

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	11.7a ^A
F strain	0	1.1	2.4	3.5	3.0	1.4	0	1.8b
F + bacterin	0	0	0.1	0.8	4.6	5.1	0	0.8c
1x bacterin	0	0.5	0.8	0.2	2.0	5.3	0	0.8c
2x bacterin	0	0	0	1.3	6.6	12.6	3.7	1.6b

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

Percent Weekly Egg Production After MG Challenge

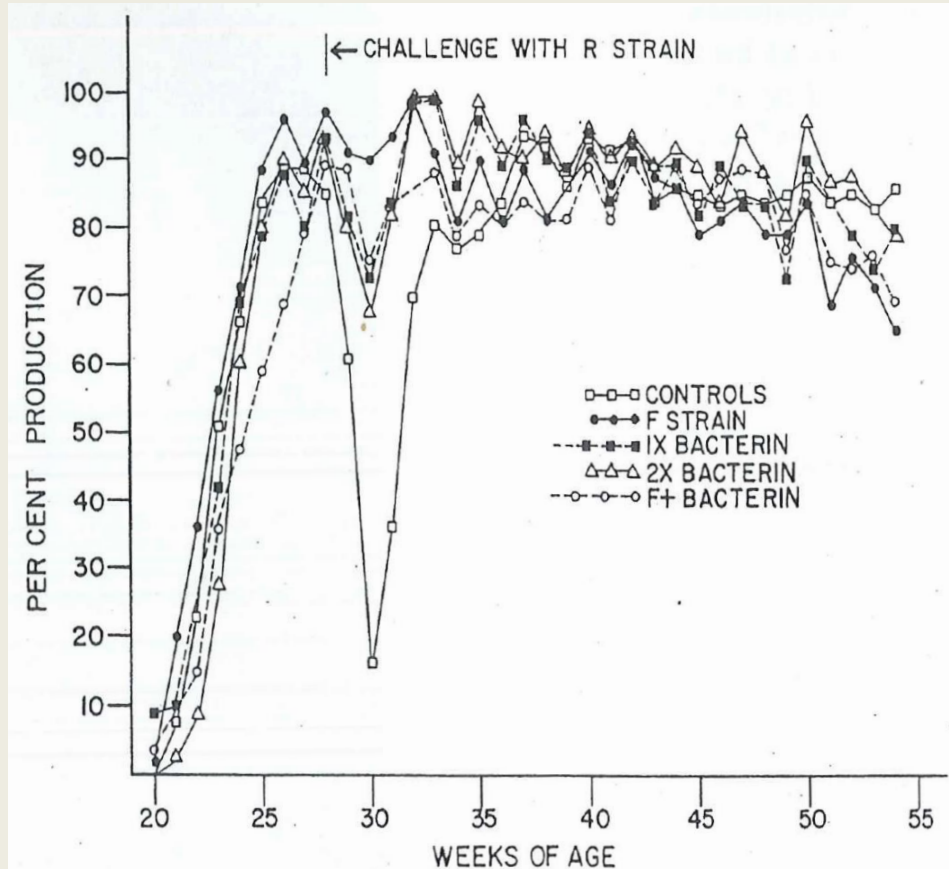
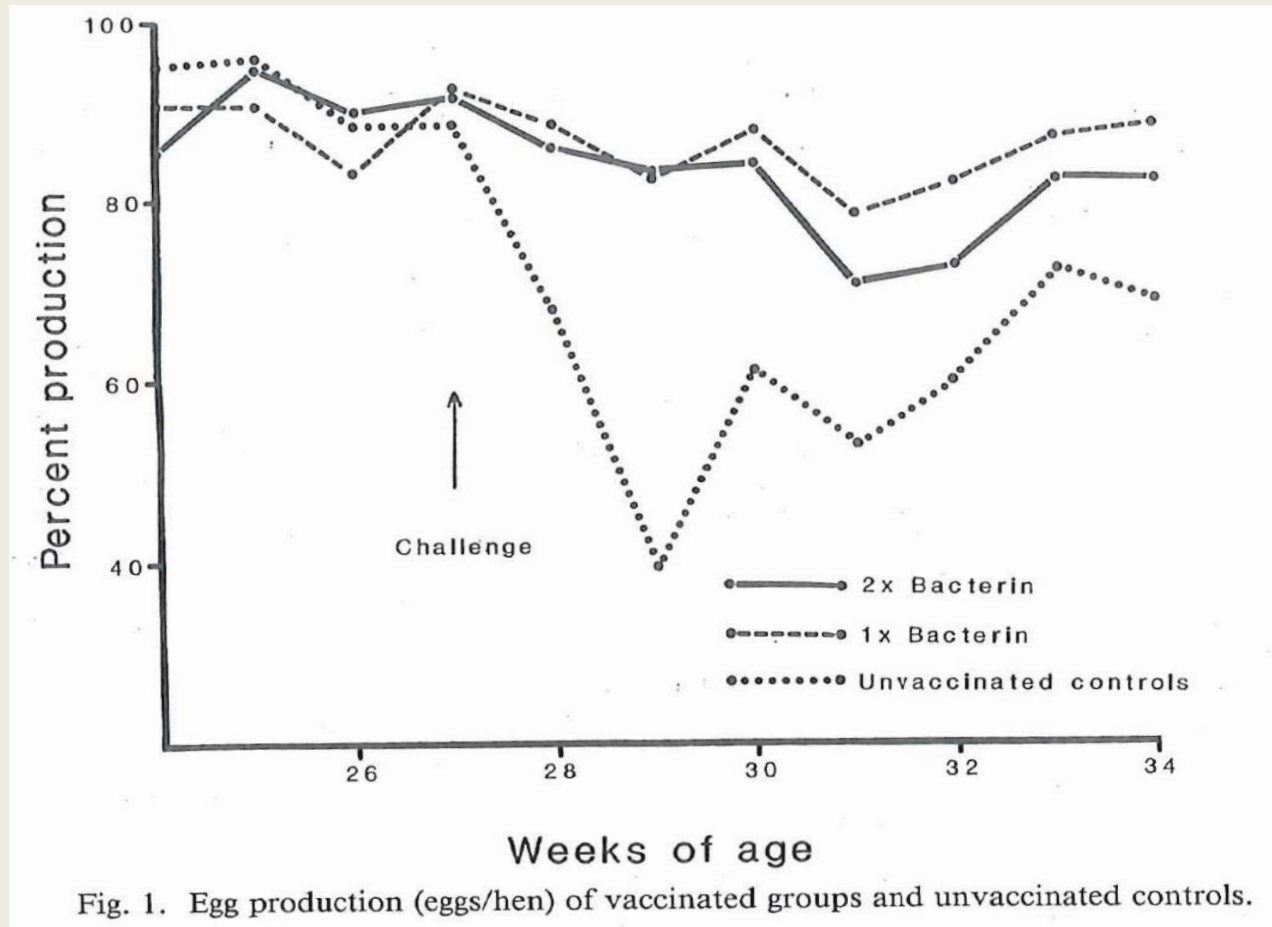


