

Experiences with *Mycoplasma gallisepticum* vaccines—killed, vectored, and vaccine combinations.

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My Background:

- I am a microbiologist by training with a research background in mycoplasma, including dissertation research on human and swine mycoplasmas.
- I work for the United States Department of Agriculture's, Agricultural Research Service.
- My research is restricted to avian mycoplasmas.
- I work predominantly with layers because of the US National Poultry Improvement Plan, which certifies broiler and other meat type birds as MG and MS free.
 - Cannot vaccinate NPIP clean birds for MG or MS.
 - The incidence of MG and MS reporting in the US is “likely” under reported to maintain NPIP clean status.



Vaccine Combinations:

What?

- Vaccine Combinations, Overlays, and Revaccination
- Chickens are vaccinated against MG with a lower efficacy vaccine at an early age
- At a later age, chickens are revaccinated against MG with a higher efficacy vaccine

Vaccine Combinations, Overlays, and Revaccination:

Why:

- Current vaccine regimen is ineffective
- Reduce harmful effects of one vaccine through prior vaccination with a milder vaccine
- Shift in poultry management policy

Pitfalls:

- Existing immune response limits response to second vaccine
- Added expense

Killed vaccines:

- First generation of MG vaccines
- Current vaccines:
 - Formalin killed virulent MG (strain may vary by manufacturer, often proprietary)
 - Mineral oil emulsion
 - Vaccination by injection
 - Develop a strong serum immune response
 - No live organism—No chance for transmission

Percent Egg Transmission of MG After MG Challenge

Treatment groups	Weeks post-challenge							Total
	0-2	3-6	7-10	11-14	15-18	19-22	23-25	
Controls	0	18.8	16.5	8.0	6.8	4.2	3.2	11.7 ^a
F strain	0	1.1	2.4	3.5	3.0	1.4	0	1.8 ^b
F + bacterin	0	0	0.1	0.8	4.6	5.1	0	0.8 ^c
1x bacterin	0	0.5	0.8	0.2	2.0	5.3	0	0.8 ^c
2x bacterin	0	0	0	1.3	6.6	12.6	3.7	1.6 ^b

^a Values within the column followed by different letters differ significantly ($\alpha < 0.05$).

Percent Weekly Egg Production After MG Challenge

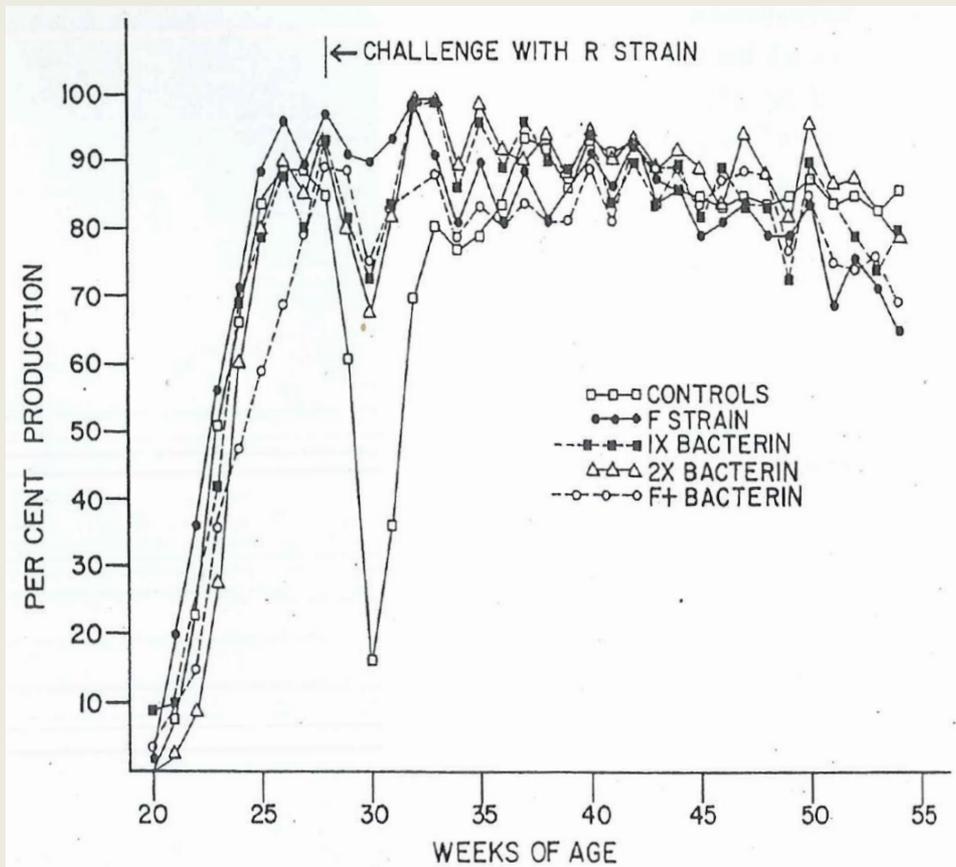


Fig. 5. Egg production (eggs/hen) of vaccinated groups and unvaccinated controls.

