STATENS VETERINÄRMEDICINSKA ANSTALT

Slutrapport

Frihet från smittsamma sjukdomar hos nöt – vägen till bättre hälsa, produktion och resistensläge.

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Utförlig sammanfattning

Bakgrund

I Sverige finns en lång tradition av förebyggande djurhälsoarbete som bland annat innefattar att systematiskt bekämpa sjukdomar som i många andra länder är endemiska. Nötkreatursssjukdomar som hanterats på detta sätt är paratuberkulos, salmonellos, bovin virusdiarré, infektiös bovin rhinotrakeit och enzootisk bovin leukos. Frihet från endemiska sjukdomar innebär fördelar för produktion och djurvälfärd och minskar behovet av antibiotikabehandlingar och därmed risken för selektion av resistenta bakterier. Infektioner med bovint respiratoriskt syncytialt virus (BRSV) och bovint coronavirus (BCV) har ännu inte bekämpats systematiskt trots att de är vanliga hos svenska nötkreatur och att det finns kunskap och diagnostisk för ett bekämpande. Inte heller har infektioner med *Mycoplasma bovis* (MB) bekämpats systematiskt men här saknas fortfarande kunskap om förekomst och epidemiologi under svenska förhållanden.

Syfte och hypotes

Projektets syfte var att dokumentera hur förekomst av BRSV, BCV och MB i besättningar med mjölkkor påverkar produktion, sjuklighet, användning av antibiotika och förekomst av antibiotikaresistens. Hypotesen var att frihet från infektionerna innebär lägre sjuklighet och som en följd därav högre produktion och lägre antibiotikaanvändning som i sin tur leder till minskad antibiotikaresistens. Avsikten var att dokumentera eventuella fördelar med frihet från BRSV, BCV och MB som underlag för diskussioner om ett eventuellt bekämpande av sjukdomarna.

Metod

I 76 mjölkbesättningar har tankmjölk och mjölk från förstakalvare vid tre tillfällen under en två-årsperiod undersökts för antikroppar mot BRSV, BCV och MB och besättningarnas status avseende sjukdomarna fastställts. Vid tre tillfällen har

Projekt har fått finansiering genom:
Stiftelsen Lantbruksforskning

avföringsprov från kalvar undersökts för förekomst av antibiotikaresistens hos tarmbakterien *Escherichia coli (E. coli)*. Släktskap mellan kinolonresistenta *E. coli* har undersökts för att utforska spridning inom och mellan besättningar (ännu inte utvärderat). Data över sjuklighet, produktion och användning av antibiotika har samlats in via enkäter till djurägarna och från Kokontrollen, KAP, Härstamningskontrollen och seminbokföringen. Samband mellan förekomst av infektionerna och sjuklighet, produktion, antibiotikaanvändning och antibiotikaresistens har undersökts med statistiska metoder.

Sammanfattning av huvudsakliga resultat

Av de 76 besättningarna var 44 fria från BRSV och 27 från BCV, 19 besättningar var fria från såväl BRSV som BCV. I övriga besättningar cirkulerade infektionsämnena en eller flera gånger under två-årsperioden. Antikroppar mot MB påvisades i 8 mjölkprov från 7 olika besättningar vilket talar för att infektionen inte var vanlig eller utbredd i besättningarna. MB utvärderades därför inte för eventuella samband med hälso-, produktions- och antibiotikaparametrar.

BRSV: Hypotesen styrktes av statistiskt signifikanta ($p < 0,05$) samband mellan närvaro av BRSV och förekomst av hosta hos ungdjur och diarré hos kalvar och ungdjur samt multiresistens och kinolonresistens hos *E. coli*. Dessutom fanns trender ($p < 0,1$) för lägre mjölkproduktion samt högre dödlighet hos kalvar. Ett signifikant samband motsade hypotesen, ospecifik feber hos kor var mindre vanligt i besättningar där BRSV cirkulerat än i de som var fria.

BCV: Hypotesen styrktes av signifikanta ($p < 0,05$) samband mellan närvaro av BCV och förekomst av diarré hos ungdjur och kor, hosta hos kalvar, ungdjur och kor samt ospecifik feber hos kor. Hypotesen styrktes också av trender ($p < 0,1$) för fler kor med klöv- och bensjukdomar och fler antibiotikabehandlingar av kor. En trend motsade hypotesen, kalvdödligheten var lägre i besättningar där BCV cirkulerat än i fria besättningar.

