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Bovine mastitis: Risk factors and isolation of Gram-negative bacteria in Western Algeria

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Electronic Supplementary Material (ESM)

Table S1. Descriptive statistics and univariate logistic regression analyses of the association of mastitis and Gram negative bacilli with different risk factors

Table S1. Descriptive statistics and univariate logistic regression analyses of the association of mastitis and Gram-negative bacilli with different risk factors

| Risk factors | Category | Total examined | CMT results | | P-value | Clinical mastitis | | P-value | Gram-negative bacilli prevalence (%) | | P-value |
|----------------------------------|---------------------|----------------|-------------------|-------------------|------------|-------------------|-------------------|--------------|--------------------------------------|-------------------|--------------|
| | | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | |
| Herd size | <27 | 54 (10.3) | 24 (44) | 30 (56) | | 7 (13) | 47 (87) | | 22 (71) | 9 (29) | |
| | 27–57 | 115 (21.9) | 50 (43) | 65 (57) | 0.566 224 | 10 (9) | 105 (91) | 0.356 050 | 29 (48) | 31 (52) | 0.007 808* |
| | >57 | 355 (67.7) | 173 (49) | 182 (51) | | 49 (14) | 306 (86) | | 154 (69) | 68 (31) | |
| Control of newly arrived animals | yes | 390 (74.4) | 181 (46) | 209 (54) | 0.823 354 | 55 (14) | 335 (86) | 0.009 155* | 170 (72) | 66 (28) | 0.112 546 2* |
| | no | 112 (21.4) | 50 (45) | 62 (55) | | 5 (4) | 107 (96) | | 33 (60) | 22 (40) | |
| Type of farming | intensive | 292 (55.7) | 139 (48) | 153 (52) | | 53 (18) | 239 (82) | | 126 (66) | 66 (34) | |
| | semi intensive | 184 (35.1) | 98 (53) | 86 (47) | 0.000 315* | 13 (7) | 171 (93) | <0.000 1* | 71 (64) | 40 (36) | 0.592 276 |
| | extensive | 48 (9.2) | 10 (21) | 38 (79) | | 0 (0) | 48 (100) | | 8 (80) | 2 (20) | |
| Dry cow therapy | no | 81 (15.5) | 42 (52) | 39 (48) | | 0 (0) | 81 (100) | | 34 (81) | 8 (19) | |
| | yes, systematically | 240 (45.8) | 103 (43) | 137 (57) | 0.199 316* | 31 (13) | 209 (87) | 0.000 394* | 93 (69) | 41 (31) | 0.007 488* |
| Construction materials | yes, targeted | 203 (38.7) | 102 (50) | 101 (50) | | 35 (17) | 168 (83) | | 178 (57) | 59 (43) | |
| | breeze block | 468 (89.3) | 229 (49) | 239 (51) | | 61 (13) | 407 (87) | | 185 (64) | 105 (36) | |
| | brick | 30 (5.7) | 13 (43) | 17 (57) | 0.011 650* | 5 (17) | 25 (83) | 0.117 640 4* | 16 (89) | 2 (11) | 0.592 276 |
| Stable hygiene | others | 26 (5) | 5 (19) | 21 (81) | | 0 (0) | 26 (100) | | 4 (80) | 1 (20) | |
| | clean | 198 (37.8) | 87 (44) | 111 (56) | | 16 (8) | 182 (92) | | 78 (76) | 25 (24) | |
| | middle | 231 (44.1) | 114 (49) | 117 (51) | 0.514 379 | 35 (15) | 196 (85) | 0.051 896* | 84 (56) | 65 (44) | 0.004 233* |
| Cleaning solution | poor | 95 (18.1) | 46 (48) | 49 (52) | | 15 (16) | 80 (84) | | 43 (70) | 18 (30) | |
| | biocide | 337 (64.3) | 156 (46) | 181 (54) | | 54 (16) | 283 (84) | | 149 (71) | 61 (29) | |
| Litter | soap | 11 (2.1) | 1 (9) | 10 (91) | 0.022 186* | 0 (0) | 11 (100) | 0.004 883* | 0 (0) | 1 (100) | 0.004 012* |
| | water | 176 (33.6) | 90 (51) | 86 (49) | | 12 (7) | 164 (93) | | 56 (55) | 46 (45) | |
| Boot bath | yes | 355 (67.7) | 159 (45) | 196 (55) | 0.142 271* | 52 (15) | 303 (85) | 0.055 945* | 125 (59) | 86 (41) | 0.001 279* |
| | no | 169 (32.3) | 88 (52) | 81 (48) | | 14 (8) | 155 (92) | | 80 (78) | 22 (22) | |
| Boot bath | yes | 34 (6.5) | 15 (44) | 19 (56) | 0.851 556 | 5 (15) | 29 (85) | 0.907 425 | 17 (85) | 3 (15) | 0.098 247* |
| | no | 490 (93.5) | 232 (47) | 258 (53) | | 61 (12) | 429 (88) | | 188 (64) | 105 (36) | |