Fig. 5. Egg production (eggs/hen) of vaccinated groups and unvaccinated controls.

Glisson and Kleven, 1983

Percent Weekly Egg Production After MG Challenge



Sasipreeyajan, 1987

Bacterin Versus Unvaccinated Pullets Post Placement on an MG Infected Farm

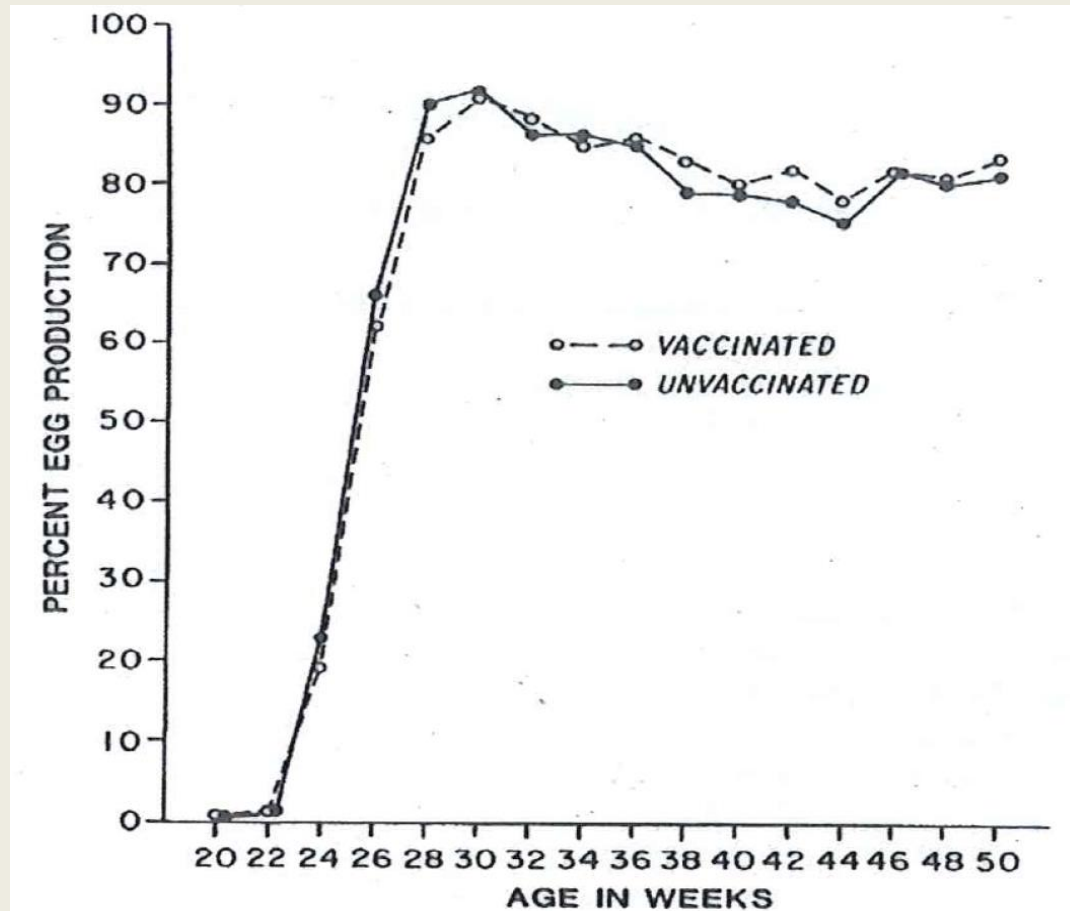


Fig. 2. Egg production of MG-free pullets on an MG-endemic ranch.

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).

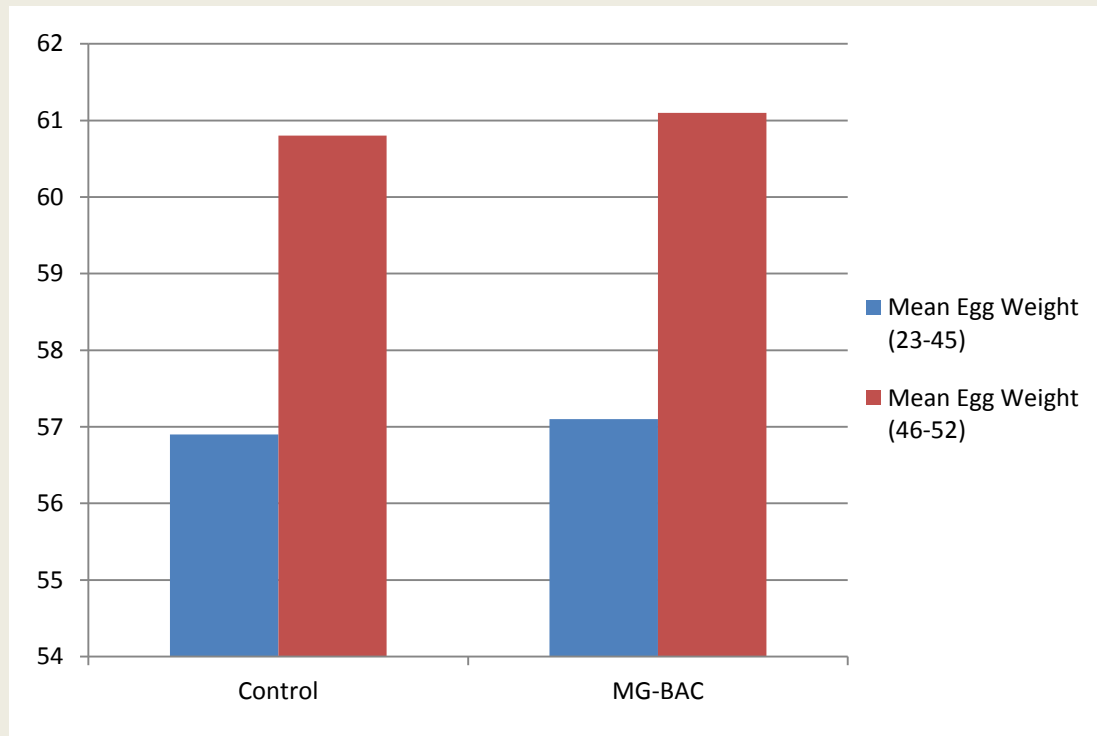
^CMacroscopically scored from 0 to 4.

