Use of bulk milk samples to detect infectious diseases of dairy cattle: Protocol for a scoping review

Diego Borin Nobrega¹, Julie French¹, and David Kelton¹
Department of Population Medicine, Ontario Veterinary College, University of Guelph

SUMMARY

Background: In spite of the growing interest in the use of aggregate samples such as bulk tank milk to detect endemic and emerging diseases of dairy cattle, there is no synthesis of the literature reporting in the testing of bulk milk as well as characteristics of tests employed. The availability of this information can be used to inform the development of surveillance programs of endemic and emerging diseases of interest.

Objectives: The objectives of this scoping review are to gather the best current information about milk-based screening or diagnostic tests of infectious diseases of dairy cattle that have been used on bulk milk samples, including detailed information about whether tests have been validated and the performance characteristics of those tests.

Design: Original studies reporting on characteristics of screening or diagnostic tests of infectious diseases of dairy cattle based on farm-level bulk milk samples will be eligible for inclusion. The review will be reported following the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (Tricco et al., 2018). Tables will be used to describe studies characteristics and to summarize results from studies.
INTRODUCTION

Disease surveillance is a critical element of an optimal functioning infectious diseases control program (Jones et al., 2008). It allows for the rapid identification of emerging and endemic diseases, supporting industry and government programs that enable the production of safe, wholesome food products and the growth of domestic and international trade. Surveillance enables contact tracing, an important tool that supports the rapid detection and mitigation of disease outbreaks.

Screening and diagnostic tests are essential to any surveillance program. Their primary purpose is to classify animals or herds according to disease status, either by identifying individuals or herds who are most likely to be disease-positive, or by confirming whether the disease is present. In dairy cattle, there is a growing interest in the testing bulk milk samples (a single sample of milk representing all cows milked on that day[s]) as part of surveillance of infectious diseases (enzootic bovine leucosis, bovine viral diarrhea, infectious bovine rhinotracheitis, coxiellosis, Johne’s disease, contagious mastitis, mycoplasmosis, neosporosis, salmonelosis). Part of the reason lies in lowering costs associated with surveillance programs; testing of bulk milk samples allows for an affordable and rapid screening of pathogens causing infectious diseases in a large population. As such, there is a growing body of literature describing characteristics of ELISA and PCR testing applied to bulk milk samples (Bartels et al., 2007, van Weering et al., 2007, Nielsen and Toft, 2014, Bauer et al., 2015, Nielsen et al., 2015, Booth and Brownlie, 2016, Nekouei et al., 2016, Pesqueira et al., 2017, Soltau et al., 2017, Tignon et al., 2017).

Despite the recent interest in the testing of bulk milk to detect infectious diseases of dairy cattle, we currently lack a comprehensive list of diseases that were tracked using bulk milk as well as characteristics of individual tests that were employed (e.g. sensitivity and specificity). The availability of such information can, in turn, be used to inform the development of surveillance initiatives that will integrate a multifaceted program aimed to mitigate the impacts of endemic and emerging infectious diseases that continue to plague the dairy industry worldwide.

OBJECTIVES

The aim of this scoping review is to gather the best current information about milk-based screening or diagnostic tests of infectious diseases of dairy cattle that have been used on bulk milk samples. A list of infectious diseases and disease-associated pathogens of dairy cattle that was tracked using bulk milk will be built, including detailed information about whether tests have been validated for use in bulk milk samples, and the performance characteristics of those tests (diagnostic sensitivity and specificity) based on peer-reviewed literature. This work will identify important knowledge gaps and serve as a reference for future development of screening tests based on bulk milk.

METHODS

Protocol and Registration
This protocol will be archived in the Atrium institutional repository at the University of Guelph (https://atrium.lib.uoguelph.ca/xmlui/handle/10214/10046). The protocol was drafted according
to the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (Tricco et al., 2018). Any deviation from the protocol will be reported in the final review.

**Eligibility Criteria**

Original studies (any design) reporting on the use or characteristics of screening or diagnostic tests of infectious diseases of dairy cattle based on individual, farm-level bulk milk samples will be eligible for inclusion. In addition, though the terms to be used in the search strategy will be in English, articles in English, Portuguese or Spanish will be eligible for inclusion. Articles must have been published in the last 35 years. This will ensure that our scoping review captures protocols and tests that have been optimized over the years.