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Table S1 to be continued

| Risk factors | Category | Total examined | CMT results | | P-value | Clinical mastitis | | P-value | Gram-negative bacilli prevalence (%) | | P-value |
|--------------------------------|---------------------------|----------------|-------------------|-------------------|------------|-------------------|-------------------|------------|--------------------------------------|-------------------|--------------|
| | | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | |
| Type of litter | straw | 324 (61.8) | 153 (47) | 171 (53) | 0.003 598* | 52 (15) | 303 (85) | 0.004 44* | 122 (60) | 83 (40) | 0.001 279* |
| | sawdust | 31 (5.9) | 6 (19) | 25 (81) | | 14 (8) | 155 (92) | | 3 (50) | | |
| Litter renewal | no | 175 (33.4) | 87 (50) | 88 (50) | 0.567 760 | 16 (9) | 159 (91) | 0.000 749* | 79 (77) | 24 (23) | 0.001 200* |
| | everyday | 205 (39.1) | 97 (47) | 108 (53) | | 19 (9) | 186 (91) | | 54 (47) | | |
| | every week | 144 (27.5) | 63 (44) | 81 (56) | 0.999 999 | 31 (22) | 113 (78) | 0.005 568* | 64 (68) | 30 (32) | 0.719 108 |
| | manual | 16 (3.1) | 8 (50) | 8 (50) | | 0 (0) | 16 (100) | | 2 (25) | | |
| Milking method | mechanical | 508 (96.9) | 239 (47) | 269 (53) | 0.794 778 | 66 (13) | 442 (87) | 0.191 514* | 199 (65) | 106 (35) | 0.887 589 |
| | yes | 208 (39.7) | 100 (48) | 108 (52) | | 37 (18) | 171 (82) | | 39 (28) | | |
| Milking clothing | no | 316 (60.3) | 147 (47) | 169 (53) | 0.949 014 | 29 (9) | 287 (91) | 0.572 525 | 107 (61) | 69 (39) | 0.108 064* |
| | yes | 445 (84.9) | 209 (47) | 236 (53) | | 52 (12) | 393 (88) | | 91 (35) | | |
| Presence of milking parlour | no | 79 (15.1) | 38 (48) | 41 (52) | 0.087 535* | 14 (18) | 65 (82) | 0.852 302 | 35 (67) | 17 (33) | 0.008 646* |
| | yes | 337 (64.3) | 149 (44) | 188 (56) | | 21 (11) | 166 (89) | | 74 (38) | | |
| Hand prewashing | no | 187 (35.7) | 98 (52) | 89 (48) | 0.984 092 | 45 (13) | 292 (87) | 0.619 664 | 85 (71) | 34 (29) | 0.036 033 0* |
| | yes | 33 (6.3) | 15 (45) | 18 (55) | | 5 (15) | 28 (85) | | 1 (5) | | |
| Using milking gloves | no | 491 (93.7) | 232 (47) | 259 (53) | 0.434 883 | 61 (12) | 430 (88) | 0.408 876 | 186 (63) | 107 (37) | 0.125 112* |
| | yes | 451 (86.1) | 209 (46) | 242 (54) | | 55 (12) | 396 (88) | | 98 (37) | | |
| Cleaning equipment | no | 73 (13.9) | 38 (52) | 35 (48) | 0.209 029* | 11 (15) | 62 (85) | 0.209 029* | 39 (80) | 10 (20) | 0.701 574 |
| | yes | 313 (59.7) | 140 (45) | 173 (55) | | 43 (14) | 270 (86) | | 70 (38) | | |
| Teat probing | no | 211 (40.3) | 107 (50) | 107 (50) | 0.209 029* | 23 (11) | 188 (89) | 0.408 876 | 92 (71) | 38 (29) | 0.125 112* |
| | systematically | 467 (90.8) | 224 (47) | 252 (53) | | 62 (13) | 414 (87) | | 100 (35) | | |
| Washing udder prior to milking | sometimes when it's dirty | 42 (8) | 21 (50) | 21 (50) | 0.209 029* | 4 (10) | 38 (90) | 0.837 962 | 18 (72) | 7 (28) | 0.701 574 |
| | no | 6 (1.1) | 2 (33) | 4 (67) | | 0 (0) | 6 (100) | | 1 (50) | | |