Percent Weekly Egg Production After MG Challenge

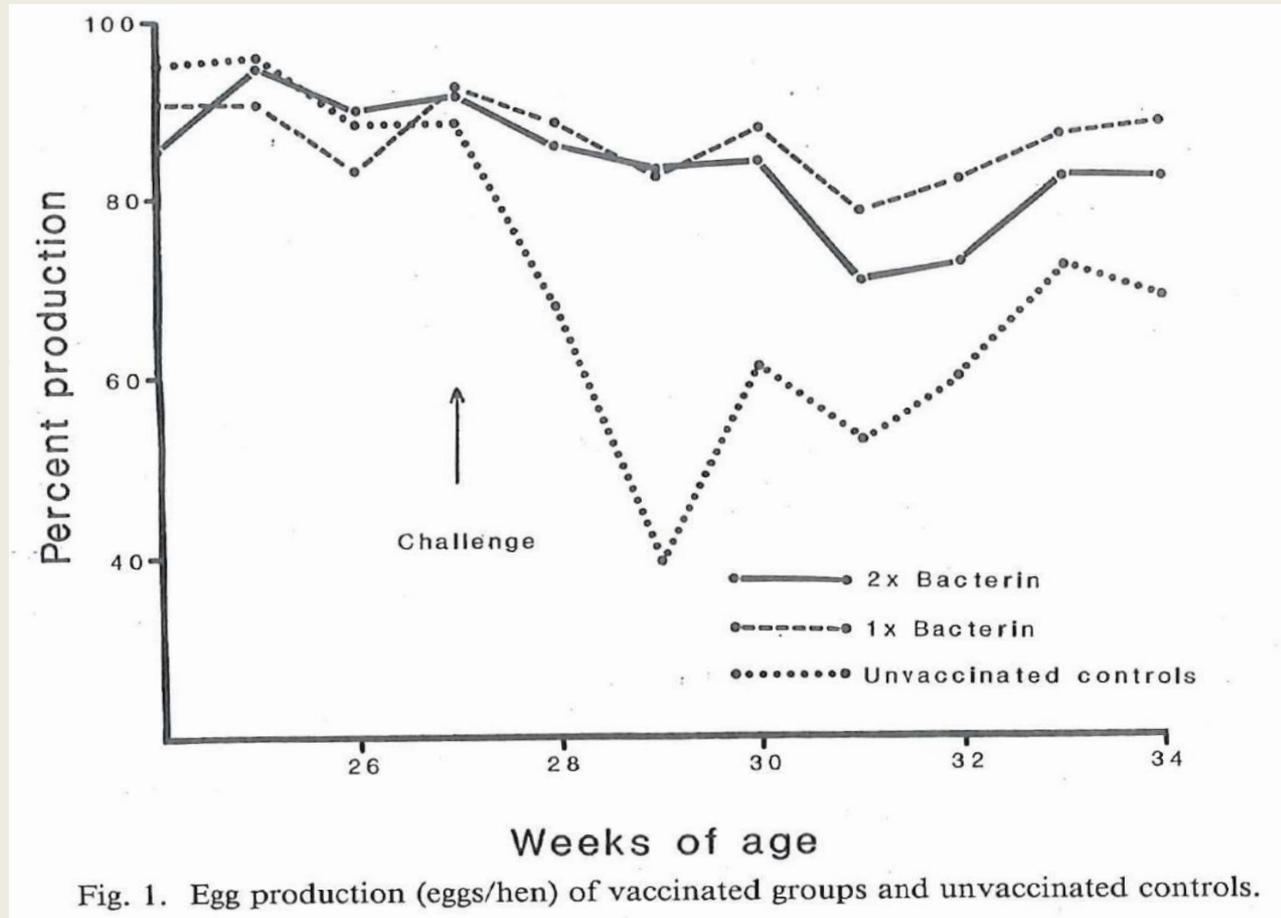


Fig. 1. Egg production (eggs/hen) of vaccinated groups and unvaccinated controls.