Nytta för näringen

Resultat från denna och tidigare studier tyder på att frihet från BRSV och BCV i mjölkbesättningar har positiv påverkan på såväl djurhälsa som produktion. Detta talar för etablering av kontrollprogram och sådana har startats i Norge. Att mjölkbesättningar kan hållas fria framgår av denna studie där en stor andel av besättningarna upprätthöll BRSV och/eller BCV-fri status under hela den tvååriga studieperioden. En nytto-kostnadsanalys skulle dock vara värdefull som underlag för vidare diskussioner.

Frihet från BRSV och BCV i mjölkbesättningar skulle sannolikt även gynna besättningar som föder upp kalvar till slakt. I dessa är luftvägs- och tarmsjukdomar orsakade av BRSV och BCV vanliga och ofta introduceras smittämnena med kalvar som förmedlats från mjölkbesättningar.

De smittskyddsåtgärder som krävs för kontroll av BRSV och BCV skulle troligen minska spridningen även av andra infektionsämnen och antibiotikaresistenta bakterier. Förbättrad djurhälsa skulle sannolikt på sikt minska behovet av antibiotikabehandlingar och därmed selektionstrycket för uppkomst och spridning av antibiotikaresistens.

Introduction

Background

Respiratory and enteric diseases in Swedish dairy cattle.

Bovine respiratory syncytial virus (BRSV) and bovine corona virus (BCV) are important pathogens affecting dairy and beef cattle worldwide (1, 2). BRSV causes respiratory tract disease and BCV enteritis with respiratory tract involvement. Affected cattle are often treated with antibiotics due to secondary bacterial infections. Both infections are common in Swedish cattle (2, 3) and impact both animal welfare and production (4, 5). Research in Sweden and Norway has addressed the epidemiology of both infections and control programs based on biosecurity measures have been suggested (6, 7). Such a program was launched in Norway 2016 (8).

Mycoplasma bovis (*M. bovis*) is a bacterium affecting cattle worldwide causing respiratory disease and mastitis as well as other clinical signs (9). The bacterium has been detected in Swedish cattle in association with respiratory disease and mastitis (10) but its epidemiology and importance in Sweden is largely unknown.

Antibiotic resistance

Effective antibiotics are vital in human and animal healthcare, but overuse and misuse has fuelled emergence of antibiotic resistance (AMR) which is recognized as a global threat to human healthcare (11). AMR in animal bacteria can spread to humans and thus impact human healthcare (12) but may also cause setbacks for food production by emergence animal diseases for which effective treatments are lacking (13, 14).

To mitigate AMR, several actions are proposed (15, 16). Specifically stressed in the EU action plan, and recognized also by OIE (14), is improved animal health. The rationale for this is that healthy animals don't need antibiotics and the selection pressure for AMR is thereby reduced. To show that improved animal health counteracts AMR would be a strong impetus for preventive measures, but such studies are lacking.

Aims and hypotheses

To support decisions on control of BRSV, BCV and MB, it would be valuable to document the possible benefits for herd health, productivity, antibiotic consumption and AMR. The aim of this project was therefore to investigate if occurrence of BRSV, BCV and MB in dairy herds affects:

- Herd health status (udder health, disease morbidity, culling rate and calf mortality).
- Productivity (milk yield and fertility data).
- Incidence of antibiotic treatments (owner reported treatments).
- Antibiotic resistance (AMR in *Escherichia coli* from feces of calves).

The hypothesis of this project was that freedom from BRSV, BCV and/or MB leads to:

- Better herd health.
- Higher productivity.
- Lower incidence of antibiotic treatments.
- Lower occurrence of AMR in *E. coli* and lower prevalence of *E. coli* resistant to quinolones (QREC) and tetracyclines (TREC).

Materials and Methods

Field study

The hypothesis was tested in a field study running over a two-year period. In the autumn of 2015, 635 dairy herds were invited to the study based on BRSV and BCV antibody status in a nationwide screening of bulk tank milk in 2013 (A. Ohlson, unpublished data). Invited herds were selected to maximize the variation in antibody status to the two infections. Only herds with at least 30 lactating cows and affiliated to the Swedish official milk recording system (SOMRS) were invited. In all, 111 herds expressed an interest to participate and were enrolled in the study.