4 Table S1 to be continued

| Risk factors | Category | Total examined | CMT results | | Clinical mastitis | | Gram-negative bacilli prevalence (%) | | P-value |
|---------------------------------|-----------------------|----------------|-------------------|-------------------|-------------------|-------------------|--------------------------------------|-------------------|-------------|
| | | | positive n (%) | negative n (%) | positive n (%) | negative n (%) | positive n (%) | negative n (%) | |
| Drying of udder after washing | collective wipping | 162 (30.9) | 80 (49.4) | 82 (50.6) | 32 (23) | 110 (77) | 61 (59) | 42 (41) | 0.131 510* |
| | without wipping | 362 (69.1) | 167 (46.1) | 195 (53.9) | 34 (9) | 348 (91) | 144 (69) | 66 (31) | |
| Dropping of milk-ing clusters | individual wipping | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.542 392 |
| | yes | 92 (17.6) | 33 (36) | 59 (64) | 20 (22) | 72 (78) | 37 (70) | 16 (30) | |
| Visitors contact-ing cattle | no | 416 (79.4) | 206 (50) | 210 (50) | 46 (11) | 370 (89) | 162 (64) | 90 (36) | 0.357 059 0 |
| | yes | 58 (11.1) | 37 (64) | 21 (36) | 10 (17) | 48 (83) | 24 (51) | 23 (49) | |
| Elimination of the first jets | no | 466 (88.9) | 210 (45) | 256 (55) | 56 (12) | 410 (88) | 181 (68) | 85 (32) | 0.010 615* |
| | yes, under the animal | 294 (56.1) | 129 (44) | 165 (56) | 39 (13) | 255 (87) | 99 (59) | 69 (41) | |
| Frequency of veterinary visits | no | 230 (43.9) | 118 (51) | 112 (49) | 27 (12) | 203 (88) | 106 (73) | 39 (27) | 0.009 704* |
| | yes, in the recipient | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| Veterinary hygiene | frequent | 227 (43.3) | 113 (50) | 114 (50) | 18 (8) | 209 (92) | 83 (63) | 48 (37) | 0.234 607* |
| | very frequent | 146 (27.6) | 61 (42) | 85 (58) | 33 (23) | 113 (77) | 58 (62) | 36 (38) | |
| Separation of sick cows | rarely | 151 (28.8) | 73 (48) | 78 (52) | 15 (10) | 136 (90) | 64 (73) | 24 (27) | 0.171 917* |
| | yes | 113 (21.6) | 45 (40) | 68 (60) | 19 (17) | 94 (83) | 36 (56) | 28 (44) | |
| History of abortion in the herd | no | 411 (78.4) | 202 (49) | 209 (51) | 47 (11) | 364 (89) | 169 (68) | 80 (32) | 0.006 503* |
| | yes | 321 (61.3) | 152 (47) | 169 (53) | 51 (16) | 270 (84) | 133 (66) | 70 (34) | |
| Abortion of mastitic cows | no | 203 (38.7) | 95 (47) | 108 (53) | 15 (7) | 188 (93) | 72 (65) | 38 (35) | 1 |
| | yes | 286 (54.6) | 136 (48) | 150 (52) | 50 (17) | 236 (83) | 107 (58) | 79 (42) | |
| Abortion of mastitic cows | no | 238 (45.4) | 111 (47) | 127 (53) | 16 (7) | 222 (93) | 98 (77) | 29 (23) | 0.000 524* |
| | yes | 113 (21.6) | 56 (50) | 57 (50) | 20 (18) | 93 (82) | 44 (58) | 32 (42) | |
| Abortion of mastitic cows | no | 411 (78.4) | 191 (46) | 220 (54) | 22 (7) | 274 (93) | 161 (68) | 76 (32) | 0.143 433* |
| | yes | 113 (21.6) | 56 (50) | 57 (50) | 20 (18) | 93 (82) | 44 (58) | 32 (42) | |