Bacterin Versus Unvaccinated Pullets Post Placement on an MG Infected Farm

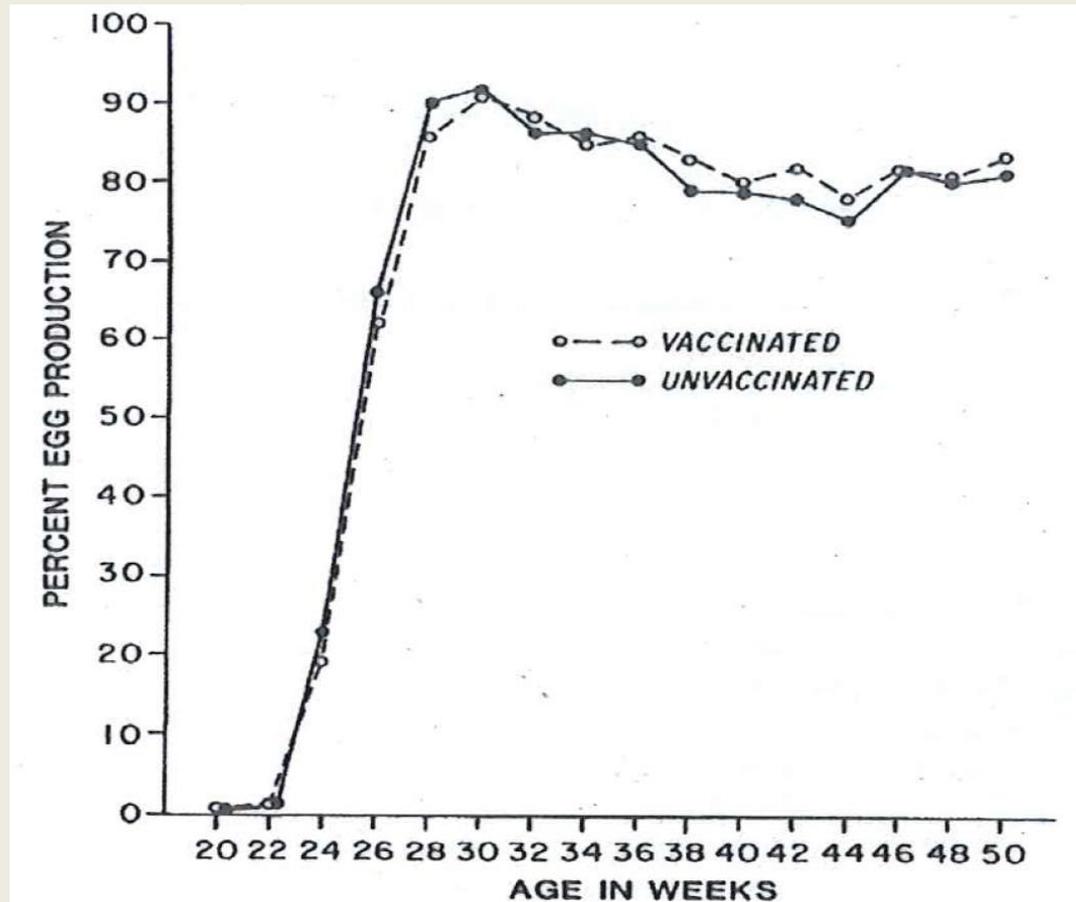


Fig. 2. Egg production of MG-free pullets on an MG-endemic ranch.

Khan, 1985

Serologic response of chickens:

15 wk of age (5 WPV), and 26.3 wk of age (16.3 WPV and 10 DPC with R-strain).^A

Age (weeks)	MG vaccine	Challenge	SPA ^{BC}	HIB ^D	ELISA ^{BE}
15	None	No	1/30 (0.0) ^c	0/30 (0.0) ^b	0/30 (0.0) ^b
	F-strain	No	18/30 (0.7) ^b	0/30 (0.0) ^b	4/30 (0.3) ^b
	MG-Bac	No	30/30 (4.0) ^a	22/30 (1.6) ^a	30/30 (1.8) ^a
26.3	None	No	2/8 (0.6) ^b	0/8 (0.0) ^c	0/8 (0.1) ^d
	F-strain	No	1/4 (1.0) ^b	0/4 (0.0) ^c	0/4 (0.2) ^{cde}
	MG-Bac	No	5/5 (4.0) ^a	0/4 (0.0) ^c	4/5 (1.6) ^{bcde}
26.3	None	Yes	15/15 (4.0) ^a	9/15 (1.4) ^{ab}	15/15 (1.7) ^{bc}
	F-strain	Yes	20/20 (4.0) ^a	11/20 (1.3) ^b	20/20 (2.6) ^b
	MG-Bac	Yes	20/20 (4.0) ^a	19/20 (1.8) ^a	20/20 (6.3) ^a

^AValues within a column and time period with a different lowercase, superscripted letter are significantly different (P # 0.05).