Sampling and laboratory analyses

Sampling of milk and faeces

Bulk tank milk (BTM), pooled milk from 3 primiparous heifers (PPM) and faeces from up to 6 calves, about 1 month old or younger, were collected when a herd entered the study (autumn 2015). The same types of samples were collected about one year later (autumn 2016) and again about two year after the start of the study (autumn 2017). Samples were collected by farm personnel and sent to SVA by mail.

Serology on milk samples

Samples of BTM and PPM were analysed for antibodies to BRSV, BCV and MB with indirect ELISAs[1] according to the manufacturer's recommendations. Herd owners were informed of their herd's BRSV and BCV status after each sampling.

BRSV and BCV infection status

For each herd, a specific period at-risk for BRSV and BCV infections was determined from the first to the third sampling. Herds were categorized for presence of viral infections in this period based on antibody status at the three samplings. Herds were considered as recently infected (RI) if antibody negative at the first or second samplings but positive in the third sampling and presumably past steadily infected (PSI) if positive in all three samplings. RI and PSI herds were presumed to have had at least one viral infection during the period. Herds were presumed to be free of infection (FREE) if antibody negative in the third sampling, regardless of the result in the first and second samplings. Assuming an average calving age of about two years, sampled primiparous cows have been in the herd for the last two years and a negative third sampling therefore indicates that no virus infection has occurred during this period.

Antimicrobial resistance of *Escherichia coli* from faeces

Faeces was collected from the rectum of calves using Amie's charcoal swabs[2]. Samples were cultured to determine the prevalence of QREC and TREC in the sample. In short, rectal swabs were vortexed in 0.9% saline and ten-fold dilutions of this suspension were plated on Petrifilm Select *E. coli* Count[3] without antibiotics and on plates with nalidixic acid (32 mg/L) or tetracycline (64 mg/L). The within-sample prevalence of QREC and TREC was calculated as the ratio of TREC and QREC colonies on plates with antibiotics to the number of *E. coli* colonies on plates without antibiotics. In addition,

[1] Svanovir BRSV-Ab and Svanovir BCV-Ab, Boehringer Ingelheim Svanova, Uppsala, Sweden; ID Screen Mycoplasma bovis Indirect, ID vet, Grabels, France

[2] Copan Diagnostics Inc., Murrieta, USA

[3] SEC plate; 3M Microbiology Products, St. Paul., MN, USA

for one randomly selected *E. coli* from each sample, minimum inhibitory concentrations (MIC) to 13 antimicrobials were determined by microdilution according to the recommendations of the Clinical and Laboratory Standards Institute (17) using VetMIC panels[4]. Epidemiological cut-off values from the European Committee on Antimicrobial Susceptibility Testing (www.eucast.org) were used to classify isolates as wild-type or non-wild-type, where the latter were considered resistant. Isolates resistant to colistin were tested by PCR for transmissible genes conferring colistin resistance and isolates resistant to cefotaxime/ceftazidime were tested by WGS for genes conferring resistance to these antimicrobials using Illumina based technologies.

Genetic relatedness of QREC within and between farms on a subset of 258 QREC-isolates from 61 of the herds was investigated by WGS. From herds where QREC were isolated at all three samplings, 2 isolates per sampling were randomly selected for sequencing and from herds where QREC were not isolated at sampling 1 but at later samplings, all isolates from samplings 2 and 3 were selected.

Collection of epidemiological data

Questionnaire

At eight occasions during the two-year study period, from November 2015 to October 2017, herd owners/farm staff responded to questionnaires on the health status of the herd. Each questionnaire covered the preceding two months but for practical reasons the summer months (May-August) were not covered. Thus, information from each herd was gathered for 16 of the 24-month study period. Questionnaires contained rating questions on the incidence of diarrhea and cough, in calves < 6 months, in young stock including bulls and cows. The incidence was rated as “none”, “a few”, “one fourth of the age group”, “half of the age group”, and “more than half of the age group”. Also included were questions on the number of cows that had experienced: mastitis/high somatic cell count, hoof or leg disorder, feed-related disorders, metritis/retained fetal membranes, fever with unknown reason, and abortion. The number of young stock and calves <6 months with hoof or leg disorders or with dullness without obvious reason were also reported, as were the number of calves <6 months with umbilical infections. In May each year, a question on vaccination against BRSV or BCV during the previous year was added. Owners/farm staff could choose to respond to questionnaires via post, a web-based platform or by telephone.

