

1 **Effect of heat stress on udder health of dairy cows**

2 Md Rezaul Hai Rakib, Man Zhou, Siyu Xu, Yang Liu, Muhammad Asfandyar Khan, Bo Han and Jian Gao

3

4 **SUPPLEMENTARY FILE**

5

6 **Supplementary Table S1.** Distribution of mastitis pathogens with incidence rate of CM in different countries

Country	Study area and sample population	Study period	Mean IRCM	Involved pathogens			Cited from
				Gram positive (% of isolates)	Gram negative (% of isolates)	Others (% of isolates)	
Argentina	2296 cows from 51 randomly selected herds in Córdoba province	March 2007 to September 2007	53.9 cases/100 cows-year	CNS (21.5) <i>Staph. aureus</i> (21.3) <i>S. agalactiae</i> (4.4) <i>S. dysgalactiae</i> (4.4) <i>Staph. intermedius</i> (3.8) <i>Enterobacter spp.</i> (2.3)	<i>E. coli</i> (2.1)	<i>Corynebacterium spp.</i> (5.2)	Dieser <i>et al.</i> , 2014
Australia	A year-round calving dairy herd near Malanda, North Queensland	January to April 2016	11.6–24.3 cases/100 cows-month	<i>S. uberis</i> (25) <i>Staph. aureus</i> (18) <i>S. dysgalactiae</i> <i>Bacillus spp.</i>	<i>E. coli</i> (27) <i>Klebsiella spp.</i> <i>Enterobacter spp.</i>	<i>Corynebacterium spp.</i>	Rowe <i>et al.</i> , 2018
Brazil	20 dairy herds of southeastern Brazil	March 2014 to January 2016	9.7 cases/10,000 quarter-days	<i>S. uberis</i> (6.1) <i>S. agalactiae</i> (5.9) CNS <i>S. dysgalactiae</i> <i>Staph. aureus</i> <i>Streptococcus spp.</i>	<i>E. coli</i> (6.6) <i>Klebsiella spp.</i> <i>Citrobacter spp.</i> <i>Enterobacter spp.</i>	Yeast <i>Prototheca spp.</i> <i>Corynebacterium spp.</i>	Tomazi <i>et al.</i> , 2018
Belgium	50 randomly selected commercial Flemish dairy herds	October 2012 to September 2013	7.4 quarter cases/10,000 cow-days	<i>S. uberis</i> (18.2) <i>Staph. aureus</i> (7.3) <i>S. dysgalactiae</i> (7.2) Non- <i>aureus</i> staphylococci (5.0) Other <i>esculin</i> -positive cocci (2.1)	<i>E. coli</i> (15.5) <i>Klebsiella spp.</i> (0.8)	<i>Corynebacterium bovis</i> (3.0) Yeast (2.0) <i>Prototheca spp.</i> (1.4)	Verbeke <i>et al.</i> , 2014