^BNo. of positive samples/No. of tested samples (SPA: \$1, HI: \$40, and ELISA: \$0.5).

^CMean agglutination grade (from 0 to 4).

^DMean titer log₁₀.

^EMean sample positive ratio.

Ferguson-Noel, 2012

Air sac lesion scores, prevalence of ovarian regression (follicle atresia), tracheal mucosa measurements, and MG isolation:

Vaccinated and nonvaccinated chickens at 16.3 WPV and 10 DPC with R-strain.^A

Challenge	Vaccine	Air sac lesion score ^{BC}	Ovarian regression ^{BD}	Tracheal mucosal thickness ^E	MG Isolation	
					Air Sacs	Oviduct
No	None	0/8 (0.0) ^a	0/8 ^a	126.3±37.9 ^d	0/8	0/8
	F-strain	0/4 (0.0) ^a	0/4 ^a	130.2±11.5 ^{cd}	0/4	0/4
	MG-Bac	0/5 (0.0) ^a	0/5 ^a	113.7±8.7 ^{cd}	0/5	0/5
Yes	None	15/15 (3.6) ^c	13/15 ^c	433.7±85.0 ^a	12/14	15/15
	F-strain	16/20 (2.1) ^b	8/20 ^b	255.4±172.8 ^{cd}	17/19	17/20
	MG-Bac	20/20 (2.5) ^b	8/20 ^b	294.4±121.3 ^{bc}	20/20	20/20

^AValues within a column with a different lowercase, superscripted letter are significantly different ($P \leq 0.05$).

^BNo. of positive samples/No. of tested samples (air sac score ≥ 1).

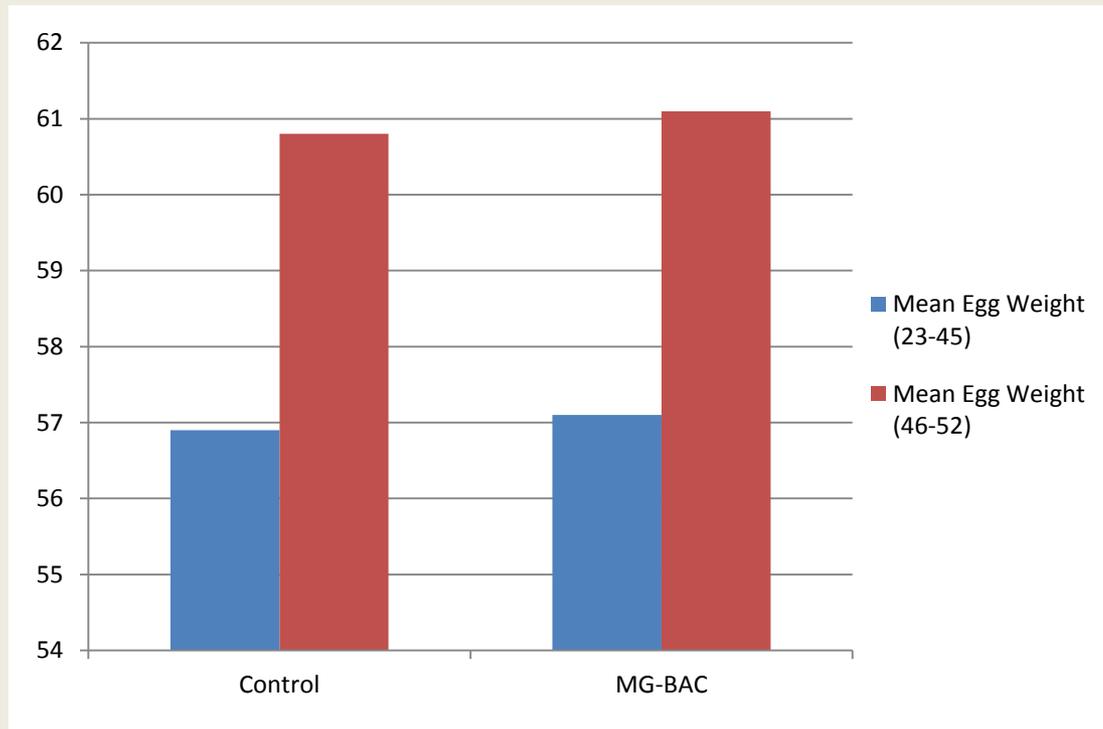
^CMacroscopically scored from 0 to 4.

^DEvaluated by gross observation.