Databases

Herd data on milk production, somatic cell count (SCC), and reproductive events (calvings, AI, and pregnancy checks), entrance and exit dates as well as reasons for exit for all animals present at any time during the study period was obtained from SOMRS

Statistical analysis

It was assumed that all outcomes may be affected by both infections and therefore, all models were run with both BRSV and BCV statuses as covariates and estimates of the effect of one infection were adjusted for the possible effect of the other. Each outcome was adjusted for the potential effects of other biological covariates. All models were reduced by stepwise backwards eliminations to only include covariates that significantly

[4] National Veterinary Institute, Uppsala, Sweden

affected the outcome or covariates that changed the estimates of remaining covariates by more than 30% (confounding).

Depending on the model type, either hierarchical mixed models considering herd and individual as random effects, or a robust variance estimator was used to account for clustering of observations at the herd or individual level. All analyses were performed in Stata 15[5]. Linear regression was conducted using the MIXED command, logistic regression using MEGLM, Cox proportional hazard using STCOX, and fractional probit regression using FRACGLM. A significance level of 5 % was used to define a statistically significant difference and a level between 5 and 10 % to define a statistical trend/tendency.

Results and discussion

Descriptive statistics

Herds

In all, 111 herds (17% of the invited) with a median herd size of 65 cows were enrolled in the study in the autumn of 2015. Eight herds did not send faecal samples at sampling 1 and were excluded from the study. An additional 16 herds dropped out at sampling 2 and 11 more at sampling 3, resulting in 76 herds that completed the whole study. The median herd size for these herds was 58 cows and they were in East (14%), South (41%) and North (45%) Sweden (NUTS1). The reasons for dropouts were not investigated.

The median herd size of Swedish dairy herds with at least 30 cows, was 68 in 2013 (A. Ohlson, unpublished data) and herds in the study can be considered representative for Swedish dairy farms regarding herd size. However, the geographical localisation of Swedish dairy farms (≥ 30 cows) in 2013 was 10%, 71% and 19% for East, South and North Sweden, respectively. Accordingly, the study was skewed towards herds in the North of Sweden. This is likely due to the selection of herds for invitation which was made to maximize the variation in antibody status to BRSV and BCV. More herds form the North were enrolled because more herds are free of the infections in the North than in the South of Sweden. The geographical location was considered in the statistical analyses and was not assumed to affect the conclusions in the study.

Antimicrobial resistance

From the faecal samples collected (1570 calves, 103 herds), 1544 isolates of *E. coli* were obtained and tested for antimicrobial susceptibility (Table 1). Overall, 52,5% of the isolates were susceptible to all antimicrobials and 21.2% were multidrug resistant (MDR), i.e. resistant to three or more antimicrobial classes. Resistance levels are in accordance with previous data for *E. coli* from young calves in Sweden as also the observation that resistance was more common in isolates from younger calves (data not shown) (18, 19). Transmissible genes conferring resistance to third generation cephalosporins were not detected in the 22 cefotaxime/ceftazidime resistant isolates but mutations in the AmpC promoter genes were identified in 9 of the isolates. Likewise, transmissible genes (*mcr*) were not detected in the 48 colistin resistant isolates.

[5] StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC

Table 1. Antimicrobial resistance in 1544 *E. coli* isolated from faeces of calves 1-10 weeks old. MICs interpreted by ECOFFs issued by EUCAST (www.eucast.org).

		Percent resistant isolates			
Antimicrobial	**ECOFF** (mg/L)	**Sampling 1** (606 calves) (103 herds)	**Sampling 2** (493 calves) (85 herds)	**Sampling 3** (445 calves) (77 herds)	**Total** (1544 calves) (103 herds)
Ampicillin	>8	17,3%	20,7%	20,2%	**19,2%**
Cefotaxime	>0.25	1,0%	1,0%	1,3%	**1,1%**
Ceftazidime	>0.5	1,3%	1,2%	1,8%	**1,4%**
Chloramphenicol	>16	2,8%	1,8%	4,9%	**3,1%**
Ciprofloxacin	>0.06	3,5%	6,1%	5,8%	**5,0%**
Colistin	>2	2,5%	0,6%	6,7%	**3,1%**
Florfenicol	>16	0,3%	0%	0,2%	**0,2%**
Gentamicin	>2	0%	0%	0,2%	**0,1%**
Nalidixic acid	>16	5,1%	6,5%	7,0%	**6,1%**
Streptomycin	>16	34,8%	34,3%	31,9%	**33,8%**
Sulfamethoxazole	>64	25,9%	27,8%	28,1%	**27,1%**
Tetracycline	>8	15,8%	22,9%	15,7%	**18,1%**
Trimetoprim	>2	5,0%	5,7%	9,0%	**6,3%**