Canada	59 dairy farms of Southern Ontario	April 2011 to May 2012	23.7 cases/100 cow-years	CNS (21.5) <i>Bacillus spp.</i> (17.6) <i>Streptococcus spp.</i> (11.3) <i>Staph. aureus</i> (9.9) <i>Enterococcus spp.</i> (3.8) <i>S. dysgalactiae</i> (2.6) <i>Trueperella pyogenes</i> (2.2) <i>S. uberis</i> (0.6) <i>S. agalactiae</i> (0.1)	<i>E. coli</i> (9.0) <i>Enterobacter spp.</i> (3.2) <i>Klebsiella spp.</i> (2.1) <i>Pseudomonas spp.</i> (1.4)	<i>Corynebacterium spp.</i> (3.7) Yeasts (1.6) Fungus (0.6)	Levison <i>et al.</i> , 2016
Canada	41 herds across Canada	July 2007 to August 2008	26.3 cases/100 cow-years	<i>Staph. aureus</i> (22.2) <i>Streptococcus spp.</i> (16.2) CNS (7.7) <i>S. uberis</i> (6.8) <i>S. dysgalactiae</i> (2.6) <i>Bacillus spp.</i> (1.7)	<i>E. coli</i> (29.9) <i>Klebsiella spp.</i> (3.4) <i>Enterobacter spp.</i> (0.9)	<i>Arcanobacterium pyogenes</i> (2.6) Yeasts (1.7) <i>Prototheca spp.</i> (0.9)	Thompson-Crispi <i>et al.</i> , 2013
Canada	106 dairy farms of 10 provinces	November 2003 to July 2005	23 cases/100 cow-year	<i>Staph. aureus</i> (21.7) <i>S. uberis</i> (13.3) CNS (10.7) <i>S. dysgalactiae</i> (8.4) <i>Enterococcus spp.</i> (4.7) <i>Streptococcus spp.</i> (4.4) <i>Bacillus spp.</i> (2.2) <i>S. agalactiae</i> (0.3) <i>Staphylococcus spp.</i> (0.3)	<i>E. coli</i> (17.6) <i>Klebsiella spp.</i> (9.1) <i>Arcanobacterium pyogenes</i> (2.6) <i>Pseudomonas spp.</i> (1.6) <i>Serratia</i> (0.8) <i>Pasteurella spp.</i> (0.1)	Yeasts (4.0) <i>Corynebacterium bovis</i> (0.4)	Olde Riekerink <i>et al.</i> , 2008
China	161 dairy herds in 21 provinces	March 2014 to September 2016	3.3 cases/100 cows-month	CNS (11.3) <i>S. dysgalactiae</i> (10.5) <i>Staph. aureus</i> (10.2) Other streptococci (8.0) <i>Enterococcus spp.</i> (5.5) <i>S. agalactiae</i> (2.8) <i>S. uberis</i> (2.1) <i>Bacillus hemolysis</i> (1.2) <i>Trueperella pyogenes</i> (0.9) <i>Aerococcus viridans</i> (0.6)	<i>E. coli</i> (14.4) <i>Klebsiella spp.</i> (13.0) <i>Pseudomonas spp.</i> (1.4) <i>Pasteurella spp.</i> (0.2)	Yeasts (1.0) <i>Corynebacterium bovis</i> (0.6)	Gao <i>et al.</i> , 2017
China	175 milk samples of cows with clinical and	June 2012 to July 2013		<i>Trueperella pyogenes</i> (28.6) <i>Staph. aureus</i> <i>Aerococcus viridans</i>	<i>E. coli</i> <i>Enterobacter aerogenes</i>	<i>Prototheca zopfii</i>	Alkasir <i>et al.</i> , 2016

	subclinical mastitis from Beijing, Tianjin and Shandong provinces			<i>Nocardia asteroides</i>			
Czech Republic	Five Holstein dairy farms in the Czech Republic	1996 to 2003	0.94 cases/cow/year				Wolfova <i>et al.</i> , 2006
Denmark	Danish Cattle Data Base and a management questionnaire from 2, 146 herds in three Danish regions	July 1993 to June 1994	44.7 cases/100 cow-years				Sato <i>et al.</i> , 2008
Denmark	The Danish Cattle Database	June 1993 to July 1994	36-48 cases/100 cow-years				Bartlett <i>et al.</i> , 2001
Ecuador	250 dairy cows of 5 farms located around the cities of Santa Rosa and Machala of El Oro Province		11.6 cases/100 cow-year	Coagulase-positive staphylococci (25.8) CNS (20.4) <i>Streptococci</i> (9.7) <i>Bacillus spp.</i> (7.5)	<i>E. coli</i> (33.3) <i>Klebsiella spp.</i> (3.2)		Amer <i>et al.</i> , 2018
Ethiopia	20 dairy farms in & around Haramaya district		6.77 cases/100 cow-year	<i>S. agalactiae</i>			Lakew <i>et al.</i> , 2019
Germany	80 dairy farms of Brandenburg, Germany	July 2001 to October 2002	26.4 cases/100 cow-year	<i>S. agalactiae</i> (29.0) CNS (9.1) <i>Staph. aureus</i> (5.7) <i>S. uberis</i> (1.0)		<i>Corynebacterium bovis</i> (7.3)	Tenhagen <i>et al.</i> , 2006
India	Jammu region	June 2012 to July 2013	11.5 cases/100 cow-year 5.76 cases/100	<i>Staph. aureus</i> (60.87) CNS (13.04) <i>S. dysgalactiae</i> (8.69) <i>S. uberis</i> (4.35)	<i>E. coli</i> (13.04)		Bhat <i>et al.</i> , 2017