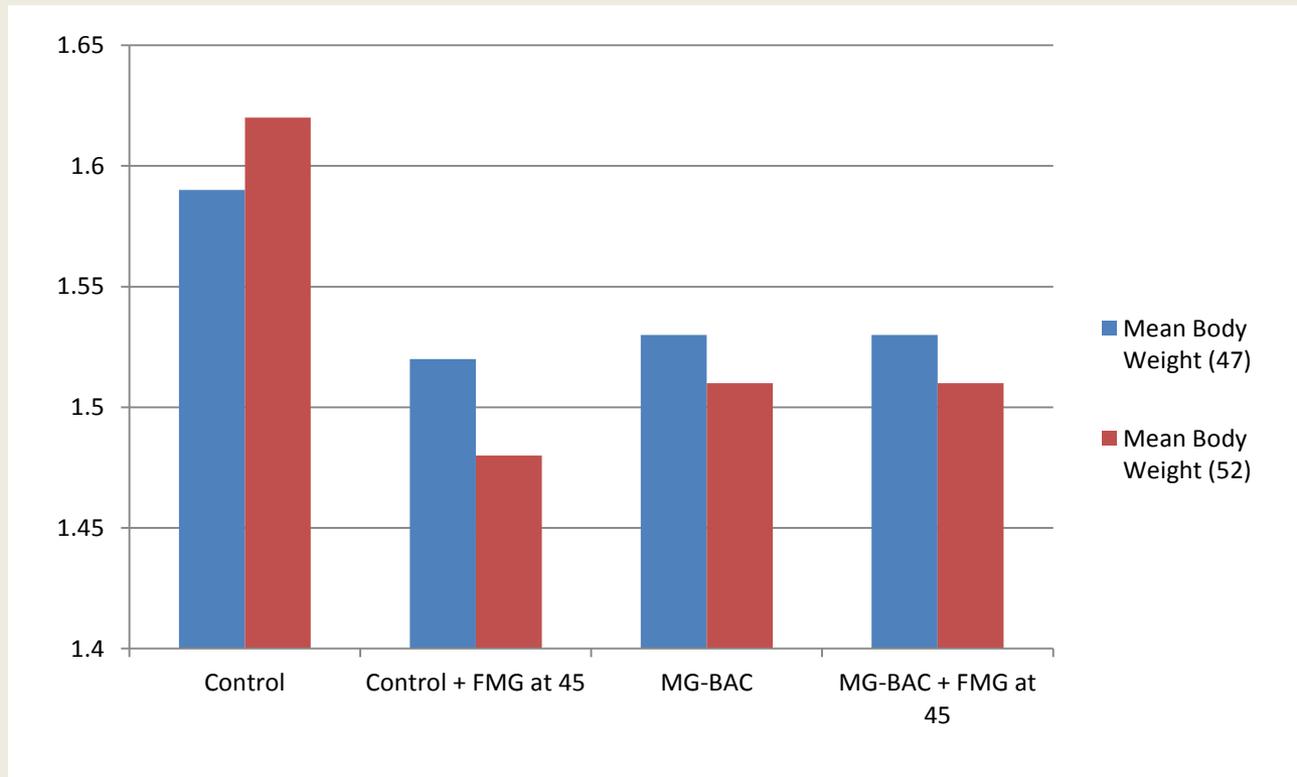
^EMean thickness for the group in micrometers \pm SD.