MB, BRSV and BCV serology

Of the 111 herds enrolled, 87 provided milk samples at samplings 1 and 2 and 76 of these also for sampling 3.

Mycoplasma bovis. Of all 548 milk samples from 111 herds collected and analysed, 8 samples from 7 herds (6.3%) were positive for antibodies to MB. In 1 herd, PPM samples were positive but BTM samples negative at samplings 1 and 2 but both PPM and BTM were negative at sampling 3. In 5 herds, PPM was positive at a single occasion in each herd but all other PPM and BTM samples were negative. In 1 herd, BTM was positive at sampling 3 but all other PPM and BTM samples from the herd were negative. The results indicate that MB was not widespread or continuously present in the herds and MB status was not evaluated for associations with outcome parameters.

BRSV and BCV. In the first PPM sampling, 30% of the 76 herds that completed the whole study were both BRSV and BCV antibody positive, 32 % were only BCV positive, 9 % were only BRSV positive, and 29 % were negative to both viruses. For samplings 2 and 3, the corresponding figures were 38%, 32%, 5%, and 25%, and 32%, 33%, 11% and 25%, respectively. Based on the antibody status in PPM samples, herds were categorized as FREE, RI and PSI during the two-year period (Table 2).

Table 2. Categorization of herd infection status during the study period according to the herd antibody status in each milk sampling for BRSV and BCV in the 76 herds that completed the study.

Milk sampling occasion Antibody positive (+) or negative (-)					
1	**2**	**3**	**Herd status**[1]	**BRSV** (number of herds)	**BCV** (number of herds)
-	-	-	FREE	29	14
+	-	-	FREE	8	4
+	+	-	FREE	3	9
-	+	-	FREE	4	0
-	-	+	RI	2	5
+	-	+	RI	4	0
-	+	+	RI	11	10
+	+	+	PSI	14	31
			VACCINATED	1	3

[1]FREE=free of infection, RI=Recently infected. PSI=Presumably past steady infected.

Regarding BRSV, 44 (58%) of the 76 herds were considered FREE, throughout the two-year study period, 14 (18%) PSI and 17 (22%) RI. Regarding BCV, 27 (36%) herds were considered FREE, 31 (41 PSI and 15 (20%) RI. Nineteen herds (25%) were considered free from both BRSV and BCV throughout the two-year study period.

Questionnaire data

The response rate to the questionnaires of the 76 herds that completed the whole study was 98% and the proportion of herds that fully completed the questionnaires was 76%. The questions on incidence of hoof or leg disorders and dullness in young stock had more than 10% missing values and were excluded from further analyses.

Of the 76 herds that completed the whole study, 22% and 12% reported at least once that half or more of the calves had diarrhoea and cough, respectively. The corresponding figures for young stock were 16% and 8%. For cows, 29% and 24% of the herds reported at least once that one fourth or more of the cows had diarrhoea and cough, respectively. All herds had at least one case of udder disease during the questionnaire period (16 months). Hoof-or leg disorders was reported at least once in 75 of the herds, metritis/retained foetal membranes in 63 herds, feed-related disorders in 60 herds, abortions in 57 herds and fever without obvious reason in 33 herds. Fifty-nine of the herds reported at least one case of dull calf without obvious reason, hoof- or leg disorders in calves in 35 herds and umbilical infection in 28 herds.

Antimicrobial treatment of cows was reported at least once during the questionnaire period (16 months) in 73 of the 76 herds, of calves in 58 herds, and of young stock in 23 herds. Dry cow antimicrobial treatments were conducted in 65 of the76 herds. Further descriptive statistics for questionnaire data are shown in Table 3.

Table 3. Descriptive statistics of outcome parameters based on questionnaire data for the 76 herds that completed the study. Response rates for each question are indicated.