Testing of individual, farm-level, unprocessed bulk milk samples including ones collected for regulatory and payment purposes, will be eligible for inclusion if samples were obtained prior to any comingling (e.g. collected directly from bulk tanks), regardless of sample provider (e.g. dairy cooperative, farm, milk haulers, milk processors). Where information regarding the comingling status of samples is unclear, it will be assumed that samples were collected directly from the bulk tanks on farms, prior to loading on tankers or to any comingling. Samples collected at wholesale, retail outlets, from milk silos at collection centres or dairy processing plants, as well as commingled milk samples will not be considered. Additionally, analysis of milk filters will not be eligible. Testing of bulk milk for non-specific markers of infections (e.g. somatic cell count, lactoferrin) and typical quality indicators (e.g. total, coliform, standard plate, psychrotrophic, mesophilic, lipolytic, proteolytic, streptococcal, thermoduric or thermophilic bacterial count, presence of non-specified spores, yeasts and moulds, toxins, antibiotic residues and heavy metals) will not be considered.

Studies will eligible for inclusion if at least one of the following applies:

1) Bulk milk samples were tested for presence of infectious diseases of dairy cattle and the testing is described in the methodology. The test aimed the detection of at least one of the following:
   a) pathogens that are typically associated with infectious diseases of dairy cattle (as defined below);
   b) specific markers associated with these infections such as antibodies.

2) The study investigated, directly or indirectly, the use of bulk milk testing to determine herd-disease status for any given pathogen or disease. This investigation was carried out in one of the following ways:
   a) The diagnostic characteristics of bulk milk testing to detect herds of varying disease status (diagnostic sensitivity and / or specificity) were estimated and reported;
   b) Data to estimate the sensitivity and / or specificity of bulk milk testing to detect herds of varying disease status were available for a sufficient number of herds;
   c) Associations (correlation, regression modelling or R² values) between bulk milk and animal-level testing results (e.g. within-herd prevalence of infected animals, average animal-level testing results) were reported.
Studies that qualify for inclusion under the second criteria will be referred to as validation studies regardless of whether they also satisfy the first criteria. Remaining studies will be classified as detection studies. At any study, bulk milk samples will not necessarily be tested for presence of diseases as an original investigation in order to remain eligible. For instance, studies could use historical data from bulk milk-based surveillance programs to estimate characteristics of bulk milk testing or use bulk milk testing results as part of disease modelling. Further, studies using protocols that include a combination of different samples (e.g. bulk milk and milk filter testing) will be eligible for this category if reporting on bulk milk testing results individually. Minimum data required to estimate sensitivity and specificity at the herd-level will necessarily include at least one of the following: 1) bulk milk and animal-level testing results from same herds reported on a herd basis for at least 15 herds (defined based on a preliminary screening of the returned hits); or 2) bulk milk testing results reported on a herd basis for at least 15 herds of known disease status based on eligibility criteria. Case reports, case series or studies wherein characteristics of bulk milk testing can be estimated based on a limited number of tested herds (<15) will not be eligible for estimation. Literature eligible for this category include: studies reporting on the performance of bulk milk testing at different thresholds of infected animals within herds, studies reporting on the sensitivity and specificity of bulk milk-based diagnostic tests using latent class models, studies describing correlations between bulk milk and herd-average ELISA results, studies reporting on the validation of bulk milk testing using animal-level testing results to define herd disease status, studies estimating the within-herd prevalence of diseases, performing bulk milk tests and reporting results on a herd basis for a sufficient number of herds, and studies describing characteristics of bulk milk testing based on testing of samples from herds of known disease status.