Ferguson-Noel, 2012

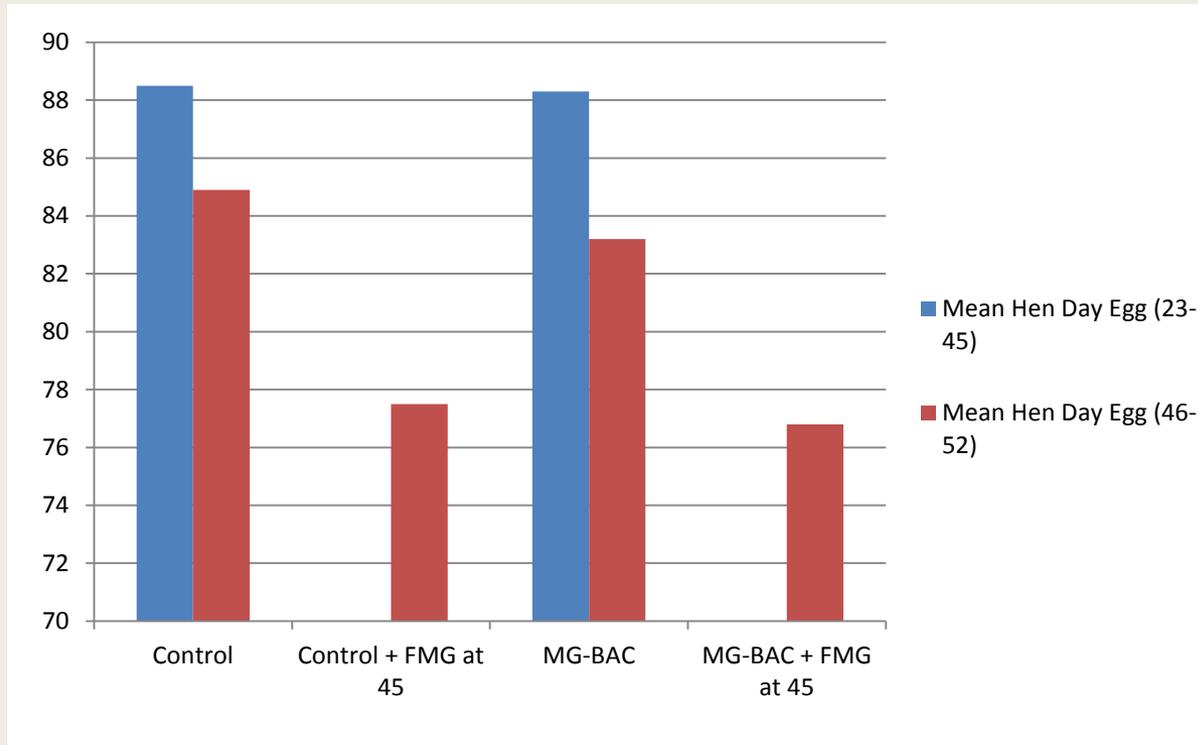
Mean Egg Weight: Bacterin at 10 weeks and F-strain (Combination) at 45 weeks.



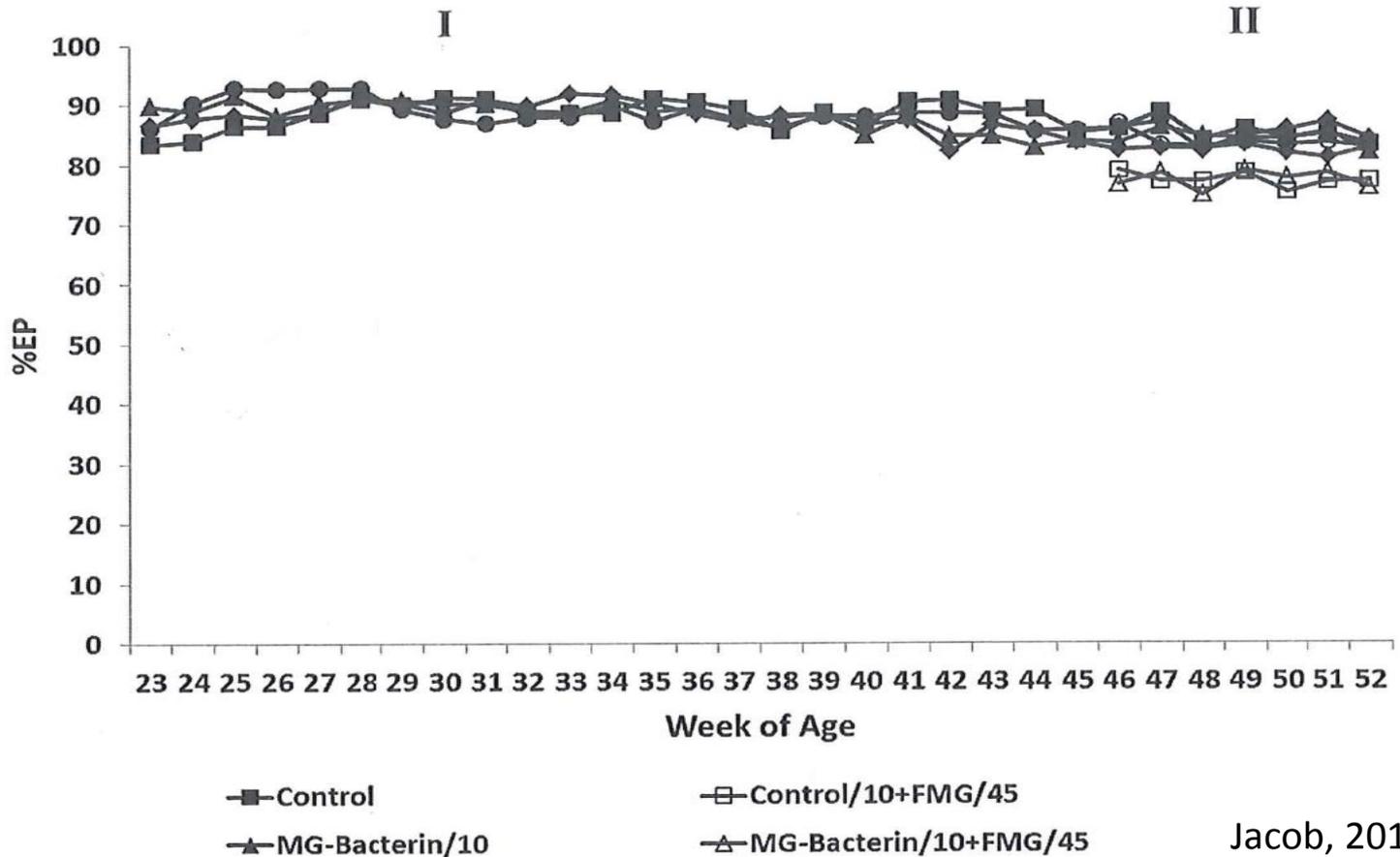
Mean Body Weight: Bacterin at 10 weeks and F-strain (Combination) at 45 weeks. Results at 47 and 52 Weeks.