			quarter-year				
Iran	Holstein-Friesian dairy herd in Mashhad area	April 2005 to March 2008	30 cases/100 cow-years				Moosavi <i>et al.</i> , 2014
Israel	7 dairy herds in the central part of Israel	September 1989 to December 1993	4.2-126.8 cases/100 cows-year	<i>S. dysgalactiae</i> (10.7) CNS (7.7) <i>Staph. aureus</i> (6.4) <i>S. uberis</i> (1.9)	<i>E. coli</i> (51.2) <i>Klebsiella spp.</i> (1.0)	<i>Arcanobacterium pyogenes</i> (4.9) <i>Corynebacterium spp.</i> (1.3)	Shpigel <i>et al.</i> , 1998
Italy	A large Italian dairy farm with 1050 to 1200 Holstein cows milked daily	January 2014 to December 2015	41 cases/100 milking heifers-year				Vitali <i>et al.</i> , 2016
Jordan	Farm Records Register of the Jordanian Ministry of Agriculture	July 1991 to August 1992	5.0 cases/100 cow-months	CNS (16) <i>Staph. aureus</i> (14)			Lafi <i>et al.</i> , 1994
Mexico	Tropical hot region of Tierra Caliente, Guerrero		20.5 cases/100 cow-years	<i>Staphylococcus spp.</i> (2.5)	<i>Proteus vulgaris</i> (37.5) <i>Salmonella spp.</i> (25.5) <i>Enterobacter aerogenes</i> (12.5) <i>E. coli</i> (10) <i>Proteus mirabilis</i> (7.5) <i>Klebsiella pneumoniae</i> (5)		Olivares-Pérez <i>et al.</i> , 2015
Netherlands	Randomly selected 240 dairy farms with conventional milking system	January to December 2013	32.5 cases/100 cow-years				Santman-Berends <i>et al.</i> , 2015
Netherlands	Based on BTSCC in The Netherlands	December 1992 to June 1994	0.26 cases/365 cow-days	<i>S. dysgalactiae</i> <i>S. agalactiae</i> <i>S. uberis</i>	<i>E. coli</i> <i>Klebsiella spp.</i>	<i>Corynebacterium bovis</i>	Barkema <i>et al.</i> , 1998

