Critical appraisal – Randomised controlled trial questions

Introduction	
Are the aims clearly stated?	Yes. 1. To follow heifers into adulthood to describe the effect of feeding heat treated colostrum on the risk of infection for *Mycobacterium avium* spp. *paratuberculosis* (MAP) in adult cows 2. To investigate the effects of heat treated colostrum on the milk production and longevity within the herd
Methods	
Is the study design suitable for the aims?	Yes
Which population was studied?	Dairy heifers from birth to approximately 5 years old (3rd lactation) in Minnesota and Wisconsin, United States of America. It included 6 farms, which had to have had at least one MAP positive animal (based on either faecal culture or serum ELISA) within the previous 3 years and be willing to adhere to the study protocol. The farms selected were a convenience sample, based on location.
Were the treatments randomly allocated? If yes, how was the randomisation done?	Treatment assignment was done systematically by birth order
Were the groups comparable prior to intervention?	No; there were the following differences: - Five farms used oesophageal tube for colostrum administration, whilst one farm used a nipple bottle followed by an oesophageal tube if any was not consumed - Five farms gave a single feed of colostrum. One farm offered a second feed 12 hours later via a nipple bottle - Calves were housed in either individual hutches (n=3) or individual pens within a barn (n=3)

	- Five farms used group maternity pens, with one farm using individual maternity pens - Four farms housed pre-weaned calves on site, whilst the other two used off site units - Five farms fed pasteurised whole milk and one farm fed commercial milk replacer - Five farms enrolled only heifers; one farm enrolled both heifer and bull calves with bull calves leaving the study after weaning
Was the person who administered the interventions blinded?	There are no details of blinding mentioned for the administration of colostrum by the farm personnel or whether the remaining data collection was performed blinded to the colostrum feeding status.
Is it clear what measurements were carried out in the study?	Yes
Were the correct measurements chosen? Do they reflect (or are they strongly related to) the outcome of interest?	The measurements chosen were ones that are used commonly in clinical practice to determine the MAP status of animals and are strongly related to the outcome of interest.
Were previously established validated methods used to make the measurements? (e.g. Glasgow pain score, International Units etc)	Yes. Validation information and references were included for faecal culture of MAP, serum ELISA for MAP and colostrum PCR for MAP.
What outcomes were measured?	1. Colostrum: the presence of MAP via PCR 2. Adult MAP status using both serology and faecal culture on an annual basis 3. Recorded on farm data including any deaths and culling, calving and conception dates, milk production and dry off dates.
Are the outcomes clinically relevant?	Yes
Were the outcomes assessed blind?	No details given

Are the statistical methods described?	Yes
Was the statistical significance level stated?	Yes – P<0.05
Was the sample size justified?	Yes
Was ethical approval obtained?	Yes
Are the methods described in enough detail that you could repeat them?	Yes
Results	
Were the basic data adequately described?	Overall cow performance characteristics were reported but as aggregated results (e.g. mean, SD and range acorss the 6 farms). More information on an individual farm basis may have useful for looking at between herd variation. The numbers receiving each treatment on each farm, along with the number of cows tested at each time point and how many tested positive are provided. However in terms of any other measurements of outcomes are reported in terms of whether they were fed pastuerised colostrum or not.
Do the numbers add up? Are all subjects accounted for?	Whilst the results state the number of animals which were culled or sold between enrollment and the study conclusion, there are 69 animals which are not in the final testing round and were not culled or sold; there is no explanation of what happened to these animals.

Was the statistical significance (p value) stated in the results? Is this consistent with the methods? (It should be stated in the sample size or power calculation)	Yes
Were any side effects of the intervention reported if applicable?	No
What were the main findings/key results?	Heat treating colostrum showed no significant effect on the future risk of MAP status for either the serum ELISA or faecal culture or both tests combined, over a three year period. Heat treating the colostrum did increase the IgG content but had no impact on longevity within the herd and milk production over the first two lactations.
Discussion and conclusion	
What do the main findings/key results mean?	It is difficult to interpret the findings of this study because: 1. 70% of the enrolled animals (n=644 out of 924) were not present at the end of the study, resulting in a small sample size (n=280). 2. The number of animals receiving MAP-PCR positive colostrum was 15% (69 calves for fresh colostrum, 70 calves for heat treated colostrum) 3. The test sensitivities and specifities: a. PCR: sensitivity 25 to 75% depending upon MAP concentration, specificity 89% b. ELISA: 51.4% sensitivity, 99.3% specificity 4. The feeding of whole pasteurised milk of unknown MAP status on five of the farms complicates whether heat treatment prevents the transmission of MAP from colostrum

	Therefore the lack of significance found between feeding heat treated or fresh colostrum may reflect inadequate power in the final study or the use of tests with poor sensitivity for detecting MAP. It is difficult to interpret whether the PCR MAP positive colostrum was truly positive due to the poor sensitivity of the PCR at low infectious doses (25% for 10^1 to 10^3 cells/ml); therefore the prevalence of MAP positive colostrum fed to the calves is unknown. It could be possible that heat treating the colostrum has no effect on future MAP status, due to the multiple other possible methods of transmission of MAP to the calf, for example faeco-oral transmission and in utero transmission. Without controlling for these other possible transmission factors, it is not possible to understand whether heat treating colostrum impacts on the future MAP status of a heifer.
Are the negative findings discussed? How are the negative findings interpreted?	The authors discussed the negative findings but they have not necessarily considered aspects of the study design may have impacted on the results for example the small number of animals per group and the lack of uniformity between the groups
Does the discussion reflect the results?	Yes
Interpretation	
What are the clinical implications of this study? Are the subjects in the study similar to those in the BET/your own?	The clinical implications of this study are that the use of heat treating colostrum as the sole method of Johnes control on farm will not reduce the prevalence of MAP positive animals. Other transmission methods, such as calving practices, milk feeding management and the MAP status of

	the dam, appear to have a possible role in preventing transmission of MAP to calves. It is difficult to interpret how the heat treatment of colostrum could act in synergy with other Johnes control methods, as the study does not discuss calving practices. Heat treatment of colostrum may show beneficial effects in terms of IgG transfer and health during the preweaned period to calves fed stored pooled colostrum; however there is currently no evidence to suggest that there are any long term benefits in terms of milk production and longevity. Subjects are similar in terms of dairy calves (predominately Holsteins with some crossbreed calves); however the farm size in the study were larger and had other Johnes control methods in place, which may not apply to this farm.
General	
Who funded this study?	USDA – Cooperate State Research, Education and Extension Service (Washington DC) for the first stage of the study