Mean Hen Day Egg Production: Bacterin at 10 weeks and F-strain (Combination) at 45 weeks.



Percent Weekly Egg Production: Bacterin at 10 weeks and F-strain (Combination) at 45 weeks.



Jacob, 2013

Bacterin Vaccine: Pros and Cons

- Pros:
 - No need for a live vaccine
 - Reduced vertical MG transmission
 - Decreased MG populations in the upper respiratory tract
- Cons:
 - Increased application costs
 - Low level of protection from virulent MG challenge
 - Protection wains over time
 - Local inflamitory reactions at the injection site
 - The presence of the carrier/adjuvant (mineral oil) generally restricts it from usage in meat type poultry.

Recombinant vaccines:

- Genetically modified organisms that are created to express MG proteins.
- The only commercially available vaccine uses the Fowl Pox virus as a vector to express to MG proteins (rFP-MG).
 - The vaccine is applied and functions as a Fowl Pox vaccine.
 - It is advertised to NOT produce a detectible serum immune response to MG. Shows as MG negative by serology.
 - Zhang *et al.* showed that rFP-MG possesses a high level of safety:
 - Stable after 5 rounds of consecutive passage
 - No gross lesions following vaccinations; localized reaction at the site of inoculation typical of Fowl Pox vaccination
 - No chicken to chicken transmission

Serologic response of chickens:

15 wk of age (5 WPV), and 26.3 wk of age (16.3 WPV and 10 DPC with R-strain).^A

Age (weeks)	MG vaccine	Challenge	SPA ^{BC}	HIB ^D	ELISA ^{BE}
15	None	No	1/30 (0.0) ^c	0/30 (0.0) ^b	0/30 (0.0) ^b
	F-strain	No	18/30 (0.7) ^b	0/30 (0.0) ^b	4/30 (0.3) ^b
	rFP-MG	No	0/30 (0.0) ^c	0/30 (0.0) ^b	0/30 (0.1) ^b
26.3	None	No	2/8 (0.6) ^b	0/8 (0.0) ^c	0/8 (0.1) ^d
	F-strain	No	1/4 (1.0) ^b	0/4 (0.0) ^c	0/4 (0.2) ^{cde}
	rFP-MG	No	1/5 (0.4) ^b	0/5 (0.0) ^c	0/5 (0.0) ^{de}
26.3	None	Yes	15/15 (4.0) ^a	9/15 (1.4) ^{ab}	15/15 (1.7) ^{bc}
	F-strain	Yes	20/20 (4.0) ^a	11/20 (1.3) ^b	20/20 (2.6) ^b
	rFP-MG	Yes	16/16 (4.0) ^a	7/20 (1.1) ^b	18/20 (1.6) ^{ce}

^AValues within a column and time period with a different lowercase, superscripted letter are significantly different (P # 0.05).

^BNo. of positive samples/No. of tested samples (SPA: \$1, HI: \$40, and ELISA: \$0.5).

^CMean agglutination grade (from 0 to 4).

