Vermont Veterinary Medical Association

2025
Winter CE Conference

February 1 and 2

Dr. Amelia Woolums
DVM, PhD, DACVM
Mississippi State University College of Veterinary Medicine

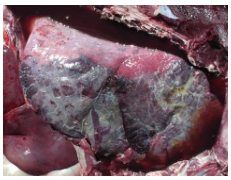
Antimicrobial Resistance in Bovine Respiratory Disease

Generously sponsored by:

DIPAOLA

Antech®
Smarter Diagnostics. Better Care.

Antimicrobial Resistance in Bovine Respiratory Disease



Amelia Woolums, DVM MVSc PhD DACVIM DACVM
Mississippi State University
amelia.woolums@msstate.edu

MISSISSIPPI STATE UNIVERSITY. COLLEGE OF VETERINARY MEDICINE

1

Background: *Mannheimia haemolytica*

- Found on normal mucosal surfaces
  - but lower numbers on healthy respiratory tract
- May "hide out" in tonsils
  - can be found in tonsils when not on nasal swabs
- 12 serotypes
  - A2: most commonly found in normal cattle
    - but causes pneumonia in sheep
  - A1 and A6: most commonly found in BRD

MISSISSIPPI STATE UNIVERSITY. COLLEGE OF VETERINARY MEDICINE

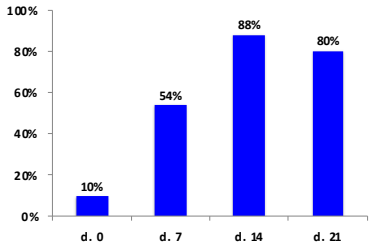
2

- In the days after cattle are shipped or co-mingled, *M. haemolytica* proliferates rapidly in the nasopharynx
- Serotypes A1 or A6 predominate over the "more normal" seroytype A2

MISSISSIPPI STATE UNIVERSITY. COLLEGE OF VETERINARY MEDICINE

3

Percent of stocker cattle positive for *Mannheimia haemolytica* on nasopharyngeal swabs collected weekly for 21 days after arrival



Woolums et al. 2018

MISSISSIPPI STATE UNIVERSITY. COLLEGE OF VETERINARY MEDICINE

4

Antimicrobials and antimicrobial resistance (AMR)

- Eleven antimicrobials labeled for treatment of BRD due to *M. haemolytica* are currently available
- Historically, AMR in *M. haemolytica* has been relatively rare

MISSISSIPPI STATE UNIVERSITY. COLLEGE OF VETERINARY MEDICINE

5

- In 461 *M. haemolytica* isolates from fatal BRD cases between 1988 – 1992:
  - most susceptible to all AM tested

    Watts et al., 1994
- *M. haemolytica* isolates collected from cattle dying of BRD between 1994 – 2002
  - stable and low rates of resistance to ceftiofur + enrofloxacin
  - resistance to tetracycline was more prevalent

    Welsh et al., 2004
- In 409 *M. haemolytica* isolates collected by nasopharyngeal swab at feedlot entry and within 30 days exit
  - 0% resistant to ceftiofur, enrofloxacin, and florfenicol
  - 4% resistant to oxytetracycline

    Klima et al., 2011

MISSISSIPPI STATE UNIVERSITY. COLLEGE OF VETERINARY MEDICINE

6

Something different…

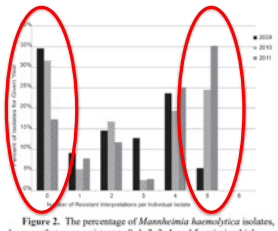


Figure 2. The percentage of *Mannheimia haemolytica* isolates, by year, that were resistant to 0, 1, 2, 3, 4, and 5 antimicrobials, respectively. Isolates in the 0 column would be considered pan-susceptible isolates. There were no isolates resistant to all 6 antimicrobials over the course of the survey.

Lubbers and Hanzlicek, 2013

- Retrospective analysis of prevalence of multidrug resistant *M. haemolytica* isolated from BRD cases
- Submissions to KSVDL, 2009-2011
- 389 isolates from 266 unique premises

- Authors acknowledge limitations
  - limited region represented (primarily KS and NE)
  - feedlot submissions overrepresented
  - didn't always have treatment history
  - no effort to determine clinical significance of isolates
- Still, these data worried some people

A clue from the recent past?