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|------------------------------------|----------------|----------------|-------------------|-------------------|------------|-------------------|-------------------|--------------|--------------------------------------|-------------------|------------|
| | | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | |
| Veterinary consultation | yes | 228 (43.5) | 101 (44) | 127 (56) | 0.291 696 | 44 (19) | 184 (81) | <0.000 1* | 86 (59) | 59 (41) | 0.043 469* |
| | no | 296 (56.5) | 146 (49) | 150 (51) | | 22 (7) | 274 (93) | | 119 (71) | 49 (29) | |
| History of infertility in the herd | yes | 240 (45.8) | 102 (42) | 138 (58) | 0.061 887* | 38 (16) | 202 (84) | 0.054 679* | 75 (54) | 65 (46) | 0.000 107* |
| | no | 284 (54.2) | 145 (51) | 139 (49) | | 28 (10) | 256 (90) | | 130 (75) | 43 (25) | |
| Tick infestation | yes | 245 (46.8) | 111 (45) | 134 (55) | 0.484 397 | 47 (19) | 198 (81) | <0.000 1* | 94 (59) | 64 (41) | 0.032 669* |
| | no | 279 (53.2) | 136 (49) | 143 (51) | | 19 (7) | 260 (93) | | 111 (72) | 44 (28) | |
| Season | summer | 385 (73.5) | 174 (45) | 211 (55) | 0.166 525* | 43 (11) | 342 (89) | 0.136 517 8* | 156 (72) | 61 (28) | 0.000 563* |
| | winter | 139 (26.5) | 73 (53) | 66 (47) | | 23 (17) | 116 (83) | | 49 (51) | 47 (49) | |
| Province | Chlef | 109 (20.3) | 45 (41.3) | 64 (58.7) | 0.093* | 26 (23.9) | 83 (76.1) | 0.000* | 56 (78.9) | 15 (21.1) | 0.000* |
| | Ain defla | 83 (15.8) | 49 (59) | 34 (41) | | 16 (19.3) | 67 (80.7) | | 28 (43.1) | 37 (56.9) | |
| | Sidi bel abbes | 196 (37.4) | 89 (45.4) | 107 (54.6) | | 6 (3.1) | 190 (96.9) | | 68 (71.6) | 27 (28.4) | |
| | Relizane | 136 (26) | 64 (47.1) | 72 (52.9) | | 18 (13.2) | 118 (86.8) | | 53 (64.6) | 29 (35.4) | |
| Cow breed | Holstein | 360 (68.7) | 162 (45) | 198 (55) | 0.071 011* | 52 (14) | 308 (86) | 0.167 348* | 141 (66) | 73 (34) | 0.958 359 |
| | Montbeliarde | 130 (24.8) | 72 (55) | 58 (45) | | 11 (9) | 119 (92) | | 54 (65) | 29 (35) | |
| Age (year) | others | 34 (6.5) | 13 (38) | 21 (62) | 0.013 347* | 3 (9) | 31 (91) | 0.167 348* | 10 (62) | 6 (38) | 0.958 359 |
| | 2–5 | 374 (71.4) | 182 (49) | 192 (51) | | 47 (13) | 327 (87) | | 151 (66) | 78 (34) | |
| | 6–9 | 145 (27.7) | 60 (41) | 85 (59) | | 19 (13) | 126 (87) | | 52 (66) | 27 (34) | |
| | >9 | 5 (1) | 5 (100) | 0 (0) | | 0 (0) | 5 (100) | | 2 (40) | 3 (60) | |
| Parity | 1 | 148 (28.2) | 53 (36) | 95 (64) | 0.008 027* | 25 (17) | 123 (83) | 0.049 108* | 59 (76) | 19 (24) | 0.088 096* |
| | 2 | 171 (32.6) | 92 (54) | 79 (46) | | 12 (7) | 159 (93) | | 63 (61) | 41 (39) | |
| | 3 | 97 (18.5) | 51 (53) | 46 (47) | | 13 (13) | 84 (87) | | 44 (69) | 20 (31) | |
| | ≥4 | 108 (20.6) | 51 (47) | 57 (53) | | 16 (15) | 92 (85) | | 39 (58) | 28 (42) | |
| Stage of lactation | 1 month | 104 (19.8) | 50 (48) | 54 (52) | 0.939 405 | 10 (10) | 94 (90) | 0.578 914 | 45 (75) | 15 (25) | 0.176 287* |
| | 2–4 months | 212 (40.5) | 98 (46) | 114 (54) | | 29 (14) | 183 (86) | | 83 (65) | 44 (35) | |
| | ≥5 months | 208 (39.7) | 99 (48) | 109 (52) | | 27 (13) | 181 (87) | | 77 (61) | 49 (39) | |

