| Section                  | Item | Description                                                                                                                                                                                                 | Page # |
|--------------------------|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| Title and Abstract       | 1    | Non-randomized and challenge model included in abstract and title                                                                                                                                          | 1      |
| Rationale                | 2    | “the objective of this study was to evaluate the degree of protection from a Salmonella Typhimurium challenge in calves fed colostrum from cows vaccinated with a Salmonella Newport SRP subunit vaccine. We hypothesized that administration of colostrum from cows vaccinated with a Salmonella Newport SRP subunit vaccine would reduce the mortality in calves fed this colostrum and challenged with Salmonella Typhimurium.” | 2-3    |
| Participants             | 3    | “Participants - All Holstein bull calves from single (not twins), normal, observed births born on the NC State Dairy Education Unit were eligible for inclusion in the study. Any calf that met these criteria was enrolled into the study until 10 calves were enrolled into each group.” | 4      |
| Interventions            | 4    | “Interventions - Calves were fed 4L of either CTL or VAX colostrum within 4 hours of birth. Randomization of treatments was achieved by mixing the bags of colostrum from the two groups of cows in the freezer, and feeders would choose the next available bag when a calf was born.” | 5      |
| Challenge model          | 4b   | “All calves were infected with $10^9$-$10^{11}$ cfu (range: $6.7\times10^9$ to $3.4\times10^{11}$) of Salmonella typhimurium between 10 and 14 days of age. The source was a clinical isolate originally obtained from a calf submitted to the Wisconsin Veterinary Diagnostic Laboratory.” | 5      |
| Objectives               | 5    | “Therefore, the objective of this study was to evaluate the degree of protection from a Salmonella Typhimurium challenge in calves fed colostrum from cows vaccinated with a Salmonella Newport SRP subunit vaccine.” | 1      |
| Primary and secondary outcomes | 6    | “Outcome assessment – The primary outcome assessed was mortality in calves after infection. Any calf that did not eat for 24 hours or was moribund was”                                                                 | 6      |
euthanized for humane reasons. Any calf that died or was euthanized prior to 4 days after infection was counted towards the mortality in that group. Secondary outcomes were also evaluated to determine if consumption of colostrum from vaccinated cows would impact the titers, clinical disease, or shedding of Salmonella in calves after experimental infection. These secondary assessments are detailed below.”

| Reflect Checklist | Sample size rationale | 7 | “Sample size was limited due to economic constraints.” | 4 |
| Randomization-sequence generation | 8 | “Calves were not randomly allocated to treatment groups prior to colostrum feeding.” | 4 |
| Randomization-allocation concealment | 9 | “Calves were not randomly allocated to treatment groups prior to colostrum feeding.” | 4 |
| Randomization-implementation | 10 | “Calves were not randomly allocated to treatment groups prior to colostrum feeding.” “Randomization of treatments was achieved by mixing the bags of colostrum from the two groups of cows in the freezer, and feeders would choose the next available bag when a calf was born. Treatments were not intentionally alternated or administered in blocks in order to prevent observers from determining which treatment was given to each calf.” | 4 |
| Blinding | 11 | “Pooled CTL or VAX colostrum was fed to calves and recorded by farm staff so that investigators who performed the inoculation and all assessments were blinded to the treatment. Records of which calf was in each group were maintained on the farm of origin, away from the lab animal facilities where the inoculation and assessments took place.” | 4 |
| Statistical methods | 12 | Included in methods | 10-11 |
| Results study flow | 13 | Included as Figure 1 | 3, 27 |
| Recruitment | 14 | “All Holstein bull calves from single (not twins), normal, observed births born on the NC State Dairy Education Unit were eligible for inclusion in the study. Any calf that met these criteria was enrolled into” | 4 |
Reflect Checklist

| Section                          | Page | Description                                                                 |
|---------------------------------|------|-----------------------------------------------------------------------------|
| Baseline data                   | 15   | This is included in Table 2                                                 |
| Numbers analyzed                | 16   | Included in the results                                                    |
| Outcomes and elimination        | 17   | “In the CTL group, 4/10 calves died or were euthanized prior to day 4, and 5/10 calves in the VAX group died or were euthanized early (Figure 2, Table 1). Neither the proportion of calves surviving nor the median survival time were significantly different between the two groups (Table 1). Survival was not associated with time to colostral feeding, birthweight, or inoculum dose.” |
| Ancillary Analyses              | 18   | Included in Results section                                                 |
| Adverse Events                  | 19   | There were no adverse events associated with the vaccination.               |
| Discussion interpretation       | 20   | The disease model is discussed in light of other published trials. Weaknesses associated with sample size are also discussed. |
| Generalizability                | 21   | A clinically relevant challenge model is used that recreated the clinical signs of salmonellosis in calves, but conclusions are limited by the sample size. |
| Overall evidence                | 22   | “Administration of the Salmonella Newport bacterin to dry cows did not provide protective immunity to calves in this study in spite of increased titers.” |

Numbers analyzed: 11-13

Total pages: 15-22

References: 12, 25, 11-13, 13-15, 12, 14-20