ICE*Pmu1*, an integrative conjugative element (ICE) of *Pasteurella multocida*: analysis of the regions that comprise 12 antimicrobial resistance genes

Geovana Brenner Michael[1,2], Kristina Kadlec[1], Michael T. Sweeney[3], Elzbieta Brzuszkiewicz[2], Heiko Liesegang[2], Rolf Daniel[2], Robert W. Murray[3], Jeffrey L. Watts[3] and Stefan Schwarz[1]*

J Antimicrob Chemother 2012; **67**: 84–90
doi:10.1093/jac/dkr406 Advance Access publication 14 October 2011

Results: The analysis of one representative *P. multocida* isolate identified an 82 kb integrative and conjugative element (ICE) integrated into the chromosomal DNA. This ICE, designated ICE*Pmu1*, harboured 11 resistance genes, which confer resistance to streptomycin/spectinomycin (*aadA25*), streptomycin (*strA* and *strB*), gentamicin (*aadB*), kanamycin/neomycin (*aphA1*), tetracycline [*tetR-tet*(H)], chloramphenicol/florfenicol (*floR*), sulphonamides (*sul2*), tilmicosin/clindamycin [*erm*(42)] or tilmicosin/tulathromycin [*msr*(E)-*mph*(E)]. In addition, a complete bla_{OXA-2} gene was detected, which, however, appeared to be functionally inactive in *P. multocida*. These resistance genes were organized in two regions of approximately 15.7 and 9.8 kb. Based on the sequences obtained, it is likely that plasmids, gene cassettes and insertion sequences have played a role in the development of the two resistance gene regions within this ICE.

- A *P. multocida* isolate from a Nebraska feedlot BRD case contained a genetic element that encoded resistance for 11 different AM
- This genetic element could be transferred from the *P. multocida* to *E. coli* or *M. haemolytica*, conferring resistance in these recipient bacteria

- Questions:
  - Can MDR *M. haemolytica* be found in live healthy cattle, or only those that die of BRD?
  - Are multi-drug resistant *M. haemolytica* present in cattle before they're treated with AM, or only after AM treatment
  - If live cattle have MDR *M. haemolytica*, are they more likely to fail treatment for BRD?

Prevalence of multi drug antimicrobial resistance in *Mannheimia haemolytica* isolated from high-risk stocker cattle at arrival and two weeks after processing[1]

E. Snyder,* B. Credille,*[2] R. Berghaus,* and S. Giguère†

*University of Georgia, Food Animal Health and Management Program, Department of Population Health, College of Veterinary Medicine, Athens 30602; and †University of Georgia, Department of Large Animal Medicine, College of Veterinary Medicine, Athens 30602

J. Anim. Sci. 2017.95:1124–1131
doi:10.2527/jas2016.1110

- Collected nasopharyngeal swabs from 169 high risk stocker cattle at arrival and 10 – 14 days later
- All cattle received tulathromycin for metaphylaxis after collection of d. 0 swab
- Eight cattle were treated for BRD (florfenicol) before d. 14, and one died
  - Second swab collected from these cattle before BRD treatment

Table 2. Frequency of antimicrobial susceptibility patterns for *Mannheimia haemolytica* isolates collected from 169 stocker calves before (Arrival) and after (second Sample[1]) metaphylactic treatment with tulathromycin. *Mannheimia haemolytica* was cultured from 27 (16.0%) calves at arrival and from 123 (72.8%) calves at second sampling. Up to three isolates were evaluated from each calf with a positive culture result

Occasion	Antimicrobial resistance[2]	No. isolates (%)
Arrival	None	59 (74.7)
	TIL	7 (8.9)
	TUL	5 (6.3)
	TIL, TUL	5 (6.3)
	ENR, GAM, TIL, TUL	3 (3.8)
	Total	79
Second sample	ENR, FLOR, GAM, TIL, TUL	253 (69.1)
	ENR, GAM, TIL, TUL	104 (28.4)
	CEF, ENR, GAM, TIL, TUL	4 (1.1)
	None	3 (0.8)
	ENR, TIL, TUL	1 (0.3)
	ENR, FLOR, TIL, TUL	1 (0.3)
	Total	366