				<i>Staph. aureus</i> CNS	<i>Pseudomonas</i> <i>spp.</i>	<i>Arcanobacteri</i> <i>um pyogenes</i>	
New Zealand	14 dairy farms from the Northland region of New Zealand	July 2005 to May 2006	0.19 cases/305 cow-days	<i>Staph. aureus</i> (23.7) <i>S. uberis</i> (23.3)			Petrovski <i>et al.</i> , 2009
Nigeria	77 cow herds located in Sokoto State, Nigeria	August to October 2011	85.33 cases/100 cow-years				Shittu <i>et al.</i> , 2012
Norway	Norwegian cattle health recording system	2005	21.28 ± 3.99 cases/100 cow-years				Østeras <i>et al.</i> , 2007
Poland	16 dairy farms from each of the 16 administrative provinces	August 2015 to July 2018	60.8 cases/100 cow-years	<i>Streptococcus spp.</i> (19.3) <i>Staphylococcus spp.</i> (7.8) <i>Enterococcus spp.</i> (1.7)		<i>Prototheca spp.</i> (8.3) Yeasts (1.8) <i>Corynebacterium spp.</i> (0.8)	Jagielski <i>et al.</i> , 2019a
Saudi Arabia	A dairy farm with a capacity of 11,200 dairy cows in Eastern Region of Saudi Arabia	For 60 days during winter	1.8 cases/100 cow-months 21.6 cases/100 cow-years	<i>S. uberis</i> (28.8) <i>S. dysgalactiae</i> (10.8) <i>Staph. aureus</i> (9.8) <i>S. agalactiae</i> (3.8) CNS (3.5)	<i>E. coli</i> (18.5) <i>Klebsiella oxytoca</i> (2.0)	<i>Corynebacterium spp.</i> (2.8)	Fadlelmula <i>et al.</i> , 2009
South Korea	24 dairy herds located in Gangwon, Gyeonggi, Chungbuk, Jeonnam, and Jeonbuk Provinces	January 2008 to December 2008	28.3 cases/100 cows-year	CNS (40.7) <i>Staph. aureus</i> (12.2) <i>S. uberis</i> (5.3) <i>Enterococcus spp.</i> (4.8) <i>Aerococcus spp.</i> (2.6)	Gram-negative bacilli other than <i>E. coli</i> (19.5) <i>E. coli</i> (4.5)	<i>Corynebacterium spp.</i> (1.2)	Nam <i>et al.</i> , 2010
Spain	25 Spanish herds of total 2,593 cows	April 2005 to December 2006	24.76 cases/100 cow-years				Pérez-Cabal <i>et al.</i> , 2008

Sweden	Field study	2002–2003	15-18 cases/100 cow-years	<i>S. aureus</i> <i>S. dysgalactiae</i> CNS <i>S. uberis</i>	<i>E. coli</i>	<i>Arcanobacterium pyogenes</i>	Waller <i>et al.</i> , 2009
Tanzania	87 randomly selected smallholder dairy herds in the Dar es Salaam region	July 2003 to March 2005	43.3 cases/100 cow-years				Kivaria <i>et al.</i> , 2007
United Kingdom	Seven commercial dairy herds in the southwestern UK	September 2010 to January 2012	46 cases/100 cow-lactations	<i>S. uberis</i> (19.9) CNS (5.7) <i>Staph. aureus</i> (2.5) <i>Enterococcus spp.</i> (2.3) <i>S. dysgalactiae</i> (2.05) <i>Bacillus spp.</i> (1.4) <i>Trueperella pyogenes</i> (1.4)	<i>E. coli</i> (20.5)	Yeasts (1.8)	Bradley <i>et al.</i> , 2015
Uruguay	Paysandú and Río Negro districts of West Littoral Region	NA	1.2 cases/100 cow-months	<i>Staph. aureus</i> (37.5) CNS (7.5) <i>S. agalactiae</i> (5) <i>S. uberis</i> (2.5) <i>Enterococcus spp.</i> (2.5)	<i>E. coli</i> (12.5)		Giannechini <i>et al.</i> , 2002
USA	109 primiparous Holstein dairy cows at University of Wisconsin Madison Marshfield Research Station	January 2013 – December 2013	1.36 cases/1,000 quarter day	CNS <i>Streptococcus spp.</i> <i>Staph. aureus</i>	<i>E. coli</i> <i>Klebsiella spp.</i>	Yeast <i>Corynebacterium spp.</i>	Rowbotham & Ruegg, 2016

