at least as good as water spray.
3. IBV vaccine delivered in a combined suspension with coccidia vaccine in GP remained stable in suspension and reached abundant 5- and 7-day post vaccination viral load numbers, assessed via RT-PCR performed on chick intrachoanal cleft swabs. Vaccine virus load was commensurate with protective levels of vaccine, indicating GP can permit delivering the IBV and coccidia vaccines used in this study in combination when mixing and application are performed correctly.