^DEvaluated by gross observation.

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

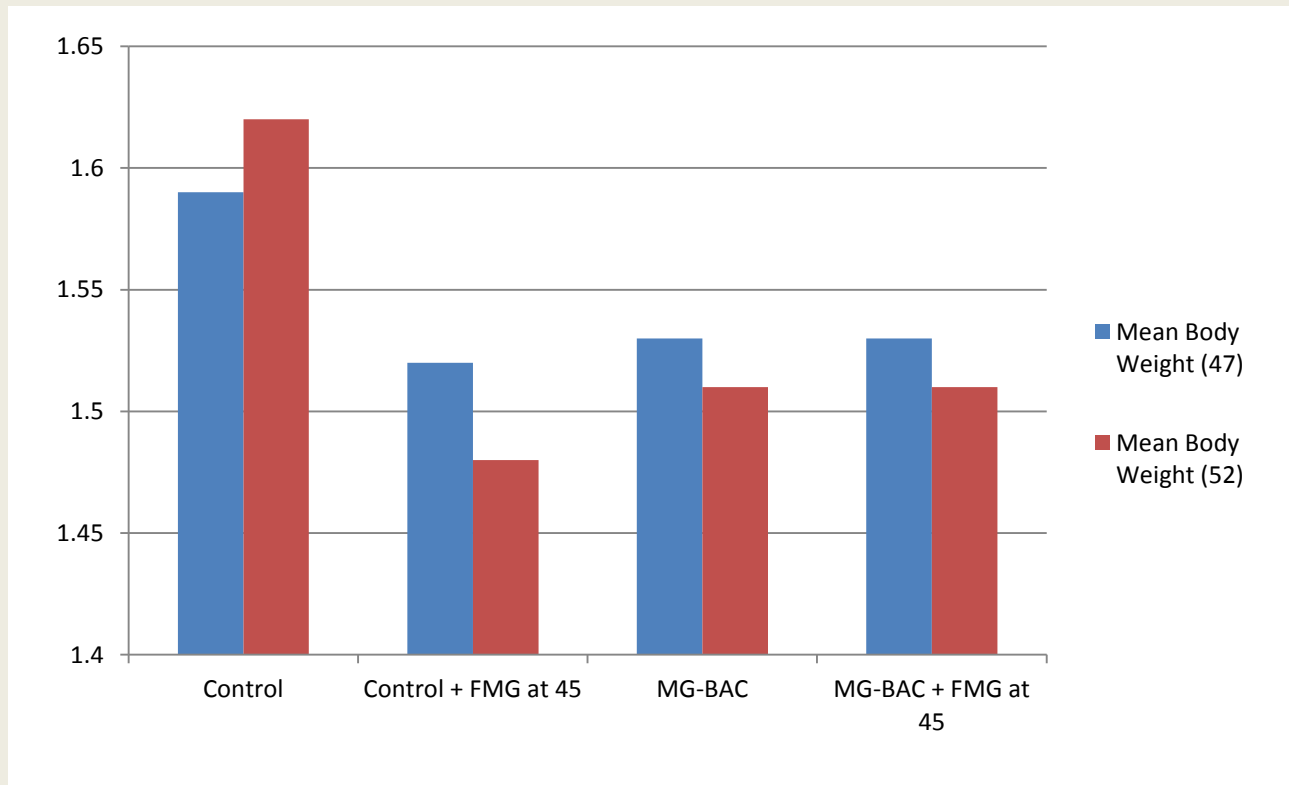
Ferguson-Noel, 2012

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

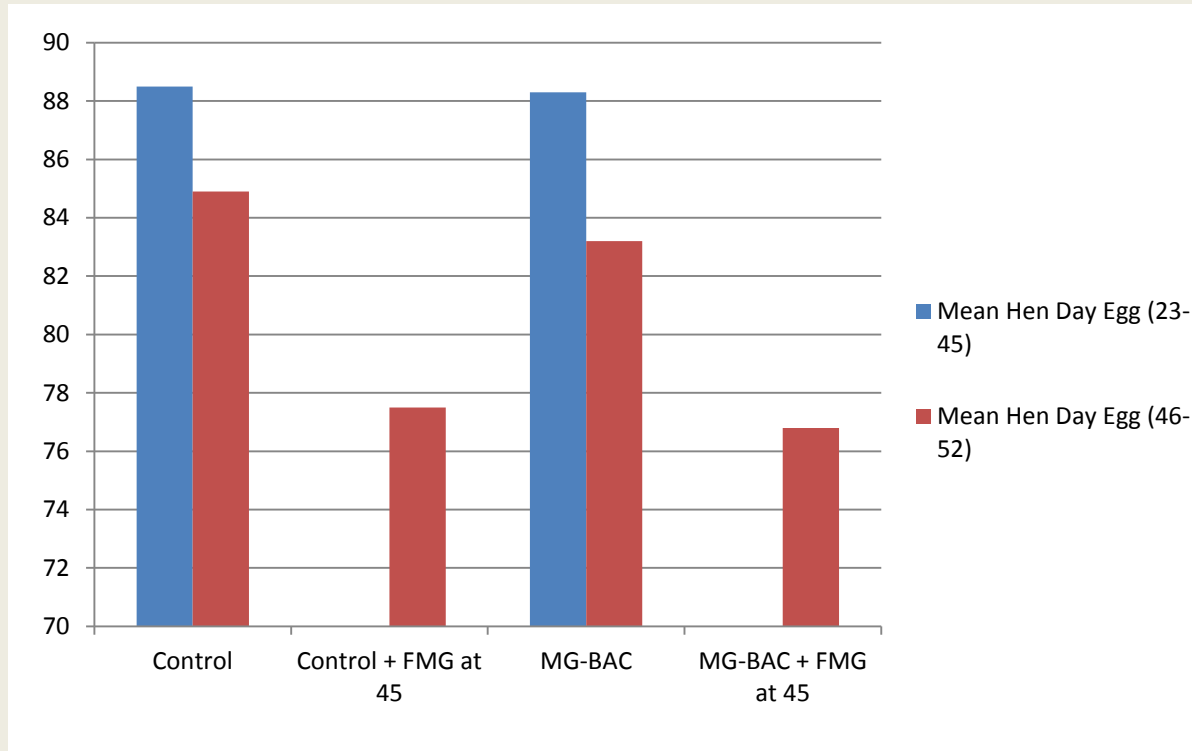


