Identification of Candidate Plant-derived Antimicrobial for Treatment of Bovine Mastitis

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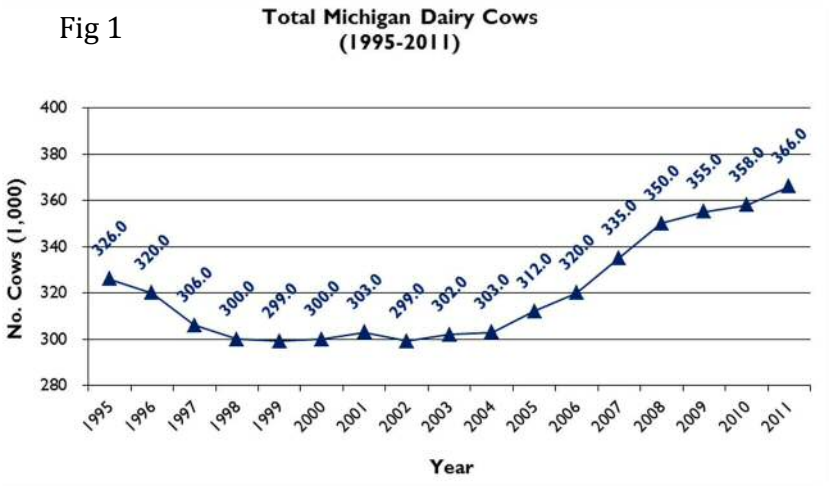
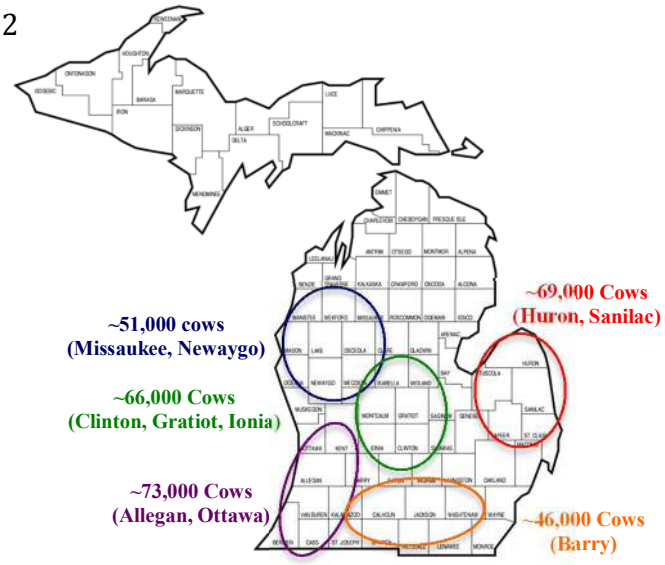
Nationwide, the total number of dairy cows has steadily declined while Michigan has increased 22.4% from 1999 to 2011 (Thomas, 2011, Fig 1). This shift occurred due to existing farms expanding their herd size through renovation of their milking parlor and housing stalls to improve profitability, while also decreasing labor (Hadley et al. 2002). Despite the fact that the average state herd size was determined to be 169 cows, farms managing fewer than 100 cows have been exiting the market. Where between 2006 and 2011, 370 commercially licensed farms in Michigan went out of business or merged as a result of plummeting milk prices and elevated operational costs (Cullens, 2011). Nonetheless, in 2012 the economic contribution of on-farm receipts accounted for $1.675 billion dollars, which was invested back into local communities in the form of wages, feed and equipment purchases (Durst, 2014).

Fig 1



Michigan is now ranked as the seventh leading state for total dairy milk production, with the average cow producing 24,116 lbs annually. There are five distinct regions within Michigan where roughly 85% of the state's total 366,000 cows are housed (Thomas, 2011, Fig 2). The top ten counties with the largest dairy herds can be found within the lower peninsula of the state: Huron, Sanilac, Allegan, Clinton, Ionia, Gratiot, Missaukee, Barry, Newaygo, and Ottawa.