Outcome parameters	Response rate	Descriptive statistics (unit)				
Occurrence of diarrhea and cough[1]		**None**	**A few**	**One fourth**	**Half**	**More than half**
Calf diarrhea (% of ratings)	98%	25	57	11	3	4
Young stock diarrhea (% of ratings)	96%	84	13	1	1	1
Cow diarrhea (% of ratings)	96%	65	30	1	1	3
Calf cough (% of ratings)	98%	49	43	7	1	0.3
Young stock cough (% of ratings)	97%	71	25	3	1	0
Cow cough (% of ratings)	97%	78	17	3	1	1
Occurrence of other diseases		**P10**	**P25**	**Median**	**P75**	**P90**
Udder disease (% of cows in the herd)	97%	0	1.5	2.9	5.3	8.3
Non-specific fever – cows (% of cows in the herd)	94%	0	0	0	0	0.8
Hoof and leg-disorders – cows (% of cows in the herd)	97%	0	0	2.1	3.5	5.2
Feed-related disorders – cows (% of cows in the herd)	95%	0	0	0	1.1	2.4
Metritis (% of cows in the herd)	96%	0	0	0	1.3	2.3
Abortions (% of cows in the herd)	95%	0	0	0	0.3	1.5
Umbilical infection – calves (% of calves in the herd)	95%	0	0	0	0	0.9
Hoof and leg-disorders – calves (% of calves in the herd)	95%	0	0	0	0	2.0
Dullness without reason – calves (% of calves in the herd)	95%	0	0	0	1.8	4.5
Antimicrobial treatments		**P10**	**P25**	**Median**	**P75**	**P90**
Lactating cows (% of lactating cows in the herd)	97%	0	0	2.1	3.9	7.1
Dry cows (% of dry cows in the herd)	92%	0	0	6.2	17.9	32.4
Young stock (% of young stock in the herd)	95%	0	0	0	0	0
Calves (% of calves in the herd)	96%	0	0	0	3.1	5.7

[1]Used as binary variables in the statistical analyses, such as no diarrhea/cough versus diarrhea/cough in a few or more·

SOMRS data

For the 76 herds completing the study the median (interquartile range) milk yield for the 127 624 test days was 32 (26-38) kg ECM/cow and SCC 61 (26-166) thousands of cells per ml milk. Of the 11 995 calves aged 1-59 days, 2% died during the study period, (mortality rate at 0.04 deaths per 100 cattle days). Of the 10 984 calves aged 60-179 days, 1% died (mortality rate 0.01 deaths/100 cattle days). Of the 11 790 young stock, aged 180-455 days, 0.8 % died (mortality rate 0.005 deaths/100 cattle days) and of the 9 669 cows, 40 % were culled (mortality rate 0.09 deaths/100 cattle days). The median (interquartile range) of age at first calving for 6 846 cows in the 76 herds was 26.0 months (24.6-27.9) and the median (interquartile range) of calving interval for 8047 cows was 12.4 months (11.4-13.9 months). For the started breeding of 13 115 cows and heifers, 49% were considered reproductive failures, i.e. having more than one breeding (natural or artificial) per confirmed pregnancy.

Effect of infection status

For outcomes where at least a statistical trend ($p<0.1$) was observed, the direction of the effect of BRSV and BCV infection status is shown in Table 4. Adjusted values, predicted probabilities, and hazard and odds ratios are presented in the text below.

BRSV. Compared to FREE herds, PSI herds had a lower milk yield per cow (29.7 kg ECM/day vs. 31.9 kg ECM/day, p=0.087), less cows with non-specific fever (0.079% vs. 0.41%, p=0.002), a higher within-sample prevalence of QREC (4.9 % vs 1.2%, p=0.025), a higher probability of diarrhoea in calves (Odds ratio 1.5, p=0.086) and young stock (Odds ratio 2.3, p=0.095), and of cough in young stock (Odds ratio 5.8, p=0.005).

Compared to FREE herds, RI herds had a higher mortality for calves under the age of 2 months (hazard ratio 1.4, p=0.062) and for calves 2-6 months for RI (hazard ratio 1.6, p=0.075), a higher mean within-sample prevalence of QREC (5.0 % vs 1.2%, p=0.012), more MDR *E. coli* (Odds ratio 1.8, p=0.038) and a higher probability of diarrhoea in calves (Odds ratio 1.7, p=0.013) and young stock (Odds ratio 2.6, p=0.037).