Snyder et al., 2017

75% were susceptible to all AM on d. 0

97% were resistant to ENR, GAM, TIL, TUL on d. 10 – 14

69% were resistant to FLO

1% were susceptible to all AM on d. 10 - 14

13

- In high risk stocker cattle given tulathromycin for metaphylaxis, prevalence of MDR *M. haemolytica* shedding increased sharply between d. 0 – d. 14
- This was not related to an unusually high rate of morbidity or mortality over the same period

14

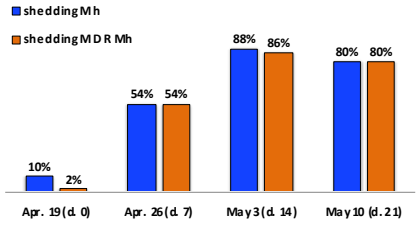
AMR in Mississippi stocker cattle

- In a similar stocker trial we found essentially the same results
- 50 high risk bulls and steers treated with tildipirosin at arrival, with other processing

Woolums et al., 2018

15

Percent of cattle shedding Mh and MDR Mh after arrival

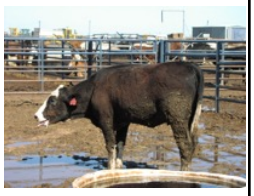


MDR: resistant to drugs in 3 or more classes of antimicrobials

Woolums et al., 2018

16

- Notably, mortality and treatment failure rates did not seem unusual in either the GA or MS stocker study
- **Question**: was widespread AMR due to long acting macrolide metaphylaxis, or other factors?
  - E.g.: treatments for BRD or other diseases after metaphylaxis?
- **Need a group not receiving metaphylaxis to know**



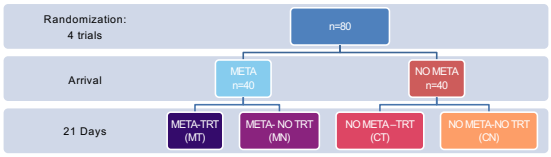
17

Clinical trial: effects of tulathromycin metaphylaxis on health and AMR in stocker cattle

- **Objectives:**
- Determine the effect of macrolide metaphylaxis on:
  - Antimicrobial resistance in *M. haemolytica*
    - Phenotype (culture & sensitivity)
    - Metagenome (18S)
    - Resistome (Target-enriched AR gene shotgun sequencing)
  - Health and production outcomes
    - Morbidity
    - Mortality
    - Weight gain

18

Study Design



Cattle: black heifers, mixed source, auction market derived, 400 – 500 lb (180 – 225 kg)

Managed on grass: approx 4 head per acre

Supplemented with free choice Accuration with no RX3 additive (Purina)

19

Methods-Cattle Processing

- META:
  - 2.5 mg/kg SC tulathromycin (Draxxin)
- All animals:
  - Dewormed: fenbendazole (Safeguard) and doramectin (Dectomax)
  - Vaccinated: Clostridial (Vision 7) and Viral Respiratory (Pyramid 5)
  - Ear notched for BVD-PI testing (acELISA)
  - Blood collected for concurrent research projects
  - Temperature taken

20

BRD Case Definition

- Clinical Score:
  - 0-Normal
  - 1-Mild
  - 2-Moderate
  - 3-Severe
  - 4-Moribund/Near Death

(Adapted from Perino & Apley, 1998)

- BRD Case-
  - Animal scored 1 or 2 AND rectal temperature ≥ 104°F (40°C)

OR

  - Animal scored 3 or 4, regardless of rectal temperature

21

BRD Treatment Protocol

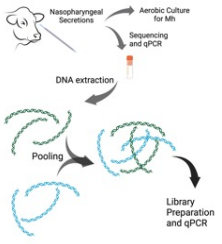
- META cattle: not eligible for treatment until day 8 of trial
- NO META cattle: eligible on day 1

- 1st treatment-ceftiofur (Excede™), 7 day PTI
- 2nd treatment-florfenicol (Nuflor™) 4 day TI
- 3rd treatment- oxytetracycline (Noromycin 300-LA™)

22

Methods-Sample Collection

- Cleaned each nostril with single use paper towel
- Guarded deep nasopharyngeal swabs
  - One from each nostril