Supplementary references to Table S1

- Alkasir R, Wang J, Gao J, Ali T, Zhang L, Szenci O, Bajcsy AC & Han B 2016 Properties and antimicrobial susceptibility of *Trueperella pyogenes* isolated from bovine mastitis in China. *Acta Veterinaria Hungarica* 64 1-12
- Amer S, Gálvez FLA, Fukuda Y, Tada C, Jimenez IL, Valle WFM & Nakai Y 2018 Prevalence and etiology of mastitis in dairy cattle in El Oro Province, Ecuador. *The Journal of Veterinary Medical Science* 80 861-868
- Barkema HW, Schukken YH, Lam TJGM, Beiboer ML, Wilmink H, Benedictus G & Brand A 1998 Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. *Journal of Dairy Science* 81 411-419
- Bartlett PC, Agger JF, Houe H & Lawson LG 2001 Incidence of clinical mastitis in Danish dairy cattle and screening for non-reporting in a passively collected national surveillance system. *Preventive Veterinary Medicine* 48 73-83
- Bhat AM, Soodan JS, Singh R, Dhobi IA, Hussain T, Dar MY & Mir M 2017 Incidence of bovine clinical mastitis in Jammu region and antibiogram of isolated pathogens. *Veterinary World* 10 984-989
- Bradley AJ, Breen JE, Payne B, White V & Green MJ 2015 An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. *Journal of Dairy Science* 98 1706-1720
- Dieser SA, Vissio C, Lasagno MC, Bogni CI, Larriestra AJ & Odierno LM 2014 Prevalence of pathogens causing subclinical mastitis in Argentinean dairy herds. *Pakistan Veterinary Journal* 34 124-126
- Fadlelmula A, Al Dughaym AM, Mohamed GE, Al Deib MK & Al Zubaidy AJ 2009 Bovine mastitis: Epidemiological, clinical and etiological study in a Saudi Arabian large dairy farm. *Bulgarian Journal of Veterinary Medicine* 12 199-206
- Gao J, Barkema HW, Zhang L, Liu G, Deng Z, Cai L, Shan R, Zhang S, Zou J, Kastelic JP & Han B 2017 Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. *Journal of Dairy Science* 100 4797-806
- Giannechini R, Concha C, Rivero R, Delucci I & Moreno López J 2002 Occurrence of clinical and sub-clinical mastitis in dairy herds in the West Littoral region in Uruguay. *Acta Veterinaria Scandinavica* 43 221-230

- Jagielski T, Krukowski H, Bochniarz M, Piech T, Roeske K, Bakula Z, Wlazlo L & Woch P 2019a Prevalence of *Prototheca spp.* on dairy farms in Poland – a cross-country study. *Microbial Biotechnology* 12 556-566
- Kivaria FM, Noordhuizen JPTM & Msami HM 2007 Risk factors associated with the incidence rate of clinical mastitis in smallholder dairy cows in the Dar es Salaam region of Tanzania. *The Veterinary Journal* 173 623-629
- Lafi SQ, Al-Rawashdeh OF, Ereifej KI & Hailat NQ 1994 Incidence of clinical mastitis and prevalence of subclinical udder infections in Jordanian dairy cattle. *Preventive Veterinary Medicine* 18 89-98
- Lakew BT, Fayera T & Ali YM 2019 Risk factors for bovine mastitis with the isolation and identification of *Streptococcus agalactiae* from farms in and around Haramaya district, eastern Ethiopia. *Tropical Animal Health and Production* 51 1507-1513
- Levison LJ, Miller-Cushon EK, Tucker AL, Bergeron R, Leslie KE, Barkema HW & DeVries TJ 2016 Incidence rate of pathogen-specific clinical mastitis on conventional and organic Canadian dairy farms. *Journal of Dairy Science* 99 1341-1350
- Moosavi M, Mirzaei A, Ghavami M & Tamadon A 2014 Relationship between season, lactation number and incidence of clinical mastitis in different stages of lactation in a Holstein dairy farm. *Veterinary Research Forum* 5 13-19
- Nam HM, Kim JM, Lim SK, Jang KC & Jung SC 2010 Infectious aetiologies of mastitis on Korean dairy farms during 2008. *Research in Veterinary Science* 88 372-374
- Olde Riekerink RGM, Barkema HW, Kelton DF & Scholl DT 2008 Incidence rate of clinical mastitis on Canadian dairy farms. *Journal of Dairy Science* 91 1366-1377
- Olivares-Pérez J, Kholif AE, Rojas-Hernández S, Elghandour MMY, Mohamed Salem AZ, Bastida AZ, Velázquez-Reynoso D, Cipriano-Salazar M, Camacho-Díaz LM, Alonso-Fresán MU & DiLorenzo N 2015 Prevalence of bovine subclinical mastitis, its etiology and diagnosis of antibiotic resistance of dairy farms in four municipalities of a tropical region of Mexico. *Tropical Animal Health and Production* 47 1497-1504