BCV. Compared to FREE herds, PSI herds had a lower mortality for calves under the age of 2 months (hazard ratio 0.7, p=0.094), more cows with hoof or leg disorders (2.8% vs. 2.1%, p=0.093), more cows with non-specific fever (0.48% vs. 0.064%, p=0.001) herds, more antimicrobial-treated cows (3.3% vs. 2.4%, p=0.080) and a higher probability of diarrhoea in young stock (Odds ratio 2.8, p=0.036) and cows (Odds ratio 3.8, p=0.005), and of cough in calves (Odds ratio 2.8, p=0.049), young stock (Odds ratio 6.3, p=0.001) and cows (Odds ratio 13.1, p=0.001).

Compared to FREE herds, RI herds had a higher probability of diarrhoea in young stock (Odds ratio 4.7, p=0.004) and cows (Odds ratio 3.8, p=0.011) and of cough in calves (Odds ratio 7.1, p=0.002), young stock (Odds ratio 7.2, p=0.003) and cows (Odds ratio 11.4, p=0.009).

Summary and Conclusions

Overall, the results support the hypothesis of better health status, higher productivity, fewer antimicrobial treatments and lower occurrence of AMR in dairy herds free from BRSV and/or BCV. Of the 28 outcome parameters, 5 supported the hypothesis for BRSV with statistical significance and 6 for BCV (Table 4). Also, 3 trends supported

the hypothesis for BRSV and 2 for BCV. In contrast, only 1 outcome parameter refuted the hypothesis for BRSV with statistical significance and 1 trend for BCV.

The study indicates a better health status in herds free from BRSV and/or BCV. Comparisons to other studies is complicated by differences in study designs but both BRSV and BCV have previously been associated to disease in cattle. Thus, BRSV has been shown to be linked to respiratory disease in dairy herds (20) and young stock (21). BRSV was also linked to higher SSC in milk, indicating udder disease (5, 22). Likewise, BCV has been linked to respiratory disease in feedlot cattle (23) and dairy cows as well as to diarrhoea in calves (24).

The effects on productivity were less apparent in this study although there was a trend for lower milk production in BRSV PSI herds. A variable negative effect on milk production was previously shown for both BRSV (5, 25, 26) and BCV (4, 24). Also, weight gain of young stock was associated with BRSV (27).

Table 4. Overview of the effect of BRSV or BCV infection on outcomes variables. Upward arrows denote a higher value and downward a lower value. Two arrows denote a statistically significant effect ($p<0.5$), one arrow a statistical trend ($0.5<p<0.1$), and a dash neither significant nor a statistical trend ($p>0.1$).

Outcome variable	BRSV		BCV	
	PSI	RI	PSI	RI
Productivity				
Milk production	↓	-	-	-
Reproductive failure	-	-	-	-
Calving interval	-	-	-	-
Age at first calving	-	-	-	-
Mortality				
Calves 1–59 days	-	↑	↓	-
Calves 60–179 days	-	↑	-	-
Young stock 180–455 days	-	-	-	-
Cows	-	-		
Morbidity				
Diarrhea calves	↑	↑↑	-	-
Diarrhea young stock	↑	↑↑	↑↑	↑↑
Diarrhea cows	-	-	↑↑	↑↑
Cough calves	-	-	↑↑	↑↑
Cough young stock	↑↑	-	↑↑	↑↑
Cough cows	-	-	↑↑	↑↑
Udder disease	-	-	-	-
Somatic cell count	-	-	-	-
Non-specific fever cows	↓↓	-	↑↑	-
Hoof- or leg disorders cows	-	-	↑	-
Feed-related disorders cows	-	-	-	-
Metritis/retained fetal membranes	-	-	-	-
Abortions	-	-	-	-
Other disease calves	-	-	-	-
Antimicrobial treatments				
Cows	-	-	↑	-
Calves	-	-	-	-
Dry cows	-	-	-	-
Antimicrobial resistance				
Within-sample prevalence of QREC[1]	↑↑	↑↑	-	-
Within-sample prevalence of TREC[2]	-	-	-	-
Multi drug resistance	-	↑↑	-	-

[1]Quinolone-resistant *E. coli;* [2]Tetracycline-resistant *E. coli*

Although respiratory disease and diarrhoea was more common in herds with BRSV and/or BCV an association between infection status and antimicrobial treatments was not documented, except for a trend of more treatments of cows in PSI BCV herds. This indicates that observed clinical signs mostly were not associated with severe disease that required antimicrobial treatment. An increased treatment incidence for respiratory disease in young stock has however previously been documented for both BCV (23) and BRSV (21).