Jacob, 2013

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

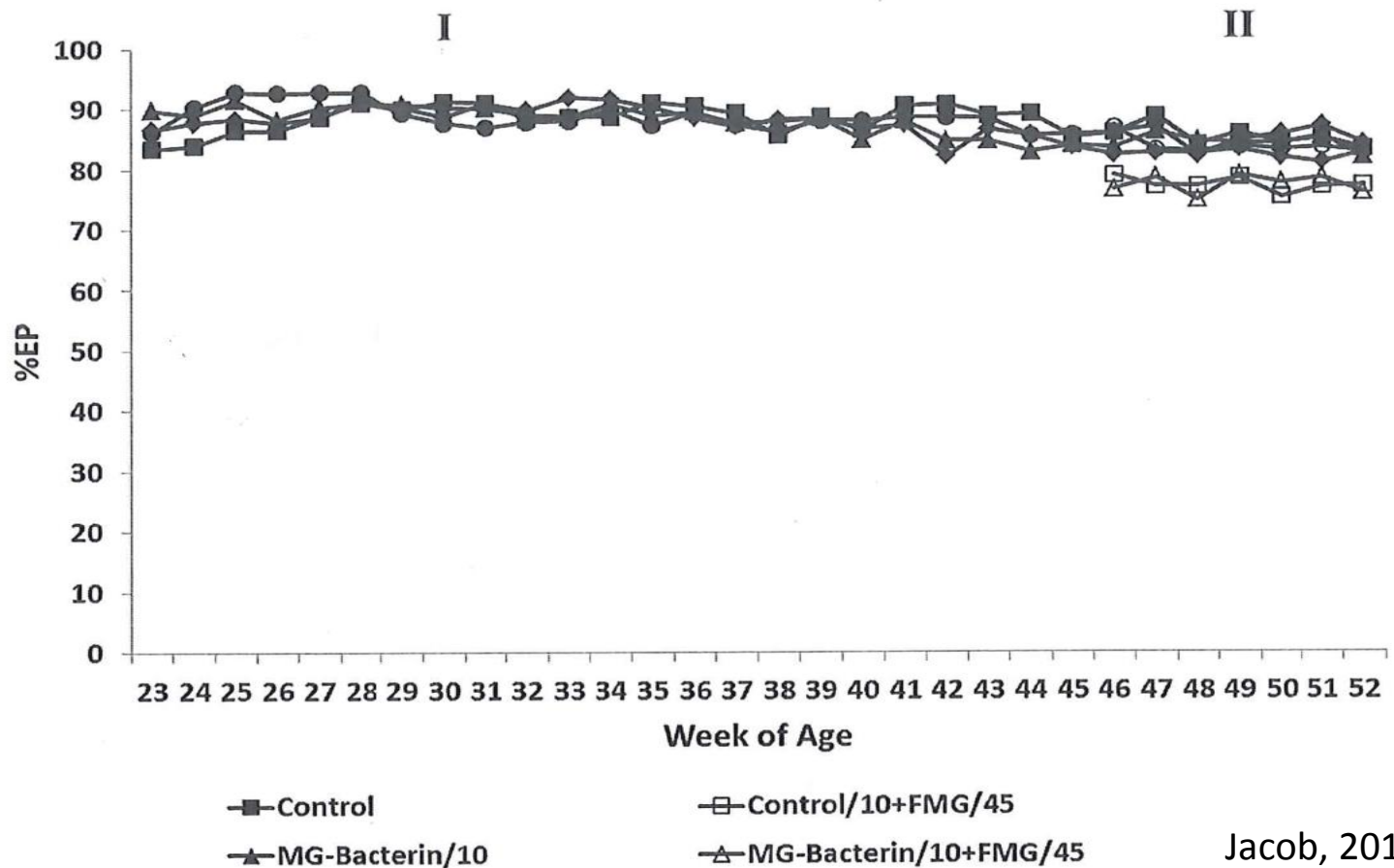


Mean Hen Day Egg Production: Bacterin at 10 weeks and F-strain (Combination) at 45 weeks.



Jacob, 2013

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).