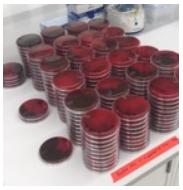


Adapted from Morley *et al.* with biorender.com

23

Methods: bacterial culture and identification

- Swabs placed in tubes with transport media
- Swabs cultured blood agar
  - *M. haemolytica* isolates selected
  - Antimicrobial susceptibility testing with Sensititre



Blood agar plates, first group

24

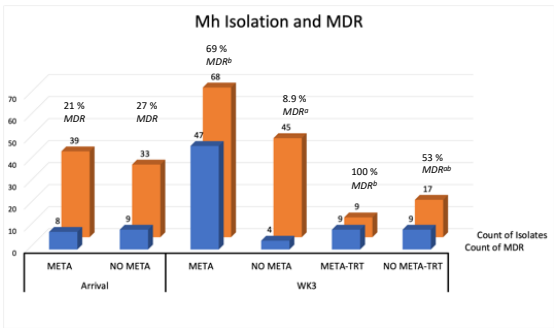
Results at 3 weeks

25

Weight gain and health at 3 weeks

Trial	Group	n	ADG (95% CI) (kg)	Animals Treated n (%)	Animals Treated (BRD) n (%)	2+ BRD Treatments n n (% of Treat)	Mortality n (%)
Fall 2019	META	41	1.48 (1.20-1.77)	11(27)	9 (22)	1 (11)	0 (0)
	NO META	41	1.00 (0.66-1.30)	9 (22)	7 (17)	0 (0)	0 (0)
	All	82	1.21 (0.83-1.65)	20 (24)	16 (20)	1 (6)	0 (0)
Fall 2020	META	42	0.54 (0.22-1.08)	8 (19)	6 (14)	0 (0)	1 (2)
	NO META	41	0.22 (-0.22 -0.71)	10 (24)	10 (24)	1 (10)	2 (5)
	All	83	0.43 (-0.11-0.97)	18 (22)	16 (19)	1 (6)	3 (4)
Spring 2021	META	41	0.86 (0.33-1.23)	4[a] (10)	4[a] (10)	1 (25)	2 (5)
	NO META	39	0.70 (0.22-1.40)	17[b] (44)	17[b] (44)	1 (6)	3 (8)
	All	80	0.83 (0.26-1.32)	21 (26)	21 (26)	2 (10)	5 (6)
Fall 2021	META	41	1.02 (0.54-1.49)	1[a] (2)	1[a] (2)	0 (0)	0 (0)
	NO META	42	0.78 (-0.02-1.13)	12[b] (29)	12[b] (29)	2 (17)	1 (2)
	All	83	0.90 (0.33-1.23)	13 (16)	13 (16)	2 (15)	1 (1)
Overall	META	165	1.02[a] (0.43-1.49)	24[a] (15)	20[a] (12)	2 (10)	3 (2)
	NO META	163	0.70[b] (0.16-1.18)	48[b] (29)	46[b] (28)	4 (9)	6 (4)
	All	328	0.89 (0.3-1.34)	75 (23)	66 (20)	6 (9)	9 (3)

26



27

Average daily weight gain at 3 weeks

Variable	Value	Estimate	Standard Error	P
Group	META	Ref	Ref	Ref
	NO META	-0.357	0.084	<0.0001*
Trial	Fall 2019	Ref	Ref	Ref
	Fall 2020	-0.813	0.118	<0.0001*
	Spring 2021	-0.472	0.122	0.0001*
	Fall 2021	-0.4782	0.117	0.0001*

28

BRD Treatment at 3 weeks

Parameters		OR	95 % (CI)	P
Group	META	Ref	Ref	Ref
	NO META	3.053	1.694-5.501	0.0002*
Arrival Weight	(kg)	0.977	0.961-0.993	0.006*

29

Mortality at 3 weeks

Variable	Value	OR	95 % (CI)	P
Group	META	Ref	Ref	Ref
	NO META	1.287	0.299-5.536	0.7351
Treated	Yes	Ref	Ref	Ref
	No	0.0758	0.015-0.383	0.002*