Table S1 to be continued

| Risk factors | Category | Total examined | CMT results | | P-value | Clinical mastitis | | P-value | Gram-negative bacilli prevalence (%) | | P-value |
|------------------------------|--|----------------|-------------------|-------------------|-------------|-------------------|-------------------|------------|--------------------------------------|-------------------|------------|
| | | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | |
| Milk production/day | <5 l | 14 (2.7) | 2 (14) | 12 (86) | | 0 (0) | 14 (100) | | 0 (0) | 2 (100) | |
| | 5–10 l | 467 (89.1) | 222 (49) | 243 (51) | 0.044 112* | 4 (9) | 39 (91) | 0.345 677 | 186 (65) | 100 (35) | 0.087 504* |
| | >10 l | 43 (8.2) | 21 (48) | 22 (52) | | 62 (13) | 405 (87) | | 19 (76) | 6 (24) | |
| Udder conformation defects | present | 284 (54.2) | 136 (48) | 148 (52) | 0.774 674 | 44 (15) | 240 (85) | 0.041 107* | 113 (63) | 67 (37) | 0.290 854 |
| | absent | 240 (45.2) | 129 (54) | 111 (46) | | 22 (9) | 218 (91) | | 92 (69) | 41 (31) | |
| Height and balance | normal | 432 (82.4) | 197 (46) | 235 (54) | 0.293 230 8 | 47 (11) | 385 (89) | 0.010 193* | 169 (69) | 75 (31) | 0.002 884* |
| | udder too low | 14 (2.7) | 7 (50) | 7 (50) | | 5 (36) | 9 (64) | | 3 (25) | 9 (75) | |
| | udder badly balanced | 78 (14.9) | 43 (55) | 35 (45) | | 14 (18) | 64 (82) | | 33 (58) | 24 (42) | |
| Quality of udder attachments | yes | 517 (98.7) | 243 (47) | 274 (53) | 0.878 597 | 64 (12) | 453 (88) | 0.216 540* | 201 (65) | 106 (35) | 0.002 884* |
| | no | 7 (1.3) | 4 (57) | 3 (43) | | 2 (29) | 5 (71) | | 4 (67) | 2 (33) | |
| Teats position | teats not aligned | 133 (25.4) | 59 (44) | 74 (56) | 0.059 995* | 21 (16) | 112 (84) | 0.423 157 | 48 (60) | 32 (40) | 0.475 000 |
| | weak suspensory ligament | 64 (12.2) | 39 (61) | 25 (39) | | 8 (12) | 56 (88) | | 31 (66) | 16 (34) | |
| | normal | 327 (62.4) | 149 (46) | 178 (54) | | 37 (11) | 290 (89) | | 126 (68) | 60 (32) | |
| Teat size | unequal | 101 (19.3) | 45 (45) | 56 (55) | 0.618 019 | 17 (17) | 84 (83) | 0.047 192* | 39 (63) | 23 (37) | 0.241 632* |
| | too short, too long, too thin or too thick | 55 (10.5) | 29 (53) | 26 (47) | | 11 (20) | 44 (80) | | 22 (55) | 18 (45) | |
| Teat shape | normal | 368 (70.2) | 173 (47) | 195 (53) | 0.618 019 | 38 (10) | 330 (90) | 0.478 602 | 144 (68) | 67 (32) | 0.241 632 |
| | conical | 7 (1.3) | 4 (57) | 3 (43) | | 2 (29) | 5 (71) | | 5 (83) | 1 (17) | |
| | cylindrical | 3 (0.6) | 2 (67) | 1 (33) | | 0 (0) | 3 (100) | | 2 (100) | 0 (0) | |
| Udder injuries | normal | 514 (98.1) | 241 (47) | 273 (53) | 0.101 183* | 64 (12) | 450 (88) | 0.000 891* | 198 (65) | 107 (35) | 0.719 108 |
| | yes | 11 (2.1) | 2 (18) | 9 (82) | | 6 (55) | 5 (45) | | 6 (75) | 2 (25) | |
| | no | 513 (97.9) | 245 (48) | 268 (52) | | 60 (12) | 453 (88) | | 199 (65) | 106 (35) | |