Fig 2



One particular issue in the dairy industry that needs to be confronted is identifying alternative treatments and management practices that are effective at decreasing the incidence of production diseases. In the past, producers have had greater incentives to boost net income by maximizing production opposed to decreasing management cost in order to improve the overall health of the herd. It can be perceived that immediate ramifications would be beneficial, but the underline impact of disease will not go unnoticed. The United States has not implemented a unified system for reporting health events on dairy farms. In

most cases, the data is confidential to individual farms or compiled within small-scale research studies spanning only a few years at most. However, Parker-Gaddis, Cole, Clay, and Maltecca (2012) examined the incidence of disease represented in data sets containing 1,427,435 treatment records filed by 759 on-farm computer systems across the United States. When observing the occurrence of 13 common production diseases, mastitis stood out with the most prominent number of cases throughout all stages of lactation (Parker-Gaddis et al. 2012, Table 1.) Also, mastitis had the highest mean lactational incidence rate (LIR) of 12.32%, which indicates then number of first occurrences of a specific health event divided by the number of lactations at risk. This value was nearly double the LIR observed in lameness (6.86%) and metritis (6.37%).

Table 1. Summary statistics of each health event of interest

	Health event 0 to 60 DIM			Health event 61 to 90 DIM			Health event 91 to 150 DIM		
Health event[1]	Herds (no.)	Cows (no.)	Total cases (no.)	Herds (no.)	Cows (no.)	Total cases (no.)	Herds (no.)	Cows (no.)	Total cases (no.)
DYST	317	123,552	5,024						
RETP	598	185,154	12,602						
KETO	211	75,458	5,280	211	74,150	36	211	74,146	27
DSAB	31	10,382	297	31	10,326	8	31	10,325	2
CALC	237	96,899	1,446	237	96,677	24	237	96,720	101
METR	600	200,875	25,525	599	192,862	385	599	193,057	1,071
DIAR	42	19,699	498	42	19,624	50	42	19,623	50
DIGE	442	152,730	5,352	442	151,591	415	442	151,652	667
RESP	457	169,561	3,013	457	169,060	277	457	169,106	384
REPR	723	225,558	7,445	720	223,709	508	721	224,142	1,850
MAST	759	233,368	26,403	759	227,624	5,174	759	228,424	8,410
CYST	699	190,227	2,519	699	190,449	2,557	707	211,338	3,690
LAME	632	205,531	7,848	631	204,090	2,521	632	204,952	4,863

[1]DYST = dystocia; RETP = retained placenta; KETO = ketosis; DSAB = displaced abomasum; CALC = hypocalcemia; METR = metritis; DIAR = diarrhea; DIGE = digestive problem; RESP = respiratory problem; REPR = reproductive problem; MAST = mastitis; CYST = cystic ovaries; LAME = lameness.

What is Mastitis?

Bovine mastitis is the most prevalent production disease in the dairy industry, accounting for annual losses of $2 billion in the USA (Varshney et al. 2004). External indicators of the disease involve inflammation of a cow's udder as a result of primarily bacterial infections. It becomes evident that a cow has mastitis when prepping the teats at

the milking parlor, because the milk will contain flakes of bacterial clumps or possibly blood. The risk of clinical mastitis increases with parity, the higher the milk yield the cow is producing, the larger the herd size and the stage of lactation during the first 50 days or at dry-off (Hamadani et al. 2013). The severity of mastitis is divided into three main categories: chronic, peracute, and subclinical. Chronic mastitis is characterized by inflammation present for months, exhibiting periodical flare-ups where somatic cell count is elevated above 3,000,000 cell/ml. *Staphylococcus aureus* is typically the culprit, because the bacteria can be transmitted from one cow to another during the milking process. Peracute mastitis occurs when there is a sudden decrease in milk yield and fever developing within the course of a day. Coliforms (particularly E. coli and Klebsiella spp.), Streptococcus uberis, Streptococus dysgalactiae and Arcanobacterium pyogenes found within the environment are able to penetrate through the streak canal and are usual suspects for peracute mastits. Wet bedding, milking improperly cleaned udders, and inadequate teat preparation can predispose the animals to exposure to these microorganisms. With subclinical mastitis, there are no visible systemic signs and minimal inflammation of the mammary gland. Coagulase negative Staphylococcus species are known to decrease milk yield without causing as detrimental effects as the other bacteria mentioned (Hamadani et al. 2013).