30

M. haemolytica isolation and MDR at 3 weeks

Outcome	Variable	Value	OR	95% CI	*p*-value
Mh isolation	TxGroup	META	1.41	0.90–2.22	0.13
		NO META	Ref	Ref	Ref
MDR *Mh* isolation	TxGroup	META	13.08	5.54–30.88	<0.0001
		NO META	Ref	Ref	Ref
	BRD Treatment x FeverAR	Interaction	–	–	0.02
		N x N	Ref	Ref	Ref[a]
		Y x Y	9.70	1.63–57.78	0.061[a]
		N x Y	0.18	0.05–0.65	0.009[b]
		Y x N	3.83	1.39–10.58	0.01[c]

31

Results: Sampling at 10 weeks

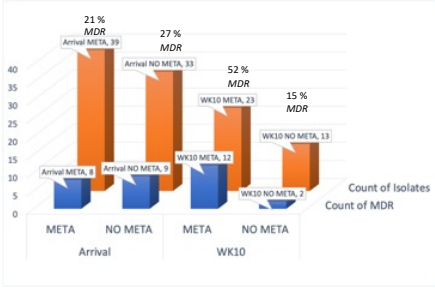
32

Weight gain and health at 10 weeks

Trial	Group	n	ADG (95% CI) (kg)	Animals Treated n (%)	Animals Treated (BRD) n (%)	2+ BRD Treatments n n (% of Treat)	Mortality n (%)
Fall 2019	META	41	0.96 (0.87-1.17)	11 (27)	9 (22)	1 (11)	0
	NO META	41	0.89 (0.72-0.96)	11 (27)	9 (22)	0 (0)	0
	All	82	0.92 (0.79-1.08)	22 (27)	18 (22)	1 (6)	0
Fall 2020	META	42	0.83 (0.64-0.93)	9 (21)	7 (17)	0 (0)	2 (5)
	NO META	41	0.65 (0.42-0.86)	12 (29)	10 (24)	1 (10)	2 (5)
	All	83	0.78 (0.49-0.91)	21 (25)	17 (20)	1 (6)	4 (5)
Spring 2021	META	42	0.76 (0.63-0.97)	5 (12)	4 (10)	1 (25)	4 (10)
	NO META	40	0.74 (0.53-0.90)	18 (45)	17 (43)	7 (41)	8 (20)
	All	82	0.76 (0.53-0.94)	23 (28)	21 (26)	8 (38)	12 (15)
Fall 2021	META	42	0.45 (0.37-0.71)	6 (14)	4 (10)	0 (0)	0
	NO META	42	0.44 (0.21-0.57)	14 (33)	14 (33)	8 (57)	3 (7)
	All	83	0.45 (0.31-0.62)	20 (24)	18 (22)	8 (44)	3 (4)
Overall	META	167	0.81 (0.55-0.97)	31 (19)	24 (14)	2 (8)	6 (4)
	NO META	164	0.67 (0.45-0.91)	55 (34)	50 (30)	16 (32)	13 (8)
	All	331	0.75 (0.48-0.93)	86 (26)	74 (22)	18 (5)	19 (6)

33

M. Haemolytica and MDR *M. haemolytica* isolation by group at 10 weeks



34

ADG at 10 weeks

Variable	Value	Estimate	Standard Error	P
Group	META	**Ref**	**Ref**	**Ref**
	NO META	-0.107	0.033	**0.001***
Trial	Fall 2019	**Ref**	**Ref**	**Ref**
	Fall 2020	-0.219	0.044	**<0.0001***
	Spring 2021	-0.196	0.047	**<0.0001***
	Fall 2021	-0.461	0.045	**<0.0001***

35

BRD Treatment at 10 weeks

Variable	Value	OR	95 % (CI)	P
Group	META	**Ref**	**Ref**	**Ref**
	NO META	2.763	1.589-4.805	**0.0003***
Arrival Weight	(kg)	0.980	0.961-0.993	**0.012***

36

Mortality at 10 weeks

Variable	Value	OR	95 % (CI)	P
Group	META	**Ref**	**Ref**	**Ref**
	NO META	1.5133	0.529-4.331	0.44
Treated	Yes	**Ref**	**Ref**	**Ref**
	No	0.0574	0.016-0.205	**<0.0001***

37

M. haemolytica isolation and MDR at 10 weeks

Outcome	Variable	Value	OR	95% CI	*p*-value
MH isolation	TxGroup	META	1.72	0.84-3.55	0.14
		NO META	Ref	Ref	Ref
MDR *MH* isolation	TxGroup	META	5.92	1.34-26.14	0.019
		NO META	Ref	Ref	Ref
Isolation of *MH* containing ICE	TxGroup	META	1.88	0.69-5.12	0.21
		NO META	Ref	Ref	Ref

38

Results: Summary

- META cattle: significantly lower proportion treated for BRD....BUT:
  - Significantly higher proportion with multi-drug resistant *M. haemolytica*
- Significantly increased multi-drug resistant *M. haemolytica* **still present at 10 weeks after metaphylaxis**

39

Significance of these results?