For the purpose of this review, the following list will be considered typical infectious diseases of dairy cattle (or pathogens typically associated with diseases): anaplasmosis (Anaplasma marginale, Anaplasma centrale), babesiosis (Babesia bigemina, Babesia bovis), besnoitiosis (Besnoitia besnoiti), bluetongue (Bluetongue virus), Bovine coronavirus, bovine respiratory disease (Mannheimia haemolytica, Pasteurella multocida, Histophilus somni), Bovine respiratory syncytial virus, bovine spongiform encephalopathy, bovine viral diarrhea (Bovine viral diarrhea virus), bovine leukosis (Bovine leukemia virus), brucellosis (Brucella spp.), coxiellosis (Q Fever, Coxiella burnetii), digital dermatitis (Treponema spp.), fasciolosis (Fasciola hepatica), foot-and-mouth disease (Foot-and-mouth disease virus), haemorrhagic septicaemia (Pasteurella multocida), heartwater (Ehrlichia ruminantium), hypodermosis (Hypoderma spp.), infectious bovine rhinotracheitis (Bovine herpesvirus-1), Johne’s disease (Mycobacterium avium subspecies paratuberculosis), leptospirosis (Leptospira hardjo), lumpy skin disease (Lumpy skin disease virus), lungworm (Dictyocaulus viviparus), Mycoplasma spp., neosporosis (Neospora caninum), parainfluenza (Parainfluenza virus 3), parasitic gastrointestinal infections (Cooperia, Haemonchus, Ostertagia or Trichostrongylus spp.), Prothoteca spp., rabies (Lyssavirus), Rift Valley fever, rotaviral diarrhoea (Bovine rotavirus), rinderpest (Rinderpest morbillivirus), Staphylococcus aureus, Streptococcus agalactiae, Salmonella Dublin, Schmallenberg virus, theileriosis (Theileria annulata), toxoplasmosis (Toxoplasma gondii), trichomoniasis (Trichomonas foetus), trypanosomiasis (Trypanosoma congoense, Trypanosoma vivax, Trypanosoma brucei), tuberculosi (Mycobacterium bovis) and vesicular stomatitis (Vesicular stomatitis virus). Studies comparing 2 or more diagnostic tests based on bulk milk testing regardless of herd-disease status, bulk milk-based surveys designed to estimate herd-level
prevalence of pathogens or diseases, studies using bulk milk testing to determine or confirm the presence, level or occurrence of diseases or infections on farms, and studies reporting on the analytical sensitivity, optimization or repeatability of bulk milk-based testing protocols will be eligible for inclusion if reporting on the presence of typical infectious diseases of dairy cattle.

The rationale for introducing the two eligibility criteria for bulk milk testing is to focus on the use of bulk milk as part of active disease surveillance systems on farms, while also generating a database of tests and methods that were used to screen bulk milk samples. As such, studies reporting on the microbiota of bulk milk, classic foodborne pathogens (e.g. Listeria spp., Campylobacter spp., Salmonella Typhimurium) or on the occurrence of pathogens other than ones depicted above but still prevalent in dairy cows as cause of disease (e.g. those causing environmental mastitis such as E. coli and Klebsiella spp.), will be eligible as long as the validation of bulk milk testing in terms of its characteristics to detect diseased animals and thus define herd disease status can be accomplished. Data from such studies will necessarily allow for a clear definition of what constitutes a disease-positive herd.

Studies using spiking of bulk milk to establish limits of detection or to estimate laboratorial test characteristics (e.g. analytical sensitivity and specificity) will be considered if reporting on presence of pathogens typically associated with diseases of dairy cattle. In contrast, studies using simulated bulk milk (e.g. pooling of cow-level samples) rather than actual bulk milk samples will not be considered, as one of the overarching goals of this scoping review is to aid with the development of disease control programs based on testing of bulk milk samples that are routinely collected as part of quality assurance in countries with a modern dairy industry. Finally, studies will necessarily describe the methodology used for bulk milk testing for each eligible pathogen or disease. Microbiome and related studies (e.g. 16S rRNA metagenomic sequencing) will not be considered.

**Information Sources**
The following databases will be screened through the University of Guelph McLaughlin library: 1) Agricola (via ProQuest), 2) CAB Abstracts (via CABI interface), 3) Web of Science (all databases), 4) ProQuest dissertation and thesis, and 5) SCOPUS. In addition, the search strategy will be enhanced by screening relevant conference proceedings published in the following specialized databases: 1) International Veterinary Information Service, and Searchable Proceedings of Animal Conferences. Proceedings of other relevant conferences (ParaTB Forum, International Colloquium on Paratuberculosis) were also screened for potential studies. Grey literature will be identified by screening websites of relevant animal health agencies (e.g. OIE, USDA’s National Animal Health Monitoring System, Canadian Animal Health Surveillance System), animal disease surveillance programs (cattle-related programs listed in the European Commission National Veterinary Programmes and national-level surveillance programs identified during the abstract and full-text review processes) and the Diagnostics for Animal database, which contains information on ~90% of the global animal health diagnostic market, for peer-reviewed literature. Info sheets, webpages or handbooks of each cattle-related diagnostic kit will be inspected for potential peer-reviewed literature to be included. Testing laboratories will not be contacted to request further validation data or access to restricted content. Results from the grey literature search will be used to build a list of available tests and disease control programs that were based on the testing of bulk milk samples (English material only).
Search
Rather than building an exhaustive list of all dairy cattle infectious diseases and to potentially identify neglected diseases that have been detected using bulk milk, a broad search strategy was designed based on four main concepts: 1) bulk milk, 2) dairy cattle, 3) test, and 4) target (Table 1). The search will be conducted using keywords related to the four concepts. In order to validate our search strategy, results compiled from a CABI database search were compared to a list of references from a narrative review reporting on selected dairy cattle diseases that were tracked using bulk tank milk (Rotolo et al., 2018). In addition, it was verified whether the search strategy captured a list of 10 relevant articles pre-selected by DK (Table 2).