Mastitis is a major economic burden on producers, who must endure the cost of veterinary treatment, increased labor, and premature culling of the animal. Likewise, producers have reduced revenue as a result of discarded milk, recurrence of infection, and the critical impact of decreased milk yield and composition in subsequent lactations. On

average 375kg of milk are lost per cow with each clinical case of mastitis (Seegers et al. 2003). Hence, there is a pressing demand for new antimicrobials to act as a safeguard against the emergence of antibiotic resistance. Meanwhile, organic farms are relying on homeopathic remedies due to the shortcomings in the commercial availability of effective non-antibiotic therapies (Ruegg 2009).

In vitro experimentation with Manuka Honey

Our laboratory at Michigan State University's (MSU) Department of Large Animal Clinical Sciences has been investigating alternatives to antimicrobials in particular; Manuka honey, over the last year. This specific honey is derived from the Manuka bush (*Leptospermum scoparium*) in New Zealand. The active component methylglyoxal can inhibit a broad spectrum of bacteria and dissipate biofilm formation (Maddocks et al. 2012). Manuka honey has been under investigation in many human medicine studies, one case in point where it was revealed to assist in growth of granular tissue and collagen in 8 patients with leg ulcerations (Gethin & Cowman, 2005).

We compared the minimum inhibitory concentration of Manuka honey at 1-12% w/v honey in agar with a commercial antibiotic (cephalothin) on 46 clinical mastitis isolates. Preliminary results indicate that at 8% Manuka honey growth of *E. coli* and *Streptococcus* species with high cephalothin resistance could be inhibited.

Research clubs

Involvement in research earlier in one's college career places undergraduates at the forefront of new discoveries. The Animal Science Undergraduate Research Student Association provides an opportunity for students to conduct research alongside faculty

mentors to develop independence and collaborate to address a research question Memberships in this organization create a sense of community for students to cooperate and succeed in addressing a common research inquiry. Outreach activities attempt to reshape how the general public perceives “industrial agriculture”.

References

Cullens, F. (2011). Dairy Heard Management: *Michigan's Dairy Industry in 2011: Key numbers and trends.* Retrieved from http://www.dairyherd.com/dairy-news/Michigans-Dairy-Industry-in-2011-Key-numbers-and-trends-160589465.html

Durst, P. (2014). Dairy Heard Management: *Michigan milk production moves to no. 7.* Retrieved from http://www.dairyherd.com/dairy-news/Michigan-milk-production-moves-to-no-7-254345111.html

Gethin, G., & Cowman, S. (2005). Case series of use of Manuka honey in leg ulceration. *International Wound Journal.* 2, 10-15.

Hadley, G.L., Harch, S.B. & Wolf, C.A. (2002). Managerial and Financial Implications of Major Dairy Farm Expansions in Michigan and Wisconisin. *J. Dairy Sci.* 85(8), 2053-2064.

Hamadani, H., Khan, A.A., Banday, M.T., Ashraf, I., Handoo, N., Bashir, A., & Hamadani, A. (2013). Bovine Mastitis - A Disease of Serious Concern for Dairy Farmers. *International Journal of Livestock Research*. 3(1), 42-55.

Maddocks, S.E. et al. (2012). Manuka honey inhibits the development of Streptococcus pyogenes biofilms and causes reduced expression of two fibronectin binding proteins. *Microbiology* 158, 781-790.

Parker-Gaddis, K.L., Cole, J.B., Clay J.S., & Maltecca, C. (2012). Incidence validation and relationship analysis of producr-recorded health event dara from on-farm computer systems in the United States. *J. Dairy Sci*. 95:5422-5435.

Ruegg, P.L. (2009). Management of mastitis on organic and conventional dairy farms. *J Anim Sci.* 87:43-55.

Seegers, H. et al. (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet Res.* 34, 475-491.

Thomas, C. (2011). Overview of U.S. and Michigan Dairy Industries. Michigan State University Extension. Powerpoint Presentation. Retrieved from http://www.google.de/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&ved=0CDQQFjAC&url=http%3A%2F%2Fdairyteam.msu.edu%2Fuploads%2Ffiles%2Foverview_us_michigan_dairy_industries.pptx&ei=3ZjVU4CUO4X74QSI-IDIAg&usg=AFQjCNGq7AEfctUixZQYILadCcWgz1hxlQ&sig2=-eWYgDoV7DeScMCGaqj09Q&bvm=bv.71778758,d.bGE

Varshney, J.P. et al. (2004). Evaluation of homeopathic complex in the clinical

management of udder diseases of riverine buffaloes. *Homeopathy*. 93(17).