- Tulathromycin metaphylaxis was associated with decreased treatment for BRD
  - Health benefits: consistent with much published literature
  - Somewhat surprising given AMR of *M. haemolytica*
  - Could beneficial **non-antimicrobial effects** explain?
- Tulathromycin metaphylaxis was associated with increased prevalence of MDR *M. haemolytica* that persisted at least 10 weeks
  - Concerning for long term impact, given potential for transmission

40

Final thoughts

- Macrolide metaphylaxis led to improved health while promoting increased prevalence of multi-drug resistance, due to genetic elements we know bacteria can share
- This is an ethical dilemma
- Possible new directions:
  - Improve methods to target metaphylaxis?
    - By individual cattle, or cattle cohorts?
  - Find ways to induce protective health effects of metaphylaxis that don't also generate AMR?

41

Final thoughts

- Antimicrobial resistance is a complex problem
- Relationships and outcomes we assume aren't always supported by data
  - Help spread the word
  - Help gather more data to confirm true impacts and outcomes

42

43

Summary

- AMR and MDR in *M. haemolytica* and other BRD agents used to be rare
- In the past 5 years, reports indicate that BRD bacteria can carry genetic elements encoding MDR
- But we don't always see high prevalence of MDR agents when AM are used extensively

MISSISSIPPI STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE

44

Summary

- Recent surveys of high risk stocker cattle managed conventionally with metaphylaxis: prevalence of MDR *M. haemolytica* nasopharyngeal shedding can increase rapidly
  - Genetically diverse *M. haemolytica* can carry similar AMR genes
  - **Negative impact on morbidity or mortality has not been clearly evident**

MISSISSIPPI STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE

45

Questions remaining

- Does high prevalence of MDR *M. haemolytica* increase risk for treatment failure or death?
- How does prevalence of MDR Mh increase so rapidly?
  - Do MDR bacteria or the genetic elements encoding MDR transmit rapidly between cattle?
- Do highly prevalent MDR *M. haemolytica* increase risk for generation of other MDR pathogens?
  - are there microbial reservoirs of resistance genes?
-

MISSISSIPPI STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE

46

Acknowledgements, MSU study

- Mississippi State University
  - Brandi Karisch
  - Bill Epperson
  - Dave Smith
  - John Blanton
  - Frank Austin
- University of Georgia
  - Ray Kaplan
- USDA ARS US NPRL
  - Jonathan Frye
  - Charlene Jackson
  - Sushim Gupta
  - Lari Hiott
  - Tiffanie Woodley
- UC Irvine
  - Michael McClelland

MISSISSIPPI STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE

47

Acknowledgements, MSU study

- Project funding
  - Mississippi Agricultural and Forestry Experiment Station (MAFES) Revolving Livestock Fund
  - MSU Dept. of Animal and Dairy Science
  - MSU Dept. of Pathobiology and Population Medicine
  - USDA ARS (Frye and Jackson labs)
- Technical assistance
  - Larry Ballard and Angela Knight

MISSISSIPPI STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE

48

49

Table 2. Demographics of study population.

	No. of Cattle	% of Cattle
Risk status of cattle		
Low risk	2,420	44.0
Medium risk	832	15.1
High risk	1,356	24.7
Very high risk	890	16.2
Arrival season of cattle		
Winter (Jan–Mar)	876	15.9
Spring (Apr–Jun)	851	15.5
Summer (Jul–Sept)	1,623	29.5
Fall (Oct–Dec)	2,148	39.1
Pen size		
<101	459	8.4
101–200	1,858	33.8
201–300	1,409	25.6
301–400	1,173	21.3
>400	599	10.9

Noyes et al., JVIM 29:705, 2015

50