- Østeras O, Solbu H, Refsdal AO, Roalkvam T, Filseth O & Minsaas A 2007 Results and evaluation of thirty years of health recordings in the Norwegian dairy cattle population. *Journal of Dairy Science* 90 4483-4497
- Pérez-Cabal MA, Yaici S & Alenda R 2008 Clinical mastitis in Spanish dairy cows: incidence and costs. *Spanish Journal of Agricultural Research* 6 615-622
- Petrovski KR, Heuer C, Parkinson TJ & Williamson NB 2009 The incidence and aetiology of clinical bovine mastitis on 14 farms in Northland, New Zealand. *New Zealand Veterinary Journal* 57 109-115
- Rowbotham RF & Ruegg PL 2016 Associations of selected bedding types with incidence rates of subclinical and clinical mastitis in primiparous Holstein dairy cows. *Journal of Dairy Science* 99 4707-4717
- Rowe SM, Tranter WP & Laven RA 2018 Effect of pre-milking teat disinfection on clinical mastitis incidence in a dairy herd in Northern Queensland, Australia. *Australian Veterinary Journal* 96 69-75
- Santman-Berends IMG, Lam TJGM, Keurentjes J & van Schaik G 2015 An estimation of the clinical mastitis incidence per 100 cows per year based on routinely collected herd data. *Journal of Dairy Science* 98 6965-6977
- Sato K, Bartlett PC, Alban L, Agger JF & Houe H 2008 Managerial and environmental determinants of clinical mastitis in Danish dairy herds. *Acta Veterinaria Scandinavica* 50 4
- Shittu A, Abdullahi J, Jibril A, Mohammed AA & Fasina FO 2012 Sub-clinical mastitis and associated risk factors on lactating cows in the Savannah region of Nigeria. *BMC Veterinary Research* 8 134
- Shpigel NY, Winkler M, Ziv G & Saran A 1998 Clinical, bacteriological and epidemiological aspects of clinical mastitis in Israeli dairy herds. *Preventive Veterinary Medicine* 35 1-9
- Tenhagen BA, Koster G, Wallmann J & Heuwieser W 2006 Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. *Journal of Dairy Science* 89 2542-2551

- Thompson-Crispi KA, Miglior F & Mallard BA 2013 Incidence rates of clinical mastitis among Canadian Holsteins classified as high, average, or low immune responders. *Clinical and Vaccine Immunology* 20 106-112
- Tomazi T, Ferreira GC, Orsi AM, Gonçalves JL, Ospina PA, Nydam DV, Moronib P & dos Santosa MV 2018 Association of herd-level risk factors and incidence rate of clinical mastitis in 20 Brazilian dairy herds. *Preventive Veterinary Medicine* 161 9-18
- Verbeke J, Piepers S, Supré K & Vlieghe SD 2014 Pathogen-specific incidence rate of clinical mastitis in Flemish dairy herds, severity, and association with herd hygiene. *Journal of Dairy Science* 97 6926-6934
- Vitali A, Bernabucci U, Nardone A & Lacetera N 2016 Effect of season, month and temperature humidity index on the occurrence of clinical mastitis in dairy heifers. *Advances in Animal Biosciences* 7 250-252
- Waller KP, Bengtsson B, Lindberg A, Nymanb A & Unnerstad HE 2009 Incidence of mastitis and bacterial findings at clinical mastitis in Swedish primiparous cows- Influence of breed and stage of lactation. *Veterinary Microbiology* 134 89-94
- Wolfova M, Stipkova M & Wolf J 2006 Incidence and economics of clinical mastitis in five Holstein herds in the Czech Republic. *Preventive Veterinary Medicine* 77 48-64