^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
	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.

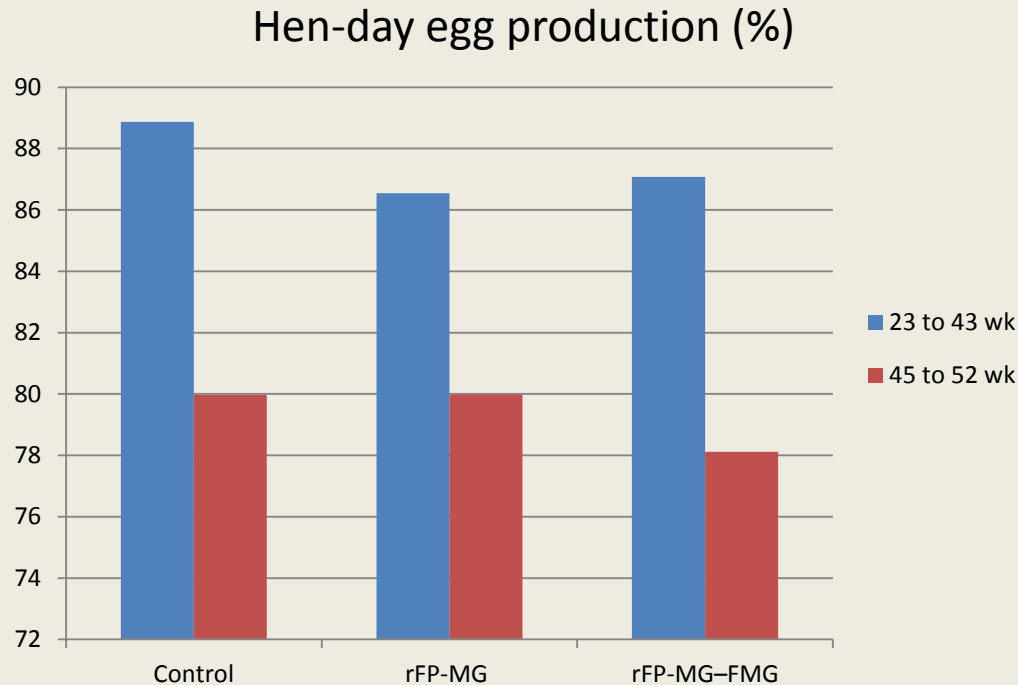
^DEvaluated by gross observation.

^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