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Ferguson-Noel, 2012

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Challenge	Vaccine	Air sac lesion score ^{BC}	Ovarian regression ^{BD}	Tracheal mucosal thickness ^E	MG Isolation	
					Air Sacs	Oviduct
No	None	0/8 (0.0) ^a	0/8 ^a	126.3±37.9 ^d	0/8	0/8
	F-strain	0/4 (0.0) ^a	0/4 ^a	130.2±11.5 ^{cd}	0/4	0/4
	rFP-MG	0/5 (0.0) ^a	0/5 ^a	125.7±22.1 ^{cd}	0/5	0/5
Yes	None	15/15 (3.6) ^c	13/15 ^c	433.7±85.0 ^a	12/14	15/15
	F-strain	16/20 (2.1) ^b	8/20 ^b	255.4±172.8 ^{cd}	17/19	17/20
	rFP-MG	20/20 (3.5) ^c	19/20 ^c	389.7±165.9 ^{ab}	20/20	20/20

^AValues within a column with a different lowercase, superscripted letter are significantly different ($P \leq 0.05$).

^BNo. of positive samples/No. of tested samples (air sac score ≥ 1).

^CMacroscopically scored from 0 to 4.

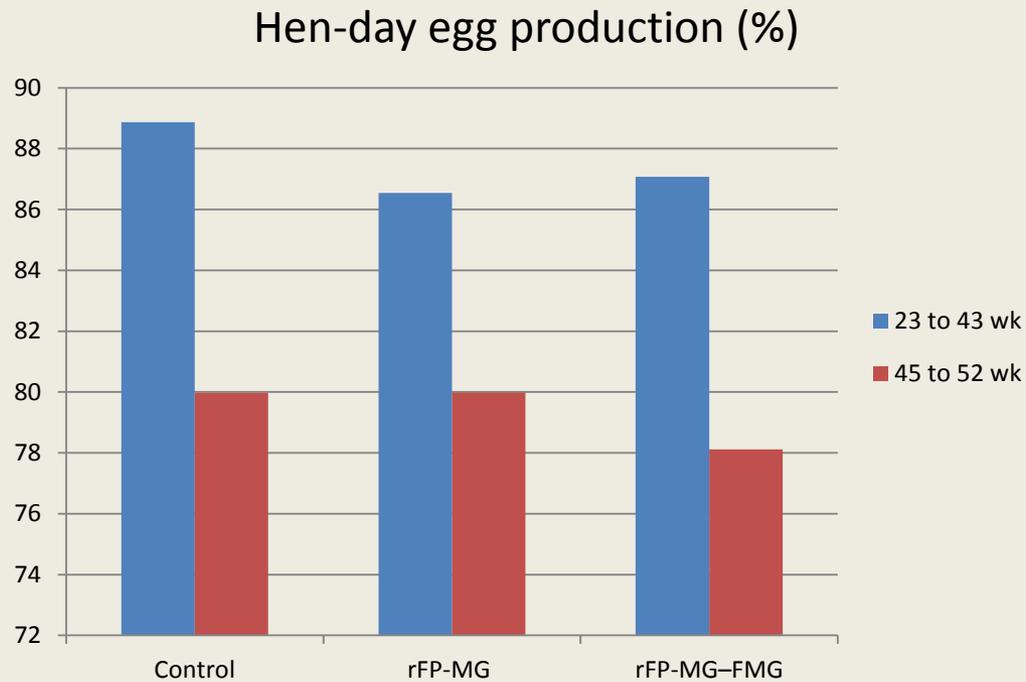
^DEvaluated by gross observation.

^EMean thickness for the group in micrometers \pm SD.

Ferguson-Noel, 2012

Egg production comparison:

Control, rFP-MG vaccination at 10 weeks, and rFP-MG vaccinated with F-strain (Combination) at 45 weeks.



Leigh, 2013

Recombinant MG Vaccine: Pros and Cons

- Pros:

- No need for a “live” vaccine, although the viral vector is viable in the host.
- Does not produce a serological immune response
 - Negative for serological MG diagnostic tests
- Two vaccines for the price of one (Fowl Pox and Mycoplasma gallisepticum)
- No known negative impact on poultry. “Safe”

- Cons:

- Increased application costs
- Negligible protection from highly virulent MG challenge
- Local inflammatory reactions at the injection site
- Potential regulatory hurdles or social stigma due to the “recombinant” nature of the vaccine

Future Directions:

- Bacterin Vaccines:

- Different adjuvants are being studied that could result in an improved immune response and improved protection
- Low possibility that these will come to market. Little interest in bacterin based vaccines from vaccine manufacturers.

- Recombinant Vaccines:

- Bacterial cell surface expression of exogenous *M. gallisepticum* antigens
 - May have similar problems to rFP-MG due to limited number of MG antigens presented
- Synthetic MG vaccine
 - Creation of a synthetic *M. gallisepticum* vaccine that colonizes the host and stimulates a protective immune response but lacks genes that result in host pathology
 - Technology to create the vaccine exists, and work is progressing
 - Development of synthetic vaccines requires a better understanding of how MG infection results in protection or pathology