Results from a search string using the CABI database are represented below (Table 1).

**Table 1.** Results of a search to identify studies reporting on testing of bulk milk samples conducted in the CABI database on December 17, 2020.

<table>
<thead>
<tr>
<th>Number</th>
<th>Terms</th>
<th>Hits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>“bulk milk” OR bulk-milk OR bulk-tank* OR “bulk tank*” OR “dairy cooler*” OR “milk silos” OR “milk cooler*” OR “pooled milk” OR “milk vat*” OR “tank milk”</td>
<td>8,744</td>
</tr>
<tr>
<td>2</td>
<td>bovine OR cattle OR cow OR cows OR dair* OR herd* OR farm*</td>
<td>1,711,774</td>
</tr>
<tr>
<td>3</td>
<td>diagnos* OR screen* OR detect* OR kit* OR tool* OR assay* OR test* OR reaction* OR testkit* OR culture* OR isolate* OR analys* OR analyz* OR assess</td>
<td>6,673,049</td>
</tr>
<tr>
<td>4</td>
<td>disease* OR illness* OR infection* OR infectious OR antibod* OR virus OR viral OR viruses OR bacteria* OR bacterium OR fungi OR fungus* OR yeast* OR mold* OR isolate* OR protozoa OR pathogen* OR parasit* OR strain* OR micro-organism* OR microorganism* OR agent OR organism* OR germ OR sequence* OR gene</td>
<td>6,238,203</td>
</tr>
<tr>
<td>5</td>
<td>#1 AND #2 AND #3 AND #4</td>
<td>3,480</td>
</tr>
<tr>
<td>6</td>
<td>#4 AND yr:[1985 TO 2021])</td>
<td>2,519</td>
</tr>
<tr>
<td>#</td>
<td>Author (Year)</td>
<td>Article title</td>
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<tr>
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</tr>
<tr>
<td>1</td>
<td>Arcangioli et al. (2011)</td>
<td>Prevalence of <em>Mycoplasma bovis</em> udder infection in dairy cattle: preliminary field investigation in southeast France</td>
</tr>
<tr>
<td>2</td>
<td>Armstrong and Mathew (2001)</td>
<td>Predicting herd protection against foot-and-mouth disease by testing individual and bulk tank milk samples</td>
</tr>
<tr>
<td>3</td>
<td>Astobiza et al. (2012)</td>
<td>Estimation of <em>Coxiella burnetii</em> prevalence in dairy cattle in intensive systems by serological and molecular analyses of bulk-tank milk samples</td>
</tr>
<tr>
<td>4</td>
<td>Balmer et al. (2014)</td>
<td>Serosurveillance of Schmallenberg virus in Switzerland using bulk tank milk samples</td>
</tr>
<tr>
<td>5</td>
<td>Bartels et al. (2007)</td>
<td>Factors associated with variation in <em>Neospora caninum</em> bulk-milk S/P ratios in initially bulk-milk negative testing Dutch dairy herds</td>
</tr>
<tr>
<td>6</td>
<td>Bauer et al. (2015)</td>
<td>Estimated herd prevalence and sequence types of <em>Coxiella burnetii</em> in bulk tank milk samples from commercial dairies in Indiana</td>
</tr>
<tr>
<td>7</td>
<td>Beaver et al. (2016)</td>
<td>Implications of PCR and ELISA results on the routes of bulk-tank contamination with <em>Mycobacterium avium</em> ssp. <em>paratuberculosis</em></td>
</tr>
<tr>
<td>8</td>
<td>Booth and Brownlie (2016)</td>
<td>Comparison of bulk milk antibody and youngstock serology screens for determining herd status for Bovine Viral Diarrhoea Virus</td>
</tr>
<tr>
<td>9</td>
<td>Collins et al. (2017)</td>
<td>Schmallenberg virus: predicting within-herd seroprevalence using bulk-tank milk antibody titres and exploring individual animal antibody titres using empirical distribution functions (EDF)</td>
</tr>
<tr>
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<td>Authors</td>
<td>Title</td>
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<tr>
<td>10</td>
<td>Daly et al. (2015)</td>
<td>Comparison of Schmallenberg virus antibody levels detected in milk and serum from individual cows</td>