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|--------------------------------|----------------|-------------------------|----------------------|-------------------|------------|----------------------------|-------------------|------------|---|-------------------|------------|
| | | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | | positive n (%) | negative n (%) | |
| Udder and leg hygiene score | 1 | 151 (28.8) | 71 (47) | 80 (53) | | 5 (3) | 146 (97) | | 54 (71) | 22 (29) | |
| | 2 | 185 (35.3) | 95 (52) | 90 (48) | 0.260 386 | 14 (8) | 171 (92) | <0.000 1* | 70 (64) | 39 (36) | 0.672 039 |
| | 3 | 160 (30.5) | 66 (41) | 94 (59) | | 38 (24) | 122 (76) | | 65 (62) | 39 (38) | |
| | 4 | 28 (5.3) | 15 (54) | 13 (46) | | 9 (32) | 19 (68) | | 16 (67) | 8 (33) | |
| History of mastitis | yes | 108 (20.6) | 61 (56) | 47 (44) | | 22 (20) | 86 (80) | 0.010 159* | 45 (54) | 38 (46) | 0.016 997* |
| | no | 416 (79.4) | 186 (45) | 230 (55) | 0.037 976* | 44 (11) | 372 (89) | | 160 (70) | 70 (30) | |
| History of infertility | yes | 61 (11.6) | 22 (36) | 39 (64) | 0.087 920* | 9 (15) | 52 (85) | | 18 (58) | 13 (42) | 0.472 841 |
| | no | 463 (88.4) | 225 (49) | 238 (51) | | 57 (12) | 406 (88) | 0.737 389 | 187 (66) | 95 (34) | |
| History of abortion | yes | 11 (2.1) | 3 (27) | 8 (73) | 0.303 627 | 2 (18) | 9 (82) | 0.637 030 | 4 (80) | 1 (20) | 0.472 841 |
| | no | 513 (97.9) | 244 (48) | 269 (52) | | 64 (12) | 449 (88) | | 201 (65) | 107 (35) | |
| Cow acquisition breeding stock | buying | 406 (77.5) | 184 (45) | 222 (55) | 0.149 590* | 54 (13) | 352 (87) | 0.456 457 | 164 (69) | 74 (31) | 0.033 756* |
| | breeding stock | 118 (22.5) | 63 (53) | 55 (47) | | 12 (10) | 106 (90) | | 41 (55) | 34 (45) | |

*P-value of variable effect