Nevertheless, in the present study both multidrug resistance in *E. coli* and prevalence of QREC in faeces of young calves were associated with BRSV. Antimicrobial use is a strong driver for resistance, but resistance can spread and persist also on farms where antimicrobial use is low, for example on farms with poor biosecurity and hygiene (28). In a previous study from our group we showed that, apart from use of quinolones, poor external biosecurity and poor hygiene were associated with occurrence of QREC on dairy farms (29). The lower occurrence of antimicrobial resistance in BRSV FREE herds observed in the present study could therefore be due to a higher level of biosecurity than in BRSV positive herds.

Benefits and recommendations

The results from this and previous studies strongly indicate that efforts to reduce occurrence of BRSV and BCV in dairy herds would improve animal health and production. Available scientific evidence therefore supports efforts to establish control programs for BRSV and BCV in dairy herds. The measures and logistics needed for control are known and control programs are established in Norway, but cost benefit analyses would be valuable in discussions on possible implementation in Sweden.

The feasibility of effective control is demonstrated by the fact that 58% of the herds in the study upheld a BRSV FREE status throughout the two-year study period, 36% a BCV FREE status and 25% a FREE status for both diseases.

Additionally, control of BRSV and BCV in dairy herds is likely to be beneficial also for farms raising calves purchased from dairy herds for slaughter. In these farms respiratory tract infections caused by BRSV and BCV are common, and the infections are often introduced through shipment of calves from dairy herds.

The biosecurity measures needed for control of BRSV and BCV in dairy herds would probably also mitigate spread of other infectious agents including antimicrobial resistant bacteria. Improved animal health in cattle would likely in a longer perspective also reduce the overall need for antimicrobial treatments and thereby the selection pressure towards resistant bacteria.

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Resultatförmedling

Vetenskapliga publiceringar	*Associations between Bovine Coronavirus and Bovine Respiratory Syncytial virus infections and productivity, mortality, health status and occurrence of antimicrobial resistance in Swedish dairy herds*. Duse, Ohlson, Stengärde, Tråvén, Alenius & Bengtsson (Submitted to Journal of Dairy Science, January 2020)
	Genetic relatedness of quinolone resistant Escherichia coli from the intestinal flora of young dairy calves. Börjesson, Duse, Björklund, Myrenås & Bengtsson. (Manuscript in preparation)
Övriga publiceringar	*Många goda skäl att bekämpa virussmittor*. Duse A. Husdjur 1 2020.
	Många goda skäl att bekämpa virussmittor. Duse A. Nötkött (Inskickad januari 2020)
	Frihet från BRSV och BCV – effekter på djurhälsa och antibiotikaresistens i mjölkbesättningar. Svensk Veterinärtidning. (Inskickad januari 2020)
Muntlig kommunikation	*Frihet från BRSV och BCV i mjölkbesättningar - ett nytt forskningsprojekt vid DOA.* Bengtsson, B. SVA:s projektråd, Sigtuna 2015.
	Freedom from contagious diseases in cattle - means to improve health and reduce resistance. Duse A. Global Challenges University Alliance 8th workshop on livestock production in the post antibiotic era. Uppsala 2015
	Frihet från smittsamma sjukdomar hos nötkreatur - Vägen till bättre hälsa, produktion och resistensläge? Duse A. Växas Vårmöte. 2016.
	Frihet från BRSV och BCV – effekter på djurhälsa och antibiotikaresistens i mjölkbesättningar Tråvén, M. Idisslarmedicin-SLU/Lantbrukets Djur-SVA samarbetsmöte om forskning och undervisning, planerat våren 2020.
Studentarbete	Inget.
Övrigt	Resultat av serologiska undersökningar avseende BRSV och BCV har löpande kommunicerats till deltagande besättningar.
	Data för resistens hos *E. coli* har presenterats i övervakningsrapporten SWEDRES-SVARM 2017.