</tr>
<tr>
<td>11</td>
<td>Drew et al. (1999)</td>
<td>The detection of bovine viral diarrhoea virus in bulk milk samples by the use of a single-tube RT-PCR.</td>
</tr>
<tr>
<td>12</td>
<td>Foddai et al. (2015)</td>
<td>Challenges for bovine viral diarrhoea virus antibody detection in bulk milk by antibody enzyme-linked immunosorbent assays due to changes in milk production levels</td>
</tr>
<tr>
<td>13</td>
<td>Hanon et al. (2017)</td>
<td>Evaluation of 16 commercial antibody ELISAs for the detection of bovine viral diarrhea virus-specific antibodies in serum and milk using well-characterized sample panels</td>
</tr>
<tr>
<td>14</td>
<td>Haran et al. (2012)</td>
<td>Prevalence and characterization of <em>Staphylococcus aureus</em>, including methicillin-resistant <em>Staphylococcus aureus</em>, isolated from bulk tank milk from Minnesota dairy farms</td>
</tr>
<tr>
<td>16</td>
<td>Johnson et al. (2014)</td>
<td>A bulk milk tank study to detect evidence of spread of Schmallenberg virus infection in the south-west of Ireland in 2013</td>
</tr>
<tr>
<td>17</td>
<td>Justice-Allen et al. (2011)</td>
<td>Detection of multiple <em>Mycoplasma</em> species in bulk tank milk samples using real-time PCR and conventional culture and comparison of test sensitivities</td>
</tr>
<tr>
<td>18</td>
<td>Kramps et al. (1999)</td>
<td>A simple, rapid and reliable enzyme-linked immunosorbent assay for the detection of bovine virus diarrhoea virus (BVDV) specific antibodies in cattle serum, plasma and bulk milk</td>
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<tr>
<td></td>
<td>Authors</td>
<td>Study Description</td>
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<tr>
<td>19</td>
<td>Lanyon et al. (2014)</td>
<td>Milk as a diagnostic sample for a commercially available ELISA to identify bovine viral diarrhoea (BVD) antibodies in dairy herds</td>
</tr>
<tr>
<td>20</td>
<td>Muskens et al. (2011)</td>
<td>Prevalence of <em>Coxiella burnetii</em> infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA</td>
</tr>
<tr>
<td>21</td>
<td>Nekouei et al. (2016)</td>
<td>Diagnostic performance of an indirect enzyme-linked immunosorbent assay (ELISA) to detect bovine leukemia virus antibodies in bulk-tank milk samples</td>
</tr>
<tr>
<td>22</td>
<td>Nielsen and Toft (2014)</td>
<td>Bulk tank milk ELISA for detection of antibodies to <em>Mycobacterium avium</em> subsp. <em>paratuberculosis</em>: Correlation between repeated tests and within-herd antibody-prevalence</td>
</tr>
<tr>
<td>23</td>
<td>Nielsen et al. (2000)</td>
<td>Bulk-tank milk ELISA antibodies for estimating the prevalence of paratuberculosis in Danish dairy herds</td>
</tr>
<tr>
<td>24</td>
<td>Nielsen et al. (2015)</td>
<td>Latent class analysis of bulk tank milk PCR and ELISA testing for herd level diagnosis of <em>Mycoplasma bovis</em></td>
</tr>
<tr>
<td>26</td>
<td>Parker et al. (2017a)</td>
<td>Bulk tank milk antibody ELISA as a biosecurity tool for detecting dairy herds with past exposure to <em>Mycoplasma bovis</em></td>
</tr>
<tr>
<td>27</td>
<td>Parker et al. (2017b)</td>
<td>Comparison of culture and a multiplex probe PCR for identifying <em>Mycoplasma</em> species in bovine milk, semen and swab samples</td>
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<tr>
<td>28</td>
<td>Pesqueira et al. (2017)</td>
<td>Short communication: Correlation between within-herd antibody-prevalence and bulk tank milk antibody levels to <em>Mycobacterium avium</em> sspp. <em>paratuberculosis</em> using 2 commercial immunoassays</td>
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<td></td>
<td>Authors (Year)</td>
<td>Description</td>
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</tr>
<tr>
<td>29</td>
<td>Phuektes et al. (2003)</td>
<td>Multiplex polymerase chain reaction as a mastitis screening test for <em>Staphylococcus aureus</em>, <em>Streptococcus agalactiae</em>, <em>Streptococcus dysgalactiae</em> and <em>Streptococcus uberis</em> in bulk milk samples</td>
</tr>
<tr>
<td>30</td>
<td>Radwan et al. (1995)</td>
<td>Development of a PCR amplification assay as a screening test using bulk milk samples for identifying dairy herds infected with bovine viral diarrhea virus</td>
</tr>
<tr>
<td>31</td>
<td>Renshaw et al. (2000)</td>
<td>Comparison of virus isolation and reverse transcription polymerase chain reaction assay for detection of bovine viral diarrhea virus in bulk milk tank samples</td>
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<tr>
<td>32</td>
<td>Rodolakis et al. (2007)</td>
<td>Comparison of <em>Coxiella burnetii</em> shedding in milk of dairy bovine, caprine, and ovine herds</td>
</tr>
<tr>
<td>33</td>
<td>Slana et al. (2008)</td>
<td>On-farm spread of <em>Mycobacterium avium</em> subsp. <em>paratuberculosis</em> in raw milk studied by IS900 and F57 competitive real time quantitative PCR and culture examination</td>
</tr>
<tr>
<td>34</td>
<td>Soltau et al. (2017)</td>
<td>Within-herd prevalence thresholds for herd-level detection of mastitis pathogens using multiplex real-time PCR in bulk tank milk samples</td>
</tr>
<tr>
<td>35</td>
<td>Tasara et al. (2005)</td>
<td>Development and evaluation of a <em>Mycobacterium avium</em> subspecies <em>paratuberculosis</em> (MAP) specific multiplex PCR assay</td>
</tr>
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<td></td>
<td>Authors (Year)</td>
<td>Title</td>
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<tr>
<td>36</td>
<td>Tignon et al. (2017)</td>
<td>Characterization of three commercial ELISA kits for detection of BOHV-1 gE specific antibodies in serum and milk samples and applicability of bulk milk for determination of herd status</td>
</tr>
<tr>
<td>37</td>
<td>van Weering et al. (2007)</td>
<td>Diagnostic performance of the Pourquier ELISA for detection of antibodies against <em>Mycobacterium avium</em> subspecies <em>paratuberculosis</em> in individual milk and bulk milk samples of dairy herds</td>
</tr>
<tr>
<td>38</td>
<td>Wilson et al. (2010)</td>
<td>Herd-level prevalence of Johne’s disease in Utah and adjacent areas of the Intermountain West as detected by a bulk-tank milk surveillance project</td>
</tr>
<tr>
<td>39</td>
<td>Zanardi et al. (2014)</td>
<td>Short communication: comparing real-time PCR and bacteriological cultures for <em>Streptococcus agalactiae</em> and <em>Staphylococcus aureus</em> in bulk-tank milk samples</td>
</tr>
</tbody>
</table>
Data management and selection process
Results from the search strategy will be downloaded and stored in EndNote X9 (Clarivate Analytics, Philadelphia). Prior to screening, duplicate records will be flagged and excluded using EndNote’s algorithm for detecting duplicates. Two levels of screening will be used, and references will be reviewed by two independent reviewers. Prior to each stage, a preliminary assessment will be carried out to ensure consistency between reviewers. At the first screening level, reviewers will go over titles, abstracts and keywords of all literature identified. References will be reviewed independently by each reviewer, using the following set of questions:

1- Is the title/abstract in English, Spanish or Portuguese?
2- Is this title/abstract from an original study?
3- Does this study involve dairy cows and/or dairy herds?
4- Does this title/abstract report on the testing of bulk milk for presence of pathogens that cause diseases in dairy cows or specific marker(s) related to such infections (e.g. antibodies)?
5- Does this title/abstract imply that bulk milk samples were tested for presence of pathogens, agents, diseases or markers other than the following:
   a. quality indicator bacteria count such as total, coliform, standard plate, psychrotrophic, mesophilic, lactic, lipolytic, proteolytic, fecal streptococcal, thermuduric, thermophilic or similar bacteria count;
   b. non-specified spores, yeasts or moulds;
   c. toxins, antibiotic residues or heavy metals.

For questions 1-5, 3 answers will be possible: “yes”, “no”, or “unclear”. Studies receiving “no” to any question will be excluded and remaining ones will move forward to the second screening level. Conflicts will be resolved primarily by consensus. If consensus cannot be reached, conflicts will be solved by means of consultation with a third member of the team (DK).

The first reviewing stage was purposively designed to be as inclusive as possible so most articles reporting on the testing of bulk milk samples would be retained. References moving forward to the second level of screening will have their full texts retrieved. Reviewers will assess each full-text article for eligibility using a less permissive criteria. The following checklist will be used to review eligibility:

1- Is this study in English, Spanish or Portuguese?
2- Is this study original?
3- Does this study include dairy cows and/or dairy herds?
4- Do bulk milk samples used in this study represent individual farms (e.g. collected from bulk tanks on individual farms)? Artificial bulk milk samples (e.g. pooled milk from > 1 cow at the laboratory) will not be considered bulk milk samples.
5- Were bulk milk samples tested prior to any processing or comingling? Samples collected after loading on tankers, comingled and retail including farm markets milk samples are not eligible. Where this information is unclear, it will be assumed that samples were obtained prior to any processing or comingling.
6- In terms of pathogen or disease eligibility, at least one of the following applies:
   a. Bulk milk samples were tested for presence of typical diseases of dairy cattle (refer to the eligibility criteria);
b. This study reported on or provide sufficient data to estimate characteristics of
bulk milk testing to define herd disease status (*refer to the eligibility criteria*);

7- Is the methodology used for bulk milk testing eligible and described for at least one of the
pathogens or diseases screened? Examples include PCR, ELISA, qPCR and
bacteriological culture. *Microbiome and related techniques (e.g. metagenomic profiling,
16S rRNA sequencing of whole community), and non-specific testing (e.g. somatic cell
count, California Mastitis Test) are not eligible.*

For all questions, 2 answers will be possible: “yes” or “no”. Studies receiving “no” to any
question will be excluded. A preliminary assessment with 10 full texts will be carried out to
ensure reviewers are consistent in their evaluation. Discrepancies between reviewers will be
resolved by consensus or consulting with a third reviewer (DK), if conflicts persist. Reasons for
exclusions will be documented.

**Data extraction**
Data will be extracted from individual studies using an electronic form in Microsoft Excel
(Redmond, WA). From all studies, the following data will be extracted: author, year, country of
origin of bulk milk samples (or corresponding author’s country, if the former is not available),
test(s) used, test(s) manufacturer (if applicable) and eligible disease(s) or pathogen(s) screened.
Eligible pathogens or diseases are those that satisfy all of the conditions described in the
eligibility criteria. For validation studies, we will also extract when available, the following
characteristics:

- **General characteristics**: herd eligibility criteria, number of herds and samples tested,
sampling scheme;

- **Test characteristics**: test target, test cut-off value (e.g. S/P ratio, C_T value), testing
scheme, interpretation (e.g. parallel, series), sensitivity, specificity, metric of association
(R^2, correlation coefficient, rate of change);

- **Disease characteristics**: infectious disease or pathogen screened, scheme to assess
disease at the herd-level (e.g. animal-level testing), interval between bulk milk testing
and herd disease assessment, test used to define disease at the herd-level, cut-off value
used in the animal-level testing, disease definition at the herd-level.

Multiple rows per study will be allowed depending on use of > 1 test, disease or pathogen
screened. When results are reported at continuous breakpoints (e.g. ROC curves), data from
conditions yielding the highest accuracy or that have been described as most relevant will be
selected for extraction. In addition, for studies reporting test sensitivities and specificities using
distinct definitions of disease at the herd-level (e.g. herd with at least 1 positive cow, herd
containing 3 out of 100 positive cows), we will extract sensitivities and specificities discussed by
authors as most relevant. Additionally, we will extract the range of sensitivities and specificities
reported as well as factors associated with the test characteristics. Data from protocols based on
the combination of diagnostic methods that include testing of bulk milk samples (e.g. testing of
water and bulk milk samples concomitantly to define herd disease status) will not be extracted.

**Critical appraisal of individual sources of evidence**
For validation studies, the VETQUADAS checklist will be used to assess the quality. Briefly, the
VETQUADAS tool explores the risk of bias in 3 main domains: clarity in reporting, internal
validity and external validity. A study judged as having “low” on all 3 domains will be classified as “low risk of bias”. Studies judged "high" or "unclear" on one or more domains will be classified as “at risk of bias”.

**Synthesis of Results**
Summary tables will be used to describe general characteristics of studies (country of origin, study design, diseases or pathogens detected), tests (tests performed according to disease, analytical sensitivity and sensitivity) and diseases (tests available to detect diseases, range of sensitivities and specificities reported from studies, and a list of factors affecting the sensitivity and specificity of each test to detect diseases as reported from individual studies [e.g. percentage of infected animals per herd, S/P cut-off values, number of tests per bulk tank]). A combination of summary figures and tables will be used as needed. A summary table containing detailed information (test, disease, testing scheme, positive test definition, positive herd definition) about bulk milk-based surveillance programs detected will also be built.

**RESULTS**

**Selection of source of evidence**
A PRISMA flow diagram will be used to describe results from the search strategy and eligibility screening. We will outline reasons for study exclusion at each step. A search strategy string used in at least one database will be shared so that our search can be replicated.

**Characteristics of sources of evidence**
We will use summary tables will to describe primary characteristics of studies (diseases or pathogens detected, tests performed, country and test manufacturers). A list of tests that can be used in bulk milk according to manufacturer’s recommendations will be built. Additionally, we will provide a list of surveillance activities based on testing of bulk milk.

**Results of individual sources of evidence**
Study-level results will be reported for validation studies exclusively. Two tables will be used to report results of individual sources of evidence: a study-level and a disease-level table. In the former, we will report on the following characteristics: study, test, infectious disease, sensitivity and specificity (including range of values reported from individual studies) and herd disease definition.

The disease-level table will contain the following fields: disease definition, test(s) available to detect the disease, range of sensitivities and specificities reported across studies and a list of factors affecting the sensitivity and specificity of each test to detect diseases as reported from individual studies. More than 1 row per disease will be allowed if distinct disease definitions are used within and between studies. If any additional relevant information is found during the review process, the data synthesis elements will be modified to accommodate the new data accordingly.

**DISCUSSION**

**Summary of evidence**
The most up to date information about milk-based screening or diagnostic tests of infectious diseases that have been used on bulk milk samples will be gathered and comprehensively presented. The results obtained from this scoping review will inform strategies to implement disease surveillance programs based on bulk milk testing. Such systems will be used to provide timely and useful information that will ultimately guide decisions and actions in dairy herds.

**Limitations**
We believe the literature reporting on emerging pathogens or using relatively new specific molecular techniques (e.g. biosensors) to detect diseases will be scarce. Conversely, we expect a vast number of studies reporting on presence of foodborne pathogens that can cause disease in dairy cattle such as *S. aureus* in bulk milk. It is plausible to assume that our search strategy will not capture all literature reporting on presence of foodborne pathogens in bulk milk. Yet, we believe that most non-included studies reporting on presence of foodborne pathogens in bulk milk will employ detection methods described in at least one of studies included in the review. Additionally, based on a preliminary screening of returned hits, we believe that most literature reporting on presence of foodborne pathogens in bulk milk, including studies potentially missed by our search strategy, will not estimate or provide sufficient data for the estimation of the characteristics of bulk milk testing to detect herds with diseased animals. In that regard, we believe that our broad search as well as the fairly permissive eligibility criteria adopted will result in the inclusion of most peer-reviewed literature reporting on the validation of bulk milk testing to detect herds of varying disease status.

Furthermore, we believe that many commercially available kits will not be backed up by peer-reviewed literature, which will limit our ability to assess the accuracy of available tests. Additionally, internal validation reports might not be available without request. Finally, this review will not provide a list of all pathogens that can be tracked using bulk milk. Additional limitations will be